Applications of stable carbon and oxygen isotope analysis to some aspects of coastal environmental change.

Edward James Twiddy

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University of Durham, Department of Geography.

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Abstract.

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Two projects were undertaken to assess the possibility of using the stable isotope composition of plant cellulose in reconstructions of coastal environments. One was specific to the common reed, *Phragmites australis* (Cav.) Trinius ex. Steudal, which is often found as a macrofossil in coastal sediment sequences. Analysis of material from 3 contemporary communities at Roudsea Marsh, Cumbria, U.K., which are subject to different frequencies of inundation by the tide, did not produce coherent or consistent intra- or inter-community patterns in either $\delta^{13}C$ or $\delta^{18}O$. Local tide gauge records and data on sediment characteristics, soilwater salinity and isotopic composition, and plant habit all pointed towards significant chemical and physical differences between the three communities analysed. However, the extent of intra-community and intra-plant variation in both the isotopic ratios was such that it was impossible to assign particular values of $\delta^{13}C$ or $\delta^{18}O$ to particular communities. The possible reasons for this are discussed.

The second project focused on a species-poor saltmarsh in western Scotland at Kentra Bay, Argyll. Here the contemporary marsh is backed by an extensive raised bog (Kentra Moss), below which are late Holocene sub-fossil saltmarsh deposits. The project compared $\delta^{13}C$ values from all the species present in 4 contemporary saltmarsh communities in Kentra Bay with $\delta^{13}C$ values from bulk plant cellulose extracted from the sub-fossil saltmarsh peats below Kentra Moss. Microfossil and stomatal density data were also collected to assess the efficacy of this approach, but the sub-fossil data bore no resemblance to the contemporary data. Possible reasons for this are discussed. It is concluded that although neither project directly adds to our understanding of coastal palaeoenvironments, both highlight other possible coastal applications of this technique.

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Declaration

This thesis is the result of my own work. Data from other authors which are referred to in the thesis are acknowledged at the appropriate point in the text.

Statement of Copyright

Abbreviations used in the text.

A ; the assimilation rate of a plant.

ABA ; abscisic acid.

C_3 ; those plants which dominantly utilise Rubisco (see below) to metabolise carbon.

C_4 ; those plants which dominantly utilise PEP (see below) to metabolise carbon.

CAM ; those plants which adopt Crassulacean Acid Metabolism.

^{13}C ; carbon of isotopic mass 13.

H.A.T. ; Highest Astronomical Tide.

LOD% ; percentage of original sample mass lost on drying.

LOI% ; percentage of dried sample lost on ignition.

M.H.W.N.T. ; Mean High Water Neap Tides.

M.H.W.S.T. ; Mean High Water Spring Tides.

^{18}O ; oxygen of isotopic mass 18.

p_i ; the partial pressure of carbon dioxide within the leaf vacuole.

p_a ; the ambient partial pressure of carbon dioxide.

PDB ; PeeDee Belemnite (used as a carbon isotope standard)

PEP ; phosphoenolpyruvate carboxylase.

ppt ; parts per thousand.

Rubisco ; ribulose-1,5-bisphosphate.

SMOW ; Standard Mean Ocean Water (used as an oxygen and hydrogen isotope standard).

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Chapter 1

Aims, Scope and Limitations.

1.1 Introduction and aims.

The dynamic nature of coastal environments has been a focus of scientific study for many years, but the investigation of sea-level changes through the recent geological past has only reached a position of academic maturity in the last couple of decades. The establishment of a particular approach to field work and laboratory examination through the 1970s and 1980s has produced a thriving sub-discipline within the study of Quaternary environments. This approach concentrates on systematic data collection in the field and detailed micropalaeontological examination of sedimentary units in the laboratory, combined with radiocarbon dating of organic units found adjacent to inorganic units. The vocabulary used is objective and in itself does not imply an actual change in relative sea-level (for example, regressive/transgressive overlaps), and together this results in the collection of a set of data points (sea-level index points) with particular ages, altitudes, indicative meanings and grid references which form a picture of the tendencies of change through time in a geographical region. This technique (reviewed by Shennan & Tooley 1987) has been successfully applied in high and low latitudes to areas where sediments have accumulated to leave a stratigraphical history of the dynamism of coastal environments.

However, there are still some particularly important aspects of coastal environmental change which have not been adequately investigated. In particular, no record of the frequency and intensity of coastal storms and flooding exists for the pre-historic period, and the initial focus of this thesis was to attempt to find a novel method for determining the incidence of coastal flooding.

The examinations of the litho- and bio-stratigraphy of coastal sediments around the British coast, which have been so successful in the determination of changes in relative sea level, have not yielded a complete reconstruction of these short term inundation events. This may reflect the generally destructive nature of the waves during storm events (Hardisty 1990) which result in the movement of sediment away from the coast (Morton 1981). This means that very little marine sediment is deposited on to land areas (Englefield *et al.* 1990), making identification of these events in stratigraphic sequences of coastal sediments very difficult. Examination at the University of Cambridge of the aerial photographs taken immediately after the 1953 flood event along the coast of Lincolnshire and Norfolk confirmed that erosion had taken place along most of the coastline covered by these photographs. The only areas where marine sediments had covered the land were immediately adjacent to the points where the sea had breached the coastal defences (see also Barnes & King 1953 and Steers 1953). At these locations small fans of sediment had formed. Unless the path of flood water into a coastal lowland is controlled by some topographic feature, it is unlikely that a long series of such lithostratigraphic markers would occur in the same area.

The entrance to many of the coastal topographic basins along the coast of Western Scotland, which are the focus of investigation by members of the Environmental Research Centre, University of Durham, is constrained by local bedrock relief. The Holocene sedimentary record in these basins is being investigated using high-resolution (1mm slices) microfossil analysis, mineral magnetic analysis and X-ray examination in order to determine whether a record of flooding caused by storm events can be revealed from these locations (Dr.Frances Green, pers. comm.).

However, in many of the areas which are most affected by coastal flooding the sedimentary record is not contained within such tightly constrained topographic basins. Consequently the opportunities to examine the deposits immediately behind the point where inundation takes place are restricted, and their discovery may rely on serendipity during coring. As a result it was decided to examine other aspects of coastal storms and inundation by sea water onto low lying land which might leave a reliable mark in the sedimentary record.

The most apparent difference between sea water and fresh water is the higher level of dissolved mineral salts in sea water (Aston 1978). A storm which causes coastal lowlands to be flooded introduces sea water containing this higher concentration of salts to areas which are normally dominated by fresh water with much lower salinity levels. A persistent record of this short term change in salinity across flooded coastal lowlands could serve as the basis for establishing a chronology of past storms. However, mineral salts are concentrated in sea water because of their inherent solubility, which means that they are not necessarily reliable, persistent palaeosalinity indicators. One possible exception which has been used as a palaeosalinity indicator is the level of boron in sediments (Levinson & Ludwick 1966). This element is concentrated in sea water relative to fresh water in the ratio 500:1, and its concentration has been used by some as the basis for the classification of sedimentary strata which formed under marine conditions (Liss & Pointon 1973). However, the use of this element as a palaeosalinity indicator was criticised by Perry (1972). From the evidence in this paper it seems that boron concentrations in sediments are subject to considerable variation according to changes in the clay mineral composition of the sediment. Unless the clay mineral composition of the sediment is constant it is not possible to compare measurements of boron concentration with a view to establishing palaeosalinity. Since palaeosalinity is the proxy by which the incidence of flooding would be established, it was decided to examine alternative aspects

of coastal storms which might produce a reliable environmental record of coastal storminess.

The high salt concentration in sea water means that many plants are unable to withstand inundation by the sea. However, certain plants rely on an input of mineral salts from the sea for their survival. Periodic or aperiodic changes in storminess over several decades or centuries might result in changes in the local distribution of these plant species, and also those species which are salt-sensitive or halophobic. If changes in the range of a plant species (or whole community) are to be used as the basis for reconstructions of past storminess, the plant must rely solely on the sea for its continued existence in a particular location. If the necessary nutrients to sustain it are available from another source, changes in the abundance of this source may be the cause of changes in the range of the species. An examination of the present ranges of different plant species around the British Isles revealed that in some areas the distribution of the black rush (*Schoenus nigricans* L.) is dependent on a significant marine influence (Sparling 1967). Although found elsewhere on mineral soils, it is found on the organic-rich soils along the coasts of Western Ireland and North West Scotland because of the considerable flux of mineral salts from sea spray to these otherwise unsuitable soils . Changes in storminess might result in changes in the distribution of this species due to fluctuations in the supply of the mineral salts it requires. However, this species does not produce identifiable macrofossils and its pollen is indistinguishable from that produced by other members of the Cyperaceae. Consequently it does not leave any mark of its range in the sedimentary record which could be used as a proxy indicator of variation in the wind-driven flux of sea salts.

During the investigation of the chemical differences between sea water and fresh water, and the environmental systems which might record these differences on a time scale relevant to the study of storms, another possible approach to this problem came into focus. This was based on the different isotopic composition of sea water and fresh water

(Craig 1961a). The significant differences in the ratios of ^{18}O to ^{16}O and of ^{2}H (D) to ^{1}H in sea water and fresh water suggested that it might be possible to use these differences to identify fluctuations in the input of sea water and fresh water, such as during the inundation of coastal lowlands during storm events.

This would only be possible if a record exists which might reflect such changes in the isotopic composition of the water. Such a record would involve a system which used the water around it and could be considered a black box recorder of the isotopic composition of this water. A parallel between this study and the reconstruction of past temperature and climate using the variations in the isotopic composition of cellulose in tree rings (for example by Krishnamurthy & Epstein 1985) became apparent.

Isotope dendrochronology relies on being able to relate changes in the oxygen and hydrogen isotope composition of cellulose from tree ring material of known age to the isotopic composition of the water available to the trees at the time of growth. At inland sites this relationship is primarily controlled by the temperature at which condensation occurs in the clouds which provide water in the form of rain or snow to the trees (Friedman *et al.* 1964). However, at coastal sites this temperature-controlled relationship might be disturbed on occasion by inundation of isotopically distinct sea water directly from the sea during coastal flooding events. If cellulose from trees which are known to be living at sites subject to flooding by sea water can be shown to vary in isotopic composition during those years when the trees are known to be subject to flooding, analysis of material from sub-fossil tree rings derived from trees known to have lived in similar circumstances might provide a very accurate (annual) record of coastal storms in pre-historic times.

A suitable contemporary site was found at Roudsea Wood, Cumbria where mixed oak forest is subject to periodic inundation during high magnitude storm events, and the ancient submerged forest material which was collected from sites along the western seaboard of England and Wales by Heyworth (Heyworth 1978, 1986) was salvaged in the hope that this material would form a starting point for the production of a chronology of past storm events. However, a number of problems quickly became apparent. Firstly no consistently reliable history of inundation in the recent (*circa* 50 year) past was available at the contemporary site. Without this information it would not be possible to contrast isotopic data from cellulose laid down in years when flooding was known to have taken place with data from years when the trees were not inundated.

Secondly, the material collected by Heyworth had lost any labelling and much had fallen into small pieces. However, some was still intact, and this was put into storage.

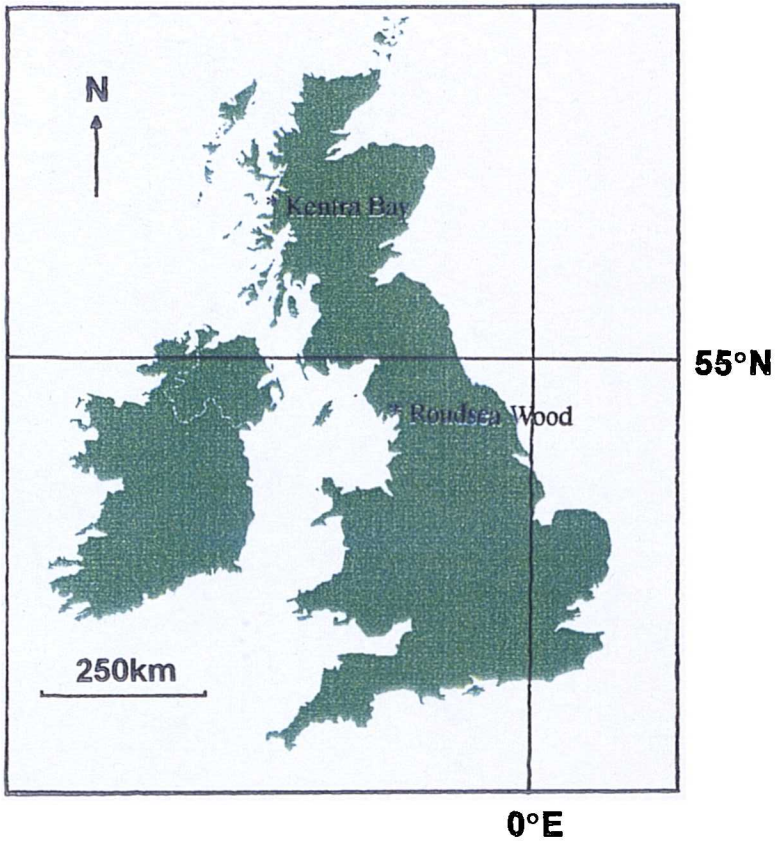
More importantly, a review of the literature on water uptake by trees and also the relatively complicated routes which metabolites can follow before being laid down as cellulose in tree rings, suggested that there might not be any record of storm events in tree ring cellulose. Studies of water uptake by trees suggest that established deciduous trees are able to change the depth at which they extract water (Megonigal & Day 1992), and in particular to decrease their intake from near-surface water sources. This means that those trees which would be the basis of a long term study of coastal flooding would, in effect, ignore the isotopically different sea water because of its salt content, and take advantage of other sources of water. In addition, those storms which occur during periods when the trees are not photosynthesising (autumn and winter) would not be recorded because no water is being taken up by the trees (Gibbs 1958).

The problems relating to the complexity of the metabolism of water and the eventual production of cellulose in the cambium are concerned with the issues raised by DeNiro & Cooper (1989). They point out that although all cellulose formed in the cambium is derived from glucose metabolised in the leaf, there is a large number of possible translocations and transformations of this primary metabolite before it is utilised in the formation of cellulose. They suggest that some post-photosynthetic modification of oxygen isotope ratios takes place, and that the relationship between the isotopic ratio of the water available to the tree and the isotopic ratio of the cellulose produced by the tree becomes more complicated with distance from the site of primary glucose metabolism. Hence isotopic ratios in leaf material are more easily explained than in shoot or stem material.

This suggested that an investigation of leaf or herb material from plants which are known to be inundated by sea water might be more appropriate. Two particular types of leaf or herb material are commonly found in cores recovered from coastal sediments. In many cores which include lithostratigraphic transitions from inorganic to organic sediments caused by changes in relative sea level, macrofossils of the common reed (*Phragmites australis* (Cav.) Trinius ex. Steudal) occur close to this transition. The occurrence of such macrofossils is used as one of a number of stratigraphic markers which have been given an "indicative meaning" (Shennan 1986; see also Shennan & Tooley 1987 for a review of the development of this term) which relates these deposits to the contemporaneous level of Mean High Water Spring Tides (MHWST) plus or minus a degree of uncertainty derived from Shennan's analysis of the location of this species in contemporary vegetation successions relative to present local MHWST. Analysis of contemporary populations of this species which are subject to varying degrees of inundation by sea water might show whether it is possible to determine the conditions under which a plant has grown from the isotopic composition of these macrofossils. At

Figure 1.1

Location of field sites



Roudsea Wood, Cumbria (Figure1.1; the same field site where the mixed oak forest is subject to inundation) *Phragmites australis* grows under three different inundation and salinity regimes and this was chosen as the location for the contemporary study of this species.

Although not related to the initial aim to investigate the incidence of flooding by coastal storms, the potential to relate particular horizons in sediment sequences to particular positions in the tidal sequence is of importance to palaeo-tidal studies. The analysis of palaeo-tidal range is another aspect of coastal environmental change that has not been fully constrained and which would benefit from a novel source of empirical data. The data from this project might also help to constrain the indicative meaning given to particular *Phragmites* peats if the isotopic composition of the material from the contemporary communities examined proves to be distinct.

The other type of sub-fossil leaf or herb material commonly found in cores of coastal sedimentary sequences, and given an indicative meaning by Shennan (1986), is the generally amorphous organic material found in buried saltmarsh peats. These deposits represent the transition from dominantly marine to dominantly terrestrial conditions and are therefore a natural focus for investigation. They are the product of all the plants which existed at the time, although the contribution from each species may not be proportional to its importance in the contemporaneous vegetation community. Consequently each species in each of a series of adjacent saltmarsh communities must be analysed and a figure for the isotopic composition of the material produced by the community as a whole derived (cf. Brenninkmeijer *et al.* 1982). From this contemporary analysis it may prove possible to divide buried saltmarsh peats into those which were formed under particular inundation regimes, and from this to relate particular sedimentary horizons to particular contemporaneous tidal levels.

The location of this study was Kentra in West Scotland (Figure 1.1). This was chosen because it offers the opportunity to analyse both contemporary spatial successions and buried temporal successions which are within a short distance of each other in an area where the Holocene sea level history has been relatively intensively studied.

During the review of previous cross-community studies of coastal vegetation utilising isotopic analysis (for example Lin & Sternberg 1993), the potential of variations in the carbon isotope ratio of plant material to indicate the extent to which a plant has been restricted by environmental factors (for example salt stress) became apparent. The theory underpinning this is discussed in Chapter 2, and following advice from researchers working in this field (Sternberg 1993, pers. comm., Switsur 1993, pers. comm., Griffiths 1993, pers. comm.) it was decided to adopt the analysis of both carbon and oxygen isotope ratios as the principal investigative method. Data from other sources which might be related to the isotopic data were also collected, and the two projects which were undertaken are described in Chapters 3 and 4 respectively.

1.2 Summary

The thesis aims to discover whether the stable isotope composition of cellulose produced by plants from different zones in the intertidal succession is significantly and consistently different. Field sites which are close analogues of situations which might be found in buried sub-fossil form were chosen to address this problem.

The two projects which were undertaken aim to establish the potential of two different types of sub-fossil deposit to elucidate sea-level histories. One project concentrates on a single species, *Phragmites australis*, macrofossils of which are often found across the transition from organic to inorganic units (and *vice versa*) in coastal

sedimentary sequences (although preservation of *Phragmites* leaves is not constant under different salinity conditions, Tanaka 1991). The project aims to determine whether the oxygen isotope ratio in the plant cellulose reflects the isotopic composition of the water available to the plants in three distinct communities. The carbon isotope ratio of the same material was also analysed to determine the effects of increasing soil salinity and incidence of tidal inundation on carbon uptake and assimilation in the three populations studied.

The second project aims to determine whether the carbon isotope values of all the plants found in adjacent saltmarsh zones combine to give significantly different and consistent values. The hope was to be able to relate bulk sub-fossil cellulose from buried saltmarsh deposits back to particular zones on the basis of this analysis of contemporary material. By analysing a contemporary marsh and sediments which have already been used to establish patterns of pollen and diatom assemblages, the efficacy of this technique can be established.

The relationship between stress (salinity and inundation) and carbon assimilation and water uptake in these projects may not be straightforward, but is likely to be far less complicated than in the study of isotope dendrochronology from which these projects were derived. Although these projects are not related to the initial aim of investigating the incidence of coastal storms and related flooding events, both of them aim to provide novel information on the relationship between particular horizons in coastal sedimentary sequences and the conditions under which they were deposited which will be useful in studies of coastal environmental change.

Chapter 2.

Stable isotopes and the theory of isotopic discrimination during cellulose synthesis.

2.1 Stable isotopes and thermodynamic fractionation.

Many elements occur as more than one stable isotope, which although chemically identical, are differentiated by the number of neutrons in the atom. These atoms are not subject to decay, and the proportion of each isotope in the global biogeochemical system is fixed. However, the different weight of each isotope means that they are often subjected to fractionation, especially during thermodynamic processes. Consequently, whilst the global proportions remain constant, the proportions of one form to another in various sinks or temporary pools varies according to the processes involved in the formation of these sinks and pools.

Elements which occur as more than one isotope include H, C, N, O, B and S. In each case the majority of the element is made up of a lighter isotope (^{1}H, ^{12}C, ^{14}N, ^{16}O...), and the heavier isotope (^{2}H / D, ^{13}C, ^{15}N, ^{18}O...) accounts for only a small proportion of the total. Changes in the proportion of the heavy isotope of carbon and oxygen (^{13}C and ^{18}O) in plant cellulose have been analysed in this study. These two forms occur in the proportions shown in Table 2.1, which gives an indication of the total mean abundance of the minor isotope. Table 2.1 also gives a measure ("delta" [δ]) of the deviation of the ratio of the minor to major isotope in a sample away from that in the standard for that element. δ is calculated as

[(ratio in sample / ratio in standard) - 1] . 1000

and hence negative values show discrimination against the heavier forms of the element. The product of dividing the two ratios is multiplied by 1000 because the proportional

change involved in most processes is so small, and therefore values of δ show differences "*per mil*" (‰). The actual extent of the changes of carbon isotopes ($\delta^{13}C$) and oxygen isotopes ($\delta^{18}O$) commonly observed in natural systems is also given. In the case of ^{13}C, the range from -40 to 0‰ covers C_3 plants (towards the lower end of the scale) through plants adopting CAM (mid-range values) to C_4 plants (values close to 0). This reflects the extent to which ^{13}C is discriminated against in these different metabolic pathways. The range of $\delta^{18}O$ values reflects the extent to which enrichment of ^{18}O takes place during photosynthesis (top end of the scale), and also the level of discrimination against the heavier isotopes during evaporative processes (lower end of the scale).

Table 2.1 The proportion of ^{13}C and ^{18}O in nature and typical ranges of δ values.

Heavy isotope	^{13}C	^{18}O
Mean fractional abundance.	0.0111	0.00204
1 δ as a fractional change of isotopic composition.	1.1×10–5	2×10–6
Usually observed ranges of δ values in nature.	–40 to 0	–30 to 30
Observed range as fractional change of isotopic composition.	4.4×10–4	1.2×10–4

Source: Handley & Raven 1992.

Some of the elements which occur as two or more isotopes are found in a variety of biogeochemical cycles, and this has facilitated the investigation of the magnitude of certain processes through the investigation of isotopic ratios (Preston 1992). The theoretical and empirical basis for this use of stable isotopes is the seminal work of Urey (1947, 1948) which was initially applied by workers such as Dansgaard (1953). Craig (1953, 1957, 1961a, 1961b) was instrumental in the standardisation of techniques as well

as studies of the natural abundance of stable isotopes. Although there are now suggestions that the original standards which he established (the Pee Dee Belemnite (PDB) for carbon isotope ratios, and Standard Mean Ocean Water (SMOW) for oxygen isotope ratios) should be set aside (Coplen 1995), they are still the prevalent standards by which δ is calculated in isotope natural abundance studies.

Since this early work there has been an expansion of the topics analysed using stable isotopes as tracers of process. These include the study of inorganic systems such as the formation of polar ice (Morgan 1982) and analysis of material from ice cores (for example by Johnsen *et al.* 1972). These have revealed patterns of climatic change on a variety of timescales based on conversions to ambient conditions through adaptations of the Rayleigh distillation formulae (Friedman *et al.* 1964). Other applications of O and H isotope ratios which use similar thermodynamic principles include the study of groundwaters (Rozanski 1985), tracking precipitation events (Lawrence & White 1984, Lawrence *et al.* 1982) and water bodies (Grebmeier *et al.* 1990). A related study is the re-creation of the oxygen isotope composition of ancient seawater from the isotopic composition of ancient oceanic crust (Holmden & Muehlenbachs 1993) and biogenic silica (Labeyrie 1974, Matheney & Knauth 1989).

All of these studies demand an understanding of the processes involved and the extent to which isotopes of different weights will be differentially transported and fixed in a variety of chemical phases. In the case of studies of O and H isotopes, the controlling influences on the isotopic composition of the source water involved are the kinetic processes acting on the water body concerned. These are principally evaporation and condensation (although see Freyer & Tans 1987 to see importance of biological controls on ^{18}O in CO_2), which result in differentiation against heavier forms and for lighter forms respectively as a function of different vapour pressures (Friedman *et al.* 1964). The result

is that freshwater is isotopically lighter than seawater (Craig 1961a), and that precipitation falling at high altitude (and hence low temperatures) is isotopically lighter than that falling closer to the source. This enables different sinks of water to be identified and the conditions under which they formed to be established. Many studies which utilise this principle include the investigation of organic systems or particular species which use the element under investigation. One notable early work which analysed stable oxygen isotopes in an organic system was by Shackleton (1969), who used biogenic calcite from planktonic foraminifera tests to reconstruct trends in ocean water isotopic composition. Although this was relatively successful, it was shown later that there is not a simple relationship between the isotopic composition of the tests and that of the contemporaneous seawater (Shackleton *et al.* 1973). The importance of this conclusion is also apparent in later studies of analogous freshwater biogenic carbonates by, for example, Lecolle (1985(and Magaritz *et al.* (1981).

However, the two projects in this study are concerned with the isotopic fractionation that is recorded in the cellulose $(C_6H_{10}O_5)_n$ formed by plants. The two isotopic ratios used (Table 2.1) are from elements that are derived by plants from different sources ($CO_2$and H_2O), and the processes which lead to differential uptake and metabolism are both kinetic and thermodynamic. Whilst this makes for a more complicated picture than studies of single source and process systems, a body of theory and empirical data has grown up to explain and quantify the processes which lead to isotopic fractionation of C and O by plants.

2.2 Stable isotopes and plants.

Plants utilise a number of elements - for example H, C, N and O - which occur as more than one isotope. The study of the isotopic ratios of these elements in plants can be used to trace processes as they vary between plants, and changes in the isotopic composition of the source of these elements (for example Bariac *et al.* 1991, Benedict *et al.* 1980, Ehleringer *et al.* 1986, Ehleringer & Cooper 1986, Heaton 1987, Kapos *et al.* 1993, Martin & Sutherland 1990, McPherson *et al.* 1993, Merwe & Medina 1989, Nordt *et al.* 1994, Petterson & McDonald 1992, Raven 1992, Rico-Gray & Sternberg 1991, Rundel *et al.* 1989, Schonwitz *et al.* 1986).

Since these elements come from a number of different sources and phases, it is possible to analyse a number of different processes by examining the isotopic ratio of elements taken up and assimilated by plants. This has been the basis of a great deal of work, and the models which have been produced to explain the isotopic discrimination found in these studies are the basis of the two projects in this thesis.

Carbon and oxygen are derived from distinct sources (Stiles & Cocking 1967) and pass along different pathways before being metabolised (Tyree & Ewers 1991). Therefore, the theories of carbon isotope and oxygen isotope discrimination and fractionation are discussed separately.

2.3 Carbon isotope uptake and discrimination by plants.

In the seventeenth century Van Helmont showed that the primary source of nutrients for plants is in the air, by growing a willow in a known weight of soil (Edwards & Walker 1983). Over a five year period the soil lost 2 ounces whilst the willow gained 164 pounds. Van Helmont ascribed this discrepancy to the utilisation of the water he had fed the tree, leaving it to later workers to show that atmospheric CO_2 is the C source for plants.

The isotopic composition of atmospheric CO_2 is not constant, varying naturally according to the trends in global biological productivity over glacial - interglacial (Coplen *et al.* 1994, Leavitt & Danzer 1992, Meyers & Horie 1993, White *et al.* 1994) and also annual timescales (Farquhar & Lloyd 1993; see also Farquhar *et al.* 1993). More recently, the ratio of ^{13}C to ^{12}C in the atmosphere has been (and continues to be) altered by the release of CO_2 from the burning of fossil fuels which have a $\delta^{13}C$ value which differs from that of the contemporary atmosphere (Penuelas & Azcon-Bieoto 1992). However, although these changes need to be taken into account when comparing material of different ages and different sites (and especially different altitudes, Korner *et al.* 1988, 1991), both of these projects are based on analyses of plants growing at the same time and within a few tens of metres of each other. Consequently the isotopic composition of the atmospheric CO_2 available to them can be considered constant, and any variations in $\delta^{13}C$ can be ascribed to processes related to C uptake and assimilation (O'Leary 1981, O'Leary *et al.* 1992).

The processes which lead to isotopic discrimination during the uptake and assimilation of C from atmospheric CO_2 can be divided into those which occur at the leaf - atmosphere boundary and those which occur inside the leaf as C is moved to the sites of

carboxylation (Keeley & Sandquist 1992, Lloyd *et al.* 1992). Since all the higher plants in this study utilise the C_3, Ribulose-1,5-bisphosphate (Rubisco) pathway (Smith & Epstein 1971, Griffiths 1992, Long *et al.* 1975, Peisker & Henderson 1992, Schuster & Manson 1990, Slocombe *et al.* 1993) the same model can be used to describe the processes involved (Machler *et al.* 1990, Rintamaki & Aro 1985). All of the processes associated with photosynthesis which involve fractionation of carbon isotopes have been studied and quantified by a number of workers, and the details of these processes are given in Table 2.2.

Table 2.2 Carbon isotope fractionations associated with photosynthesis.

Process	Fractionation (‰)
Solubility of CO_2 in water.	1.1
Hydration of CO_2.	–9.0
CO_2 diffusion in air.	4.4
CO_2 diffusion in aqueous solution.	0.7
Spontaneous hydration of CO_2.	6.9
Carbonic anhydrase-catalysed hydration of CO_2.	1.1
Carboxylation of PEP.	2.0
Carboxylation of Rubisco.	29.0

Source: O'Leary 1993; value of fractionation is positive for processes that cause depletion of ^{13}C, and negative for those that lead to enrichment relative to the starting state. PEP = phosphoenolpyruvate carboxylase (dominant in C_4 species and accounting for ~10% of C fixation in C_3 species).

Some of the fractionations in Table 2.2 are thermodynamic, others are kinetic. Thermodynamic fractionations are the product of differences in equilibrium constants for isotopic species, whilst rate constant differences result in kinetic fractionations. These processes cause fractionations of different magnitudes, thermodynamic fractionations generally being smaller than kinetic fractionations (O'Leary 1993).

The thermodynamic fractionations which are important to these projects include the diffusion of CO_2 in air and diffusion of CO_2 dissolved in aqueous solution. CO_2 entering the plant via its pores (stomata) is subject to a fractionation of 4.4‰ against $^{13}CO_2$, whilst in solution the transfer of mass is eased by the presence of water which results in a significantly smaller discrimination of 0.7‰.

For C_3 plants the fractionation associated with carboxylation is close to that of Rubisco (29‰ against ^{13}C), but Farquhar & Richards (1985) pointed out that a certain (small) proportion of C is fixed via PEP and they concluded that the total fractionation during carboxylation is closer to 27‰ against ^{13}C (an early summary is given by Troughton 1979). To this is added the fractionation of CO_2 during dissolution, giving a 28 - 30‰ fractionation compared to gaseous CO_2, the range reflecting the changing relative importance of PEP. Although this introduces a certain degree of variation, it is not as significant as the shifts in CAM species, Groenhof *et al.* 1990). Since Rubisco does not use HCO_3^- the effects of hydration on isotopic composition are not relevant to this study (Collatz *et al.* 1990).

Since air has a $\delta^{13}C$ value of –8‰ (Keeling *et al.* 1979), it is possible to calculate the potential and actual influence of these various fractionations on the isotopic composition of plant material synthesised under different conditions. The most obvious conclusion is that C_4 species, using PEP carboxylase, will show smaller differences

between the isotopic composition of source CO_2 and plant material. The value of $\delta^{13}C$ in C_4 species has also been shown to be less susceptible to variations with species and environment than in C_3 species. Consequently remains of C_4 plants are a far better source of information on past changes in atmospheric CO_2 isotopic composition than material from C_3 plants (Marino & McElroy 1991). On the other hand the $\delta^{13}C$ value of C_3 species does respond to environmental stimuli. This is because of the influence of environmental conditions on the water balance and gas transfer of the plant (Mortley *et al.* 1994, Woodrow *et al.* 1990).

The impact of environment on plant physiology in C_3 species can be qualitatively reduced to the principle that a stressed plant closes its stomata to avoid dehydration if the flux of water from the plant exceeds that entering the plant and / or cell turgor is threatened by an excess of mineral salts (Bernstein & Hayward 1958). O'Leary (1993, pp.24-25) considers two extremes:

"First, if the stomata are nearly closed, the overall CO_2 uptake rate is limited by the initial diffusion process and the internal CO_2 concentration is low. In these circumstances, the carboxylation process takes up virtually all carbon available, and the carboxylation isotope fractionation is not expressed. The isotope fractionation is small, approaching 4.4‰ at very small apertures. Thus, $\delta^{13}C$ for a C_3 plant should approach –12‰ (–8 – 4.4)."

"On the other hand, if the stomata are relatively open, the internal CO_2 concentration approaches the external CO_2 concentration, and there is a facile transfer of CO_2 between the external and internal pools. In this case the diffusional fractionation is not expressed (diffusion approaches equilibrium) and the observed fractionation approaches the carboxylation fractionation. Leaf $\delta^{13}C$ would approach –38‰ (–8 – 30)."

"Real plants, of course, show behaviour intermediate between these two extremes. The internal CO_2 concentration is perhaps half the external concentration, and the isotope fractionation is larger than the diffusional fractionation but smaller than the carboxylation fractionation. As stomatal aperture changes, the fractionation and the internal CO_2 concentration will change correspondingly."

Farquhar *et al.* 1982 produced a series of models to describe this relationship between isotopic discrimination and CO_2 partial pressure, which in its simplest form is

$$\Delta = a + (b - a) . (p_i / p_a) \qquad \textbf{(1)}$$

where Δ = discrimination in leaves of C_3 plants.

a = fractionation occurring due to diffusion in air (4.4‰) .

b = net fractionation caused by carboxylation (mainly discrimination by Rubisco, *circa* 30‰).

p_a = ambient partial pressure of CO_2.

p_i = intercellular partial pressure of CO_2.

Berry (1989) adopts Farquhar & Richards' (1984) term "Δ" to refer to isotope discrimination from a source to a product. This is defined as

$$\Delta = (R_s/R_p) - 1 \qquad \textbf{(2)}$$

where R_s and R_p are the molar abundance ratios of ^{13}C and ^{12}C in the source and the product carbon respectively. Although this links very easily to equation **(1)**, δ is retained in these analyses since values can be quickly converted from δ to Δ in contemporary samples

but, more importantly, δ is the established unit used in palaeoenvironmental studies since the value of R_s is not always known.

The important factor in determining the extent of isotopic discrimination is therefore the relationship between p_i and p_a. This is a function of stomatal conductance, and appears to lead to a direct relationship between stomatal conductance and discrimination. According to **(1)**, at $p_i / p_a = 0$ discrimination = 4.4‰, and at $p_i / p_a = 1.0$ discrimination = 30‰.

However the fit between the expected results and observed levels of discrimination for different values of p_i / p_a is not perfect, and it is not correct to assume that there is a simple stomatal control on discrimination. This would imply that the plant remains wholly operational under all conditions of stomatal conductance; Farquhar *et al.* (1989) summarise this by saying:

" it is a little dangerous to take the argument further and say that when p_i and Δ are small, stomata are necessarily limiting photosynthesis. That conclusion would only follow if the relationship between assimilation rate, A, and p_i remained linear beyond the operational point."

Nevertheless, a significant relationship between p_i / p_a and discrimination (Δ or δ) does exist, and since p_i / p_a is a function - to a certain extent - of stomatal conductance, this suggests that any influence on stomatal aperture will have an effect on δ. Since stomata respond to environmental stimuli to maintain the water balance of a plant, anything which potentially upsets this balance will lead to a change in stomatal conductance and hence δ (see also Gross *et al.* 1991).

In a coastal context the primary sources of stress which cause stomatal aperture to be reduced are salinity and inundation (Bowman & Strain 1988a, b). Both of these influences are generally at their greatest towards the seaward end of the intertidal vegetation succession, although Sternberg (1994 pers. comm.) has recorded higher levels of soilwater salinity in mangrove communities in the Florida Keys which are at higher altitude and less frequently inundated than others as a result of high evapotranspiration and low levels of freshwater input at these points. Some species are adapted to these conditions (halophytes) and have mechanisms for excluding or exuding excess salt (Cheeseman 1988, Clough & Sim 1989, Flanagan & Jeffries 1989, Flowers *et al.* 1990, Fitzgerald 1992, Guy & Wample 1983, Megonigal & Day 1992, Munns 1993, Pearcy & Ustin 1984, Salim 1989, Winter 1979, Yeo 1983, Youngman & Heckthorn 1992) and withstanding the impact of tidal inundation (Clarke & Hannon 1970, Cooper 1982, DeLaune *et al.* 1987, 1990, Hwang & Morris 1994, Knight *et al.* 1994, Marcum & Murdoch 1992, Pezeshki & DeLaune 1988, Pezeshki *et al.* 1987a, b, 1988, 1989, 1990, Rozema & Blom 1977, Treichel & Bauer 1974) . However, others are less able to cope without their metabolism being affected (Flowers *et al.* 1991, Galves *et al.* 1993, Glenn *et al.* 1992, Hester & Mendelsohn 1989, Karim *et al.* 1993, Lin & Lin 1992, Liao & Lin 1994, Meinzer *et al.* 1994, Singh 1991, Ziska *et al.* 1989, 1990, Zrenner & Stiff 1991). This commonly results in a change in stomatal aperture, leading Farquhar *et al.* (1989 p.520) to point out that, in non-halophytes:

" stomatal closure is typically associated with increased salinity. Thus it should not be surprising to note that in these species Δ decreased with increasing salinity, indicating a decrease in p_i with increasing stress. What is perhaps more intriguing is that halophytic species also exhibit a similar pattern whether in field or laboratory conditions."

Herein lies the basis of the application of stable carbon isotope analysis to plants in coastal environments (Parker 1964; see also Berry 1989, Brugnoli & Bjorkman 1992).

The two projects undertaken in this study are not the first attempt to analyse the $\delta^{13}C$ value of material from coastal plants under different stress regimes in coastal successions (DeLaune 1986, DeLaune & Lindau 1987, Chmura 1988, Chmura & Aharon 1995). However, they do attempt to identify the efficacy of this approach for palaeoenvironmental reconstruction by setting out to answer specific questions about the stable isotope composition of plants in the two field sites.

Since stomatal conductance has been isolated as an important influence on the $\delta^{13}C$ value of plant leaf material (Farquhar & Sharkey 1982; see also Ishibashi & Terashima 1995, Jones 1985, Knapp 1993, Kubiske & Abrams 1993, Meinzer *et al.* 1993, Montieth 1995, Wong *et al.* 1979, Woodward & Smith 1994), it is important to determine the controlling mechanisms on stomatal aperture, size and density.

2.3.1 Factors influencing stomatal conductance.

The net conductance of CO_2 into the chloroplast of a leaf is the product of both leaf physiognomy and physiology. Viewing the stomata as simply gates allowing gaseous transfer, these limitations can be divided into those which are a function of the number of gates per leaf area (stomatal density), the size of the gates (stomatal size) and how open the gates are (stomatal aperture).

Stomatal density and size are determined as the leaf is produced (Longstreth & Nobel 1979, Penuelas & Matamala 1990), and changes in plant water stress may influence the production of stomata (Pearson 1995 pers. comm.). They can be measured by examination under a microscope and can therefore be compared to $\delta^{13}C$ values from the same plant. This has been done in the past, and links were found in these which suggests a relationship between stomatal density and $\delta^{13}C$ (Beerling *et al.* 1993, Poole *et al.* 1995,

Woodward & Bazazz 1988). Whether this is a causal relationship remains open to question, since it takes no account of physiological variations which impact on $\delta^{13}C$ and cannot be measured after the plant has died (see Jones & Sutherland 1991).

The physiological controls on stomatal conductance are principally related to those which affect stomatal aperture (for example Aphalo & Jarvis 1993). Recent works points to the rôle of abscisic acid (ABA) in controlling stomatal aperture (Castonguay *et al.* 1993, Kicheva *et al.* 1994, Perera *et al.* 1994). Although there is some debate about the timing and mechanism by which ABA is produced (Jackson & Hall 1983, Khalil & Grace 1993, MacRobbie 1992, Nagy & Galiba 1995, Tardieu & Davies 1992, 1993, Tardieu *et al.* 1992, 1993), in saline environments (such as at the coast) it seems to be related to the root sensory system reacting to a lack of water or excess soilwater salinity (Everard *et al.* 1994, Mansfield & DeSilva 1994, Mansfield *et al.* 1990, Quick *et al.* 1992, Seeman & Critchley 1985). The production of ABA results in the closure of stomata (Trejo *et al.* 1993), although there is debate about the actual mechanism involved and the possible rôle of other compounds. There is also further research needed to fully explain the opening of stomata as guard cells relax (Dewar 1995, Lee *et al.* 1994, Rengel 1992, Ruiz & Mansfield 1994).

By closing the stomata, leaf water is retained, and cell turgor and osmotic balance is maintained (Baron 1967). This not only impacts on plant water use (which potentially influences $\delta^{18}O$ values, see Chapter 2.4), but has a significant impact on the uptake and assimilation of C (Welker 1993). By closing the stomata, the plant is limiting the flux of gaseous atmospheric CO_2. The CO_2 that is trapped in the stomatal cavity is now the only source of C available to the plant and if the assimilation rate (A) is maintained, and the production of Rubisco is not altered, then this CO_2 will be fully utilised. This situation then approaches the extreme position mentioned in Chapter 2.3 in terms of C isotope

discrimination, where there is limited discrimination against ^{13}C compared to conditions when the stomata are fully open.

In reality these conditions are unlikely, and the stomatal aperture may not fully close (van Kraalingen 1990, Schulze *et al.* 1975) and the assimilation rate is likely to change . However, the impact of ABA production and the resulting decrease in stomatal aperture is to increase the efficiency of water use (ψ), and to decrease the discrimination against ^{13}C, resulting in less negative $\delta^{13}C$ values in plant cellulose. Consequently this causes variation in $\delta^{13}C$ in plants from the same species growing under different environmental conditions.

By studying stomatal density as well as $\delta^{13}C$ in both projects it may be possible to qualitatively determine the importance of physiology and physiognomy on carbon isotope discrimination in the plants analysed (Malone *et al.* 1993). However, there will always be a question mark about the importance of ABA or related physiological mechanisms (for example Ramapogal & Carr 1991, Rasulov 1993) simply because no direct measurements of these variables is made in these projects.

2.4 Oxygen isotope fractionation during cellulose formation.

The kinetic fractionation of oxygen isotopes during evaporation and condensation outlined in Chapter 2.1, and the resulting gradient in $\delta^{18}O$ of water between fully marine, brackish and freshwater, results in isotopically distinct source waters mixing in coastal environments. This could be a source of information for palaeoenvironmental reconstructions if these differences in the isotopic composition of water are faithfully recorded in the $\delta^{18}O$ value of the cellulose of plants living across an intertidal vegetation succession, drawing water from distinct sources.

However, unlike carbon isotope analysis where the sole (in most cases) source of C is atmospheric CO_2, there are three potential sources of O which can be utilised in the formation of plant carbohydrates such as cellulose (Bauer 1977). These sources are H_2O, CO_2 and O_2 and although early work assumed, and found, a link between local $\delta^{18}O$ of soilwater (which was often then linked to the $\delta^{18}O$ of precipitation and then, using the Rayleigh distillation formulae, back to temperature) it was not until later that this relationship was formally established.

This was done by varying the isotopic composition of atmospheric CO_2 and O_2 whilst maintaining constant values of $\delta^{18}O$ in source water. By examining the $\delta^{18}O$ of plant cellulose produced under these varying atmospheric conditions, it was possible to show that the direct impacts on the $\delta^{18}O$ of cellulose of very different $\delta^{18}O$ values in atmospheric CO_2 and O_2 are negligible. This means that the variations in natural $\delta^{18}O$ recorded in plant cellulose can indeed be linked to variations in source water (Cooper & DeNiro 1989a, Ehleringer & Dawson 1992, Sternberg 1989a, b, Sternberg *et al.* 1986).

Although this work established the link between oxygen isotope ratios in cellulose and $\delta^{18}O$ values of source water, and accounted for other possible sources of O in $(C_6H_{10}O_5)_n$, it did not provide a clear understanding of the mechanism by which fractionation takes place or the extent to which it occurs. Two contrasting models were suggested at different times to explain the relationship between water and cellulose. One (Epstein *et al.* 1977) proposes that isotopic equilibration between CO_2 and oxygen takes place before incorporation into the Calvin cycle and that

"since water is in excess, oxygen isotope ratios of carbon dioxide entering the Calvin cycle will essentially be determined by the isotope ratio of the water."

(Sternberg 1989a, p.136)

This was model criticised by the work of Deniro and co-workers (Deniro & Epstein 1979, Sternberg & Deniro 1983) who proposed (and found evidence) that a link exists between oxygen isotope ratios in plant cellulose and the carbonyl hydration reaction. A number of lines of evidence support this model;

"Firstly, the oxygen incorporated from water in the carboxylation process is lost during the subsequent reduction step...Secondly, it was shown that isotope effects between water and cellulose are similar in autotrophic and complete heterotrophic metabolism (i.e. no CO_2 uptake)...Thirdly, the isotope effect associated with CO_2 equilibrium with water is highly sensitive to temperature, while no temperature effect was observed in the isotope effect between water and cellulose..."

(Yakir 1992, p.1010)

Having established a mechanism to explain the isotopic discrimination between $\delta^{18}O$ of source water and cellulose, the same group of workers found that the equilibrium isotope effect was 27‰ by using acetone as a model system. The single carbonyl oxygen present was found to reach equilibrium with water in a relatively short period of time and with a magnitude of 27‰ (Sternberg & DeNiro 1983). This matches the value later found by Yakir & DeNiro (1990) who studied aquatic plants where possible changes in isotopic composition due to evaporation are nil (Wetzel 1988). Their results showed that an ideal situation in the laboratory was mirrored in an ideal situation in the field where the plant analysed could be considered a closed system and source water $\delta^{18}O$ was fixed (see also Cooper *et al.* 1991, Sternberg *et al.* 1985).

However, terrestrial plants lose water as it passes through the plant due to evaporation (DeNiro & Cooper 1990), and this flux involves isotopic discrimination for the same reasons that affect any evaporative process. The result is that water becomes isotopically heavier as it passes up a plant (and up a canopy, Sternberg *et al.* 1989), and

consequently there is potentially a problem of intra-plant $\delta^{18}O$ variation in cellulose due to variations in leaf and stem water $\delta^{18}O$. However, Yakir (1992 p.1011) quotes unpublished data from experiments by Yakir, Marino & DeNiro showing that in maize plants, where tissue water varied by ~15‰ in different parts of the plant, the $\delta^{18}O$ values of the cellulose in the same places varied by 1.6‰ (see also Yakir *et al.* 1994). This suggests that the $\delta^{18}O$ value of cellulose reflects some form of average $\delta^{18}O$ value of plant water.

The variations in cellulose $\delta^{18}O$ noted by DeNiro & Cooper (1989) suggest that studies which utilise stem or root cellulose may have to take post-photosynthetic modification of $\delta^{18}O$ values more seriously than studies examining leaf material. This appears to be related to the number of transformations to or from starch and sucrose that the original glucose undergoes before being laid down as cellulose. Since the use of $\delta^{18}O$ in this study is restricted to the leaves of *Phragmites* it is possible to consider the isotopic composition of leaf water as the control on the cellulose $\delta^{18}O$ values (Yakir *et al.* 1991).

The increasing interest in $\delta^{18}O$ as a physiological and ecological tool (for example Yakir *et al.* 1990a, b) has enabled isotope-based studies of palaeoenvironments to utilise this tool with a great deal more certainty about the possibilities and restrictions implicit in $\delta^{18}O$ analysis.

2.5 A summary of developments in the analysis of the stable isotope composition of sub-fossil plant material in palaeoenvironmental reconstructions.

It is only relatively recently that the importance of p_i on $\delta^{13}C$ values has been appreciated and isotope-based palaeoenvironmental reconstructions have taken a detailed look at the controls on $\delta^{13}C$ in the plant material being analysed (Duponey *et al.* 1993,

Yakir *et al.* 1994). Initial efforts were concentrated in the field of isotope dendrochronology, which has the potential to produce temporally accurate data because of the production of annual tree rings. Although $\delta^{13}C$ was used as a measure of plant productivity (which has been linked to a variety of climatic conditions), often in alliance with ring width measurements (Epstein & Yapp 1976, 1977, Epstein *et al.* 1976, Pearman *et al.* 1976), the thrust of the original work was on $\delta^{18}O$ and $\delta^{2}H$ (δD), because of the relationship between ambient temperature and the isotopic composition of rainfall (Burk & Stuiver 1981, Gray & Thompson 1976, 1977, 1978, Libby & Pandolfi 1977a, b, 1978, Libby *et al.* 1976, Perry 1977, Wigley *et al.* 1978, Wilson 1978, Wilson & Grinsted 1977, Yapp & Epstein 1977, 1982a, b, Ziegler *et al.* 1976).

Two important conclusions came out of this early work. The first was that only certain fractions of bulk plant material were shown to be suitable for analysis (Wilson & Grinsted 1977), which lead to the adoption of cellulose as the primary compound used for environmental reconstructions. Cellulose was adopted because it accounts for c. 40% of the total dry weight of plant material (although this proportion varies with species and between trees, shrubs and herbs), it is relatively easily extracted, and is fairly robust. The last point is significant because the initial work assumed that, once the cellulose molecule was formed, each atom in the chain was fixed. However, it was found that the O-H bonds were relatively easily broken, which means that only carbon-bound H can be guaranteed to be *in-situ* (DeNiro 1981). Since it is necessary to nitrate the cellulose sample before using it for analysis of δD and the cryogenic separation of H is more complicated than C or O, this technique is often not used in either contemporary or palaeoenvironmental studies. The great advantage of studying δD is that H in cellulose is solely derived from H_2O (Kramer 1964, Gray & Song 1984, Krishnamurthy & Epstein 1985, Schiegl 1972, Schiegl & Vogel 1970, White et al. 1985; see Geel & Middledorp 1988 for an assessment of the use of δD and problems encountered when analysing bulk plant cellulose), although there

is some debate about the possibility of discrimination against D during water uptake (see for example Yakir & Yechieli 1995).

Since the early work by Schiegl (1974), the environmental and physiological controls on $\delta^{13}C$ in tree rings has been assessed in greater depth (Francey & Farquhar 1982, Francey et al. 1985, Freyer & Belacy 1983, Livingston & Spittlehouse 1993, Luckman & Kearney 1986, Martin *et al.* 1988, Ramesh *et al.* 1985, Stuiver & Braziunas 1987). The level of sampling resolution has also increased (Leavitt & Long 1984), and intra-annual analysis of the stable isotope composition of oak tree rings has been undertaken (Switsur *et al.* 1994). The data from these analyses show significant differences between cellulose laid down at different times of the growing season in each of δD, $\delta^{13}C$ and $\delta^{18}O$. The different sources of C utilised by trees during the growing season - for example stored carbohydrate and contemporary atmospheric CO_2 - and the difficulty of splitting the contribution of one from another and accounting for the effect of isotopic pooling, is an excellent example of the problems involved in identifying the environmental stimuli responsible for these patterns. The original idea that isotopic values recorded in tree rings might elucidate changes in temperature and atmospheric CO_2 partial pressure in simple variations of δD, $\delta^{13}C$ and $\delta^{18}O$ has been shown to be unfounded. There is undoubtedly an environmentally-driven signal within the isotopic composition of tree ring cellulose (Edwards *et al.* 1985, Leavitt & Kalim 1992, Luckman & Gray 1990), but there is not a straightforward relationship between environment and any of the δ values.

In addition, DeNiro & Cooper (1989) showed that the isotopic composition of cellulose produced in the root or shoot of a plant is not identical to that of the glucose produced during photosynthesis in the leaf. The greater the number of transformations and translocations of secondary metabolites, the more difficult it is to relate the final cellulose

isotopic composition to the conditions in the leaf. However, by focusing on the $\delta^{13}C$ and $\delta^{18}O$ values of annually-produced leaf material (as in this study and also, for example, Welker *et al.* 1993) the problems of analysing tree-ring cellulose without a complete understanding of the extent of post-photosynthetic modification of the isotopic composition of cellulose produced in the cambium is avoided (see also Kelly & Woodward 1995).

It is important not to attempt to simplify the relationship between isotope discrimination and environment (see Chapter 2.6 below), but the theoretical basis for adopting an isotope-based approach in this study does exist. This has been shown by the work of Chmura (Chmura 1988, Chmura & Aharon 1995) and a number of analyses of $\delta^{13}C$ from a variety of plant species in coastal environments have also provided evidence to support this relationship between stress and carbon isotope discrimination (for example Guy *et al.* 1989). Coupled to the evidence from mangrove communities in Florida that isotopically distinct water is being used in different communities across intertidal vegetation successions, this adds fuel to the argument that this approach may well provide an additional tool for the reconstruction of coastal environments.

2.6 Complicating factors which might influence $\delta^{13}C$ and $\delta^{18}O$ values.

2.6.1 The preservation of cellulose.

Unlike the problems of ionic translocation encountered during Long's (1991) study of *Phragmites* caused by post-depositional alteration of plant material (Tukey 1970), cellulose is assumed to be relatively stable and resilient through time. The widely-appreciated importance of using only carbon-bound hydrogen in δD analyses is the major exception to this assumption (for example Feng *et al.* 1993). However, there are a number

of studies which have shown that question the assumption that cellulose is unaltered through time. DeNiro & Hastorf (1985) showed that both $\delta^{13}C$ and $\delta^{15}N$ can undergo a measure of post-depositional alteration, and Macko *et al.* (1994) and Meyers (1994) have also found evidence for modification of $\delta^{13}C$ (see also Aller 1994, Chmura & Aharon 1995). This is obviously of critical importance to this and similar studies. However, the nature of this degradation is not fully understood - or widely accepted to be an important factor - and may be the result of a number of physical, chemical and biological processes in a range of combinations. It is difficult to link this degradation to measurable quantities such as physical resilience to pressure (Lucas *et al.* 1995).

The apparent changes in isotopic composition over time may be a function of the differential preservation of different parts of the plants analysed. If there is considerable intra-plant variation in isotopic composition and some parts of the plant are more susceptible to degradation because, for example, they have less lignin than other parts of the plant, the average isotopic composition of the remaining cellulose might change. This highlights the advantage of being able to examine particular parts of particular species when utilising this technique in palaeoenvironmental studies. This is the case in the study of *Phragmites australis*, but not in the study of the bulk organic matter in the saltmarsh peats at Kentra.

It is also possible that post-depositional alteration of $\delta^{13}C$ values can be explained by differential preservation of the six carbon atoms that make up a cellulose molecule. Since the carbon atoms in different positions in the molecule pass along different pathways before being metabolised, there is a degree of intra-molecular fractionation within cellulose molecules. This has been studied in other plant compounds, notably starch, by Schmidt (1995) who has found significant (c. 4‰) differences in the extent of discrimination involved in these pathways (see also Kennicutt *et al.* 1992). If the cellulose

molecule is not preserved in a pristine form, it could be that these intra-molecular differences will cause as-yet unaccounted for variations between contemporary and sub-fossil δ values from cellulose.

This is leading a number of workers (Sternberg 1995 pers. comm., Switsur 1995 pers. comm.) towards attempts at isolating particular parts of the cellulose molecule which are known to be derived through certain pathways. Although this may prove difficult it might be a route to realising some of the initial hopes about direct links between δ values and environment.

2.6.2 Seasonality of isotopic values.

The work of Switsur *et al.* (1994) shows that there are significant and consistent differences in the isotopic composition of cellulose formed at different times of the year. Given the seasonal patterns of water use in trees (Gibbs 1958), and the utilisation of carbohydrate for early growth in year *t* which was produced and stored during year *t* -1 (Pearson 1993, pers. comm.), this is not wholly surprising. Similar patterns have been found in analyses of herbaceous (Smedley *et al.* 1991) and aquatic vegetation (Robe & Griffiths 1992). Not only does this have an impact on the isotopic composition of the atmosphere (Keeling & Shertz 1992), but the plant cellulose also records this same pattern.

2.6.3 Variations in δ values that are not the product of environmentally-driven factors.

A number of papers have been published that include data on $\delta^{13}C$ from plant material which shows significant intra-community variation within a stand of a particular

species (for example Long *et al.* 1993). The range of $\delta^{13}C$ values found in these studies is not the result of environmental variation since all the plants occur in the same community. Zhang *et al.* (1993) have found a relationship between C isotope discrimination and genetic differentiation within *Pseudotsuga menziesii* L. plants from the same stand. This genotypic link was also found by Siddiqui *et al.* (1994) who examined species of *Terminalia*. Since there is a considerable amount of evidence which points to genotypic differentiation within species found in different saltmarsh zones, including *Phragmites* (see Chapter 3.1), this may be of considerable importance.

The extent of this inter-plant differentiation is highlighted by Kohorn *et al.* 1994 who found a 5.1‰ range in $\delta^{13}C$ in *Simmondsia chinensis* L. from an apparently homogeneous habitat. The within-plant values of $\delta^{13}C$ were consistent and $\delta^{13}C$ was correlated with the size of leaves on each plant. However, there was no relationship between plant size, leaf mass, foliage density or any measures of plant water status or instantaneous measures of water-use efficiency or gas exchange and the $\delta^{13}C$ values. These data throw a big question at the models suggested above which try to explain discrimination. Kohorn *et al.* (1994 p.1817) state that they:

" attribute this wide variability in isotopic composition in the field at least in part to genotypic diversity within the population....Sampling technique for stable carbon isotope analysis in field studies of natural populations should consider the implications of potential genetic variability."

Fortunately the methodology adopted in these projects was designed to test the extent of inter- and intra-plant variations as well as inter-community variations in $\delta^{13}C$ values. In particular, the *Armeria maritima* L. (Mill) from Kentra and the *Phragmites* leaves from Roudsea were analysed for variations between seasons, between plants and, in the case of *Phragmites*, within plants. In addition *A. maritima* was analysed both on an

individual plant basis and by pooling material from a number of plants. By adopting this approach it has been possible to answer the questions about intra- and inter-plant variability that this recent paper shows (see also Roy & Mooney 1982), and in particular Kohorn *et al.*'s (*op.cit.*) concluding remarks (p.1821) that:

"When stable carbon isotope ratios are used in ecological studies, a common methodology has been to sample leaves from several individuals and to pool foliar material prior to analysis. The range in isotopic composition that we have observed reveals that this methodology may generate misrepresentative results due to the sampling bias resulting from intra-populational variation. Some idea of the extent of within-plant and within-population variability should be included in experimental design. The morphologically "typical" individual may not necessarily represent the population."

However, this does not actually overcome the possible problems which genotypic variation may cause but simply measures the extent of it. It may be very difficult to assess the importance of genotypic variation on $\delta^{13}C$ (and possibly $\delta^{18}O$), but if specimens of the same species from different sites and ages are to be used as the basis of environmental reconstructions (for example in isotope dendrochronology), this factor will have to be considered at some stage.

2.6.4 Selective soilwater utilisation and the implications for $\delta^{18}O$ values.

The basis for the use of oxygen isotopes in this study is that there is a significant difference in $\delta^{18}O$ (and δD) between seawater and freshwater. In coastal and estuarine environments there is a strong gradient of $\delta^{18}O$ and δD values between wholly freshwater and wholly marine extremes which corresponds to levels of salinity between ~0 parts per thousand and ~35 parts per thousand. In saltmarsh communities there is a mixing of water from a number of sources - isotopically heavy, saline, marine-derived water from tidal

inundation, and isotopically light freshwater that falls directly on the marsh, flows onto, across or through the marsh from the hinterland communities or from fluvial inputs into estuarine sites (Swart *et al.* 1989). Each of these sources will feed water which is isotopically distinct into saltmarsh communities in varying extents at different times of the day (diurnal tide), month (tidal cycle) and year (seasonal climate variations; Feitzel *et al.* 1988). The result is a complicated flux of isotopically and chemically heterogeneous water.

However, there is a growing body of evidence of selectivity of water uptake by coastal plants, fuelled by the work on the isotopic relations of stem water in mangrove and hammock communities in Florida by Sternberg and co-workers (Sternberg *et al.* 1991, Ish-Shalom *et al.* 1992; see also Dawson & Ehleringer 1991 and Flanagan *et al.* 1992). There is a suggestion that certain species utilise water that does not isotopically reflect that which is immediately around them by extending their root systems landwards of the point of aerial growth to avoid highly saline waters. Given that the method of lateral extension by *Phragmites* relies on rhizomes from a core population (see Chapter 3.1), it may be that the $\delta^{18}O$ values of the leaves from shoots of *Phragmites* do not reflect the $\delta^{18}O$ composition of the soilwater around them if they are being fed along these rhizomes with water taken up from other sources.

The extent of selectivity of water utilisation has been highlighted in a recent paper by Yakir & Yechieli (1995), who studied the plants colonising the hyper-saline shorelines being exposed as the level of the Dead Sea falls. The plants found here are growing in soils with 9% salt by weight, and there are two very distinct sources of water available to them. One is the very saline lake water, and the other comes from occasional flood water from the Jordan River which reaches the Dead Sea. These are isotopically distinct, and

Yakir & Yechieli have traced δD and $\delta^{18}O$ values in root, stem and leaf water back to the two water sources. They concluded that:

"There was a clear discrepancy between the plant water and the soilwater dug out from among the roots (even if a possible deuterium effect during uptake is considered). In fact the flood water, to which all the lines lead, seems to be the likely source of water for the plants found at the study site. These results clearly indicate that the highly saline soilwater dug out along the main root systems was not used by the plants; to our knowledge, they provide the first evidence for the absence of a simple relationship between the salinity at any part of the root zone and the water actually used by the plants."

(Yakir & Yechieli 1995, p.804)

This suggests that plants at their physiological limit may utilise water selectively. This has considerable implications for the relationship between environmental $\delta^{18}O$ and $\delta^{18}O$ in cellulose, especially in coastal environments where many species are limited by the influence of inundation and soil salinity.

In order to determine whether this is occuring in the case of the *Phragmites* plants at Roudsea it would be necessary to sample the stem and leaf water before, during and after inundation by sea water. Samples of the sea water and water from all depths in the rhizosphere would also be necessary for comparisons with water extracted from the plant. Such sampling should continue over the course of a full tidal cycle and include periods of high rainfall to compare the isotopic composition of the water utilised by the plants.

It would also be necessary to determine whether water is being fed to the shoots and leaves along the rhizome network from an area subject to a different inundation regime by investigating the architecture of the rhizomes and analysing the direction of flow and isotopic composition of any water found. This was not possible due to limitations on

the number of isotopic analyses available and the restrictions placed on excavations by the classification of the site as part of a National Nature Reserve. A controlled simulation of the effect of changes in tidal inudation and rainfall using transplanted material and water of known isotopic composition and salinity might indicate whether *Phragmites* plants do selectively utilise different sources of water.

Yakir & Yechieli also mention the possibility of a "deuterium effect". This was first suggested by Lin & Sternberg (1993) who studied mangrove species in the Florida Keys. They found an apparent discrimination against deuterium during the uptake of water by analysing source water and stem water composition. This has not been investigated in other species, and although it is not an influence on $\delta^{13}C$ or $\delta^{18}O$ values it may have implications for studies of this nature, and consequently is discussed separately below.

2.7 The deuterium effect.

Whilst there is no evidence for isotopic discrimination during uptake of water against oxygen isotopes, there is some evidence that discrimination against deuterium does take place (Lin & Sternberg 1993, cf. Cooper & DeNiro 1989b). This has only been found in certain species and under certain environmental conditions, and is not accepted as the product of direct discrimination by some workers in the field (Allison *et al.* 1985). However, the evidence for this process is from coastal environments and although it is not particularly significant in terms of magnitude relative to variations in natural abundance of deuterium in different source waters (Leaney *et al.* 1985), it does raise a further question about the use of hydrogen isotopes for palaeoenvironmental studies in coastal situations. The extent of this possible discrimination is not known and neither is the variation between species or across a variety of environmental gradients (White 1993). There are also a host

of metabolic and post-metabolic problems associated with δD values which led Yakir (1992 p.1006) to say that:

"In our attempts to understand the variations in the natural abundance of stable isotopes in plants, the hydrogen isotope composition (D / H or ^{2}H / ^{1}H) presents the greatest challenge."

2.8 Summary.

Early work which utilised C and O isotope analyses in environmental reconstruction reduced the rôle of the plant to a sample black box recorder. Oxygen (and hydrogen) isotope ratios were believed to be directly related to the isotopic composition of contemporary local precipitation, and variations in carbon isotope ratios were thought to reflect trends in temperature and ambient CO_2 partial pressure. Criticism of this approach in the late 1970s and through the 1980s which has involved experimental testing of the magnitude and direction of isotopic discrimination during uptake and metabolism of C and O into a single plant fraction (cellulose) has produced a more incisive body of theory which takes into account the importance of plant physiology in determining the extent of isotopic fractionation.

Although this has made the reconstruction of palaeoenvironments through variations in isotope ratios more complicated, a significant number of new applications for isotopic analysis - especially in comparative plant ecology - have arisen. In studies of environmental reconstruction using oxygen isotope variations in cellulose the magnitude of isotopic discrimination between plant water $\delta^{18}O$ and cellulose $\delta^{18}O$ has been established as 27‰ by Sternberg and co-workers.

Carbon isotope variations in cellulose are thought to be due to changes in the relationship between p_i and p_a (internal and ambient CO_2 partial pressure respectively). This may be caused by species-specific physiological variations, by changes in p_a, or changes in p_i as a result of the response of stomatal conductance to stress. Since all the comparative analyses of contemporary $\delta^{13}C$ values of plants from different habitats in these projects are intra-specific and time of sampling is fixed (thereby keeping p_a constant), changes in $\delta^{13}C$ are assumed to be the result of variations in p_i. This is controlled by two factors, stomatal conductance and assimilation rate.

Stomatal conductance must be considered on a per leaf, areal basis. Consequently the controls on this factor are leaf size, stomatal density, stomatal size and the extent of stomatal opening and closure during the life of the leaf (see Yakir & Israeli 1995 for an assessment of the importance of irradiance on stomatal conductance). Only the physiognomy of leaf macrofossils can be measured, and consequently the rôle of stomatal opening and closure in determining $\delta^{13}C$ is never satisfactorily assessed in palaeoenvironmental reconstructions. This is important because not only is the production of ABA resulting from water stress and excess salinity a principal control on stomatal opening and closure, but it also has an impact on assimilation rate (A).

An additional complicating factor is the possibility of genotypic variation impacting on plant physiognomy and physiology to the extent that $\delta^{18}O$ and $\delta^{13}C$ values are genetically rather than environmentally controlled.

However, as the theory stands, there is still good reason to believe that both $\delta^{13}C$ and $\delta^{18}O$ values of cellulose from plants of the same species inhabiting different coastal environments will be significantly different. Work elsewhere has shown that the oxygen isotope composition of water utilised by coastal vegetation does vary along tidal

inundation gradients. Hence the basis for $\delta^{18}O$ analysis seems firm so long as the plants from different zones are taking up and utilising consistently different water through-out their life.

In terms of $\delta^{13}C$, the premise must be that A is constant and that the impact of inundation and salinity affects stomatal conductance (through density, size or opening or closure) thereby altering p_i and impacting on the extent of discrimination against ^{13}C (but see Leunig 1995). Analyses of a number of C_3 plants, including halophytes, across salinity gradients both in the field and in the laboratory support this proposition (Guy & Reid 1986a, b, Guy *et al.* 1980, 1986, 1989, Pulich *et al.* 1985, Ross *et al.* 1994) and give some hope to the aim of using $\delta^{13}C$ as a tool for environmental reconstructions. The potential problems of different C_3 species mixes in bulk plant cellulose through time will, however, have to be confronted at some stage (cf. Sukamar *et al.* 1993).

Chapter 3

A study of *Phragmites australis* (Cav.) Trinius ex. Steudal.

3.1. Background, aim and potential.

Phragmites australis ((Cav.) Trinius ex. Steudal; = *P.communis* and hereafter *Phragmites*) is a rhizomateous perennial grass, the annual aerial shoots of which reach heights of 3 metres in favourable environmental conditions (Haslam 1972, Grime *et al.* 1988). It is a species which is found throughout the temperate and sub-tropical latitudes. *Phragmites* often forms monodominant stands in the shallow margins of freshwater lakes (Vaquer & Heurteaux 1988, Kamio 1989) but is also found at the landward edge of the intertidal zone between true saltmarsh and fully terrestrial vegetation (Adam 1990, Phillips 1987, Lee 1990b, Orson *et al.* 1987, Ranwell 1974). It is often the initial coloniser of waterlogged sites (Smith and Morgan 1989) and *Phragmites* stands are recognised as a component of one of the most productive ecosystems in terms of annual primary productivity (Lee 1990a).

The significance of this species to studies of coastal environmental change is that identifiable macrofossils (Ollendorf *et al.* 1988) of it are often found in core material recovered from coastal sedimentary sequences. Long's (1991) examination of the ionic content of *Phragmites* macrofossils from different sedimentary facies attempted to utilise these remains as a source of palaeoenvironmental information, but suffered from problems of post-depositional ionic translocation (Tukey 1970). This project is aimed at determining whether the stable carbon and oxygen isotope ratios of cellulose from *Phragmites* growing in contrasting environments is both consistently and significantly different, and can

therefore be used as a palaeoenvironmental indicator in studies of coastal environmental change.

Choice of field site was governed by the need to find an undisturbed vegetation succession that offered the opportunity to study *Phragmites* at its saline limit, as well as in freshwater communities. Roudsea Wood National Nature Reserve in South Cumbria (Gray 1971, Gray & Bunce 1971) offers just this (Figure 1.1, Map 3.1, Ordnance Survey grid reference SD329817), with *Phragmites* occurring in a stunted form in a middle/upper saltmarsh community and in a nearly monodominant fringe between saltmarsh and mixed oak woodland that is subject to occasional flooding by exceptional tides. It is also found in a similar position that, since the turn of the century, has been protected from coastal inundation by an earth embankment. If the stable isotope ratios of the *Phragmites* from these three communities show significant differences then it may be possible to identify facies from these types of environments by analysing *Phragmites* macrofossil remains.

3.2 Possible complications and limitations.

The impact of different water qualities and depths on *Phragmites* has been studied for some time (Haslam 1970b), and an interstitial water salinity limit of c.20 parts per thousand has been established (Ranwell *et al.* 1964). More recently the actual mechanisms of flood and salinity tolerance have been identified (Gries *et al.* 1990, Matsushita & Matoh 1991, Ostendorp 1991, Hellings & Gallagher 1992, Matsushita & Matoh 1992). These imply rhizome-controlled limitations on water uptake which may have serious implications for the relationship between the isotopic composition of the water used by the plant and the variable composition of the water in the rhizosphere as the influence of freshwater and saltwater sources changes. The same processes which influence this project (because of the implications for water uptake) may also explain some of the data in studies of ionic

uptake by *Phragmites* (Glenn 1987, Long 1991). Of this work, the most significant is probably that of Matsushita and Matoh (1992) on enzyme-controlled sodium uptake limitation.

Whilst the uptake of water is primarily controlled by mechanisms within the rhizome, the assimilation of carbon is principally controlled by the above-ground growth of *Phragmites*. However, this is not constant through time or with age group (Haslam 1970a), and Brix (1990) points to the use of non-gaseous carbon by very young (less than two year old) plants. Although he suggests that less than 5% of total carbon demand is satisfied from sedimentary uptake, this is potentially very important given that it is necessary to be able to assume that the ratio of ^{12}C to ^{13}C in the carbon source is a constant at any particular time. If the utilization of carbon varies according to the age of the plant and certain zones are characterized by plants of particular age group, this will make analysis of data from different facies unsuitable for environmental reconstruction.

In some cases this above-ground growth is heavily influenced by a variety of management techniques, which often have different ends in mind. The impact of interference by man has been studied from a number of different aspects (Dithlogo *et al.* 1992, Cowie *et al.* 1992, Mook & Van der Toorn 1982a, b), but at Roudsea the influences are restricted to very low density seasonal grazing by sheep and the impact of the earthen sea defence. The latter has undoubtedly altered the vegetation community of what was previously upper saltmarsh, but it has effectively created an environment reminiscent of, if not identical to, a coastline undergoing a regression of sea-level and a diminishing influence by the tide. Although the construction of a bridge to carry the Carnforth to Barrow-in-Furness railway in the mid 19th Century altered the mouth of the estuary and the sediment flux from the Leven catchment has undoubtedly changed as a result of human interference, these factors do not significantly detract from the value of the site as a useful

analogue for this study. By examining the nature and salinity of the surface sediment throughout the study period in each community it should be possible to determine the degree of environmental variation within a growing season and assess the influence of these factors on the above-ground growth of *Phragmites*.

However, *Phragmites* is more compex than many other plants in terms of gaseous exchange (and hence CO_2 assimilation and H_2O and O_2 losses) since it is not wholly restricted to transfer via stomata. In particular, Brix (1989) has pointed to the rôle of dead culms in gas exchange, which is significant in this project since the dead culms are of varying proportions, strengths and densities as a result of the different stresses inherent in the three environments. A number of workers have also highlighted the significance of varying light regimes on *Phragmites* (Brix 1988, Weisner 1988, Armstrong & Armstrong 1988, Armstrong & Armstrong 1990, Armstrong *et al.* 1992). They point to the significant variation in water uptake, through flow, use and loss which, combined with effects on carbon assimilation, may be very important in this study because two of the communities are on the woodland fringe and face west whilst one is in full sun on the open saltmarsh.

Additional problems arise from the nature of plant growth, and especially the rhizome system. *Phragmites* generally reproduces and colonises vegetatively (Phillips 1987, Haslam 1972), resulting in a dense and extensive network of rhizomes which may be interlinked and are often clonal. This has certain advantages and disadvantages from the point of view of this project. An advantage is that the plants within a particular community are often closely related and hence might respond to physiological stress in the same way, resulting in similar water uptake and carbon assimilation modes in adjacent plants.

One disadvantage is that the rhizomes can extend laterally at a significant rate (up to 2 metres *per annum* is recorded at Roudsea over the period 1969-1994, Adam 1976, 1994 pers. comm.), sending up aerial shoots as they go. This extension may be into areas from which the plant is not extracting water during all the time that it is photosynthesising because of, for example, excess soil water salinity. Rather, water is fed from a core area by the rhizomes (Hara *et al.* 1993, Kuido & Ito 1992), potentially resulting in macrofossils which are autochthonous but do not reflect the conditions under which they were growing. When sampling the above-ground material it is not immediately possible to determine the extent of the rhizome network, but sampling the rhizome itself is not useful for palaeoenvironmental purposes since rhizomes from contemporary plants have been found at depths of up to 2 metres below the ground surface (Haslam 1972). It is therefore not always sound to relate a rhizome found at a particular depth to one particular surface environment. Consequently, particular parts of equivalently positioned leaves were studied so that comparisons could be made of material from the same plant (cf. Pizzolata 1993, 1994), between plants in the same zone and between zones as a whole. By adopting this approach there is a risk that the oxygen isotope composition of the cellulose of the plants at the saline limit may not reflect the composition of the soil water that characterises this environment. However, the stunted nature of the plants in this community suggests that the uptake of carbon is being restricted (see chapter 2, below, for theoretical explanation) and this may be reflected in the isotopic ratio of the carbon in the cellulose.

From the point of view of the plant, a clonal community can be very successful if it is suited to the particular environment at that time, but it may mean that a change of environment has significant effects on the extent and health of the community. Retreats of *Phragmites* have been noted as a result of environmental shifts by a number of researchers (Boar *et al.* 1989, Cizkova-Koncalova *et al.* 1992, Chatterton *et al.* 1989) and in the past this was associated with limited ecological ranges amongst the population types of

Phragmites, to the extent that sub-species were defined (Haslam 1970). However, explanations based on phenotypic plasticity have been replaced with ones based on genotypic plasticity as a result of investigations into polyploidy in *Phragmites* populations (Gorenflot 1986, Zong *et al.* 1991). The implications for this study are significant since if the three environments contain genetically different populations with, say, values of n=2 at the freshwater extreme and n=4 or n=8 at the saltwater extreme (Gorenflot 1986, Zong *et al.* 1991), variations in the assimilation of both oxygen and carbon may not be primarily controlled by environmental factors (see also Wang *et al.* 1995). The result would be the production of incomparable data sets from analysis of material taken from plants on the three zones. Although the genetic structure of the communities analysed is not investigated in this study, the fact that the community isolated from the tide was once the exact equivalent of the marsh to wood fringe population, and that the two communities once adjoined each other, offers some hope that they are genetically related. Similarly, an examination of a study of Roudsea in 1969 revealed that the marsh has expanded laterally and vertically since then, but that the absolute altitude of the seaward limit of the *Phragmites* has not changed (Adam 1976, 1994 pers. comm., Gray 1994 pers. comm.; see Chapter 3 for details). This suggests that there has not been an invasion of a more salt-tolerant form since 1969 and that the expansion over the period 1969 to 1994 is from the same population which today forms the marsh to wood ecotone.

All of these complications need to be considered, but by examining particular parts of a number of plants from each of the three communities the potential of this technique can be assessed. It is important to highlight that this is ultimately a project which is looking for tools for palaeoenvironmental reconstruction, and not one trying to explain the distribution of *Phragmites* or the particular controls on its photosynthetic and respiratory systems. However, since these processes directly influence the variables being examined,

an assessment of the potentially complicating factors of this species' physiology (Coops *et al.* 1994, Daniels 1991) is fundamental to the project.

3.3 Research design and strategy.

The research strategy was developed to answer certain key questions. These can be summarised in point form.

i) At what altitude are the three communities, and how often are they flooded by the tide?

ii) How saline is the soilwater in each community, does it vary during the growing season and does salinity appear to be a factor limiting the expansion of *Phragmites* further down the saltmarsh?

iii) Is there a significant difference in the habit (plant height, number and size of leaves) of the three *Phragmites* communities?

iv) Does stomatal density vary significantly between the three communities?

v) Does the $\delta^{18}O$ value of the soilwater in the rhizosphere differ between the three communities?

vi) Is the isotopic composition of the cellulose from equivalent parts of plants from each community consistent within each plant and within each community but also significantly different between each community?

Questions i) to v) aim to set out the environmental differences and the variations in plant physiognomy between the three communities. The key question remains whether $\delta^{13}C$ and $\delta^{18}O$ values from each zone are internally consistent (both intra-plant and intra-community) but sufficiently different to be able to unequivocally demonstrate a link between environment and isotopic ratio.

3.4 Methodologies adopted to answer these questions.

3.4.1 Measurement of altitude and relationship to tidal inundation.

The altitude of each community relative to Ordnance Datum (O.D.) Newlyn was determined by levelling from a benchmark (indicated on Map 3.1). The seaward limit of the *Phragmites* was also measured along the fringe of the lowest *Phragmites* community at Roudsea. These data were compared to tidal records from the tide gauge at Heysham, Lancashire (O.S.grid reference SD403602). Although this is 23km. away, it is within the large embayment of Morecambe Bay and will give a measure of the annual frequency of tidal inundation. Records from 1975 to 1994 were received from the Proudman Marine Laboratory, and kindly made available by Dr.Y.Zong. A correction factor was applied to account for the variation in the O.D. altitude of mean high water spring tide (MHWST) and highest astronomical tide (HAT) between Heysham and Roudsea of plus 20cm (Zong 1993).

3.4.2 Measurement of soil sample characteristics.

The salinity of the soilwater in each community through the growing season was measured by taking 5 randomly located surface soil samples from each of the three communities and the area below the limit of *Phragmites* on the saltmarsh on twelve

occasions between early April and early October 1994. These samples were brought back to the laboratory where they were dried to establish their moisture content (loss of mass on drying, LOD%) and the proportion of organic matter in the sediment (loss of mass on ignition, LOI%). Soil salinity was measured by establishing the amount of available chloride in each sample and relating this to moisture content. The exact methodology used in these analyses of the soil samples is outlined in Appendix 1.

3.4.3 Measurement of the habit of *Phragmites* in each community.

The above-ground growth of *Phragmites* in each community was measured from a number of different aspects. The density of aerial shoots was established using a purpose-built wooden 1m×1m quadrat which consists of three fixed sides and three detachable metre-long dowels. This design enables the density of the very tall *Phragmites* shoots to be measured without trying to force a fixed-sided quadrat over the top of the *Phragmites* stand. Instead the quadran is slid around the base of the *Phragmites* shoots, and the dowels are used to form the limit of the 1m×1m square and then to subdivide this into four 50cm×50cm quadrats.

Using this quadrat, the density of living shoots was measured in early October of 1994 and the height of the tallest and shortest shoot in each 50cm×50cm subdivision was also measured. At the same time fifteen shoots were cut off at their junction with the rhizome system and the height, number of leaves and length of the second leaf down from the infloresence at the top of each stem was measured. This leaf (hereafter "leaf 1") was used as the basis for intra- and inter-community analyses of stomatal density and isotopic ratios. It was chosen because it was present on all the shoots studied (many of the lower leaves had fallen or were in a state of disrepair), and it is formed in the latter part of the

growing season and consequently the input of stored carbohydrate from the rhizome is likely to be minimal (Haslam 1972).

The standardisation of sampling for intra- and inter-community analyses was also followed for intra-plant analyses. In this case the leaves 2, 4 and 6 positions below leaf 1 were chosen (hereafter leaves 3, 5 and 7 respectively).

3.4.4 The measurement of stomatal density.

A five centimetre strip from the basal portion of each leaf analysed was cut out and used for the measurement of stomatal density. In each case the underside of the leaf was analysed, and this was done for leaf 1 of all 15 shoots from each of the three communities and for leaves 3, 5 and 7 from 5 randomly chosen shoots from each sample of 15. The method used to prepare and count samples to measure stomatal density is given in Appendix 2 (see also Beerling & Chaloner 1993b), and the basis of the count was ten random views across each slide. By analysing the same part of equivalent leaves from each community it was hoped to ascertain whether there might be a link between environment and this aspect of physiognomy which has been linked to $\delta^{13}C$ values (Beerling & Chaloner 1993b, see also Chapter 2).

3.4.5 Measurement of the $\delta^{18}O$ value of soilwater from the rhizosphere in each community.

These measurements were made possible by Dr. Tim Heaton at N.I.G.L., Keyworth, and were performed in order to establish whether the water available to the three *Phragmites* communities was isotopically distinct. Only a limited number of analyses

were possible (twelve), and so these were carried out on samples taken on the same day at four set depths in soil cores from each community.

The samples were taken at 5cm, 15cm, 25cm and 35cm depth from a 75mm wide core. Living rhizomes were found at each depth in each community. The samples were wrapped in plastic-sheeting to avoid any gain or loss of the water and stored in a refrigerator prior to extraction of the water. Half of the sample was used to establish LOI%, LOD% and salinity (see Chapter 3.4.1). From the other half the water was extracted by placing each sample into a glass test tube fitted with a rubber seal. This and a second similar, but empty, tube were fitted to a glass arm which had a valve and an aperture which could be attached to a vacuum line. The valve was closed and the seals on the tubes were fixed with sprung clamps before plunging the tube containing the soil sample into a liquid nitrogen bath.

Once the sample had frozen it was removed from the bath, and the whole apparatus was evacuated to $<10^{-3}$ bar. The valve was then closed and the apparatus removed from the vacuum line. The empty tube was placed in a liquid nitrogen bath and the tube with the frozen sample was placed in a heating block at 110°C. As the sample thawed and the water evaporated from the soil it condensed and froze in the cooled tube. This process took 4-6 hours, after which the water was decanted into sure-seal vessels and put into a refrigerator prior to analysis.

The isotopic analysis used a slowly rocking, covered manifold which was kept at a constant temperature (27°C) by the circulation of warm air. The water samples were decanted into glass vessels and two water standards were added to each run. The vessels were evacuated and then filled with CO_2 of known isotopic composition. The isotopic composition of the CO_2 and H_2O samples equilibrated (Brenninkmeijer *et al.* 1983), and

once equilibrium was achieved the CO_2 was fed to a gas isotope ratio mass-spectrometer. This measured the ratio of CO_2 of masses 44, 45 and 46 from the sample manifold and compared it to the ratios from a CO_2 standard (Anon. 1989). Given the fixed temperature in the manifold it was possible to determine the $\delta^{18}O$ value of the water sample given the extent of transformation of the original CO_2 added to the vessels during isotopic equilibration.

3.4.6 Measurement of the isotopic composition of *Phragmites* cellulose.

For the purposes of inter-plant and inter-community comparisons, cellulose from leaf 1 was extracted for isotopic analysis. Intra-plant comparisons were made on the basis of data from the cellulose of leaves 3, 5 and 7 in addition to leaf 1. In all cases the whole leaf (less the 5cm strip taken for stomatal density measurements) was processed and a sub-sample of the homogenised cellulose used for the isotopic analyses.

The procedure for the isolation of cellulose is outlined in Appendix 3. This is the method adopted in all analyses of $\delta^{13}C$ and $\delta^{18}O$ in plant cellulose and is as outlined in Sternberg 1989a (see also Field 1993a, b, Robertson 1993).

Cellulose was prepared from leaf 1 of all 15 shoots sampled from each community, and from leaves 3, 5 and 7 from the same 5 shoots that were chosen for analysis of intra-plant stomatal density. However, limitations on laboratory time restricted the number of analyses of both $\delta^{13}C$ and $\delta^{18}O$, although a significant sub-sample was tested. Nine of the fifteen shoots from each community were used for tests of inter-plant and inter-community $\delta^{13}C$ from cellulose of leaf 1, and two of the five shoots used in the intra-plant analysis of stomatal density were chosen to examine intra-plant variations in $\delta^{13}C$. This made 9×3 (nine different leaf 1's from 3 communities) and 3×2×3 (leaves 3, 5 and 7 from two

different shoots in each of the three communities) or a total of 45 analyses of $\delta^{13}C$ from *Phragmites* cellulose.

The analysis of $\delta^{18}O$ was aimed at establishing whether a link existed between the $\delta^{18}O$ value of soilwater (Chapter 3.2.5) and cellulose $\delta^{18}O$ (DeNiro & Epstein 1979, DeNiro *et al.* 1988, 1990, Ehleringer & Dawson 1992). These analyses were performed during a visit to the University of Miami and a further sub-sample of the original 15 leaf 1 cellulose preparations was made. In this case six samples, each of which had been part of the nine taken for $\delta^{13}C$ analysis, were chosen for each community as the basis of an analysis of inter-plant and inter-community consistency, with other samples available to test intra-plant variations if these samples revealed data that might be of use for palaeoenvironmental studies.

The processes of conversion of cellulose to CO_2 for measurement of $\delta^{13}C$ and $\delta^{18}O$ are quite different, and are given in Appendices 4 and 5 respectively. Thereafter the analyses involve establishing the ratio of CO_2 of weights 46, 45 and 44 and calculating the deviation of the measured ratio from that of the PDB standard for $\delta^{13}C$ and SMOW for $\delta^{18}O$ (Anon 1989).

During all of these analyses standards of known isotopic composition were run alongside the samples. In Miami three standards were used. One was a graphite standard (National Bureau of Standards #21), and the other two were carbonate standards from Beta Analytic Inc. who share the mass spectrometer used. At the Isotope Geoscience Laboratories in Keyworth a carbonate standard was used. The results from these analyses are given in Table 3.1. All of the results from running these standards fall within 0.1‰ of the known value.

Table 3.1 Isotopic standards used during the analyses of *Phragmites* isotopic composition.

Laboratory	Standard used	Expected value	Result	Precision
Miami	NBS#21 (graphite)	$\delta^{13}C$ = -28.1‰ PDB	$\delta^{13}C$ =-28.08‰ PDB	0.006‰
Miami	NBS#21 (graphite)	$\delta^{13}C$ = -28.1‰ PDB	$\delta^{13}C$ =-28.18‰ PDB	0.004‰
Miami	NBS#21 (graphite)	$\delta^{13}C$ = -28.1‰ PDB	$\delta^{13}C$ =-28.15‰ PDB	0.003‰
Miami	b3scd (carbonate)	$\delta^{18}O$=22.15‰ SMOW	$\delta^{18}O$=22.13‰ SMOW	0.02‰
Miami	cstan (carbonate)	$\delta^{18}O$=29.1‰ SMOW	$\delta^{18}O$=29.1‰ SMOW	0.02‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.67‰ PDB	0.03‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.73‰ PDB	0.03‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.73‰ PDB	0.03‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.68‰ PDB	0.03‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.69‰ PDB	0.03‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.66‰ PDB	0.05‰
Keyworth	mcs-13 (carbonate)	$\delta^{13}C$ = -0.7‰ PDB	$\delta^{13}C$ = -0.74‰ PDB	0.05‰

3.5 Results of analyses on environment of growth and variations in plant physiognomy.

3.5.1 Altitude and frequency of inundation.

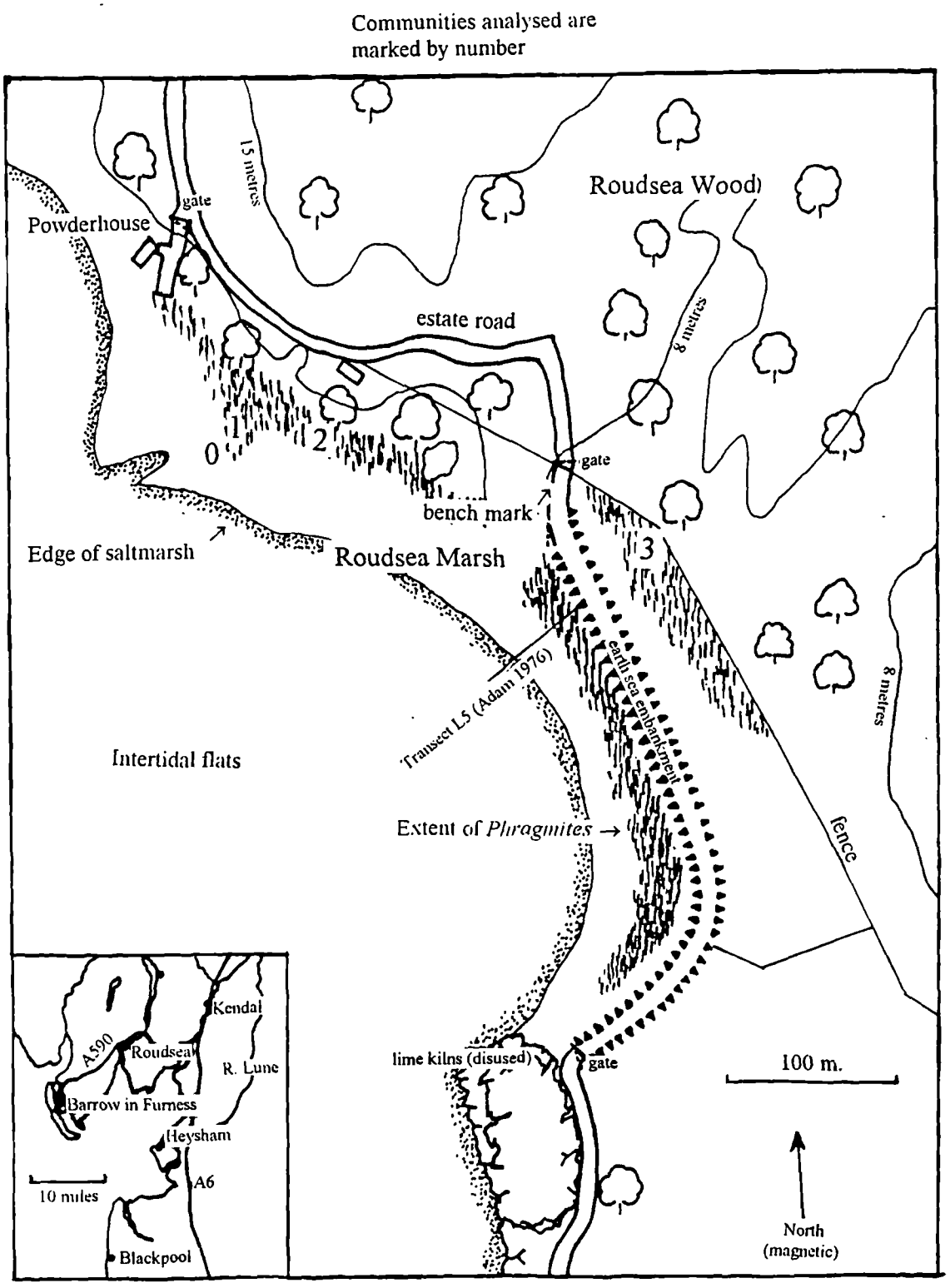
The three *Phragmites* communities are distinguished by occurring at different altitudes and being inundated by the tide at varying frequencies. The variations in environmental factors such as inundation, salinity and soilwater $\delta^{18}O$ in each zone are a function of altitude, and on this basis zones 1, 2 and 3 are defined (see Map 3.1).

Zone 1 is the community found in the middle to upper saltmarsh, adjacent to and mixing with a typical *Puccinellietum* saltmarsh vegetation community (Adam 1978, 1981).

Zone 2 is the ecotone between saltmarsh and the mixed oak forest, similar to that identified by Ranwell (1974), which is a mono-dominant *Phragmitetum* (Adam 1978, 1981) community.

The *Phragmites* in zone 3 was formerly the equivalent of zone 2, but is now isolated from flooding by the tide by a seawall. Consequently the absolute altitude of the seawall is the controlling factor in this particular zone, rather than the ground altitude of the zone itself.

Map 3.1. The location of Roudsea (inset) and the position of the *Phragmites* communities analysed.



The altitude of zones 1 and 2 and of the lowest point of the seawall were calculated relative to Ordnance Datum Newlyn from a local benchmark (levelling was rounded with a closing error of less than 2cm). The altitude of the zones (mean ± 1 standard deviation, n=10) and the seawall (minimum measured along its length) are;

Zone 1	4.76 (±0.03) metres OD
Zone 2	5.85 (±0.08) metres OD
Sea wall (minimum)	6.51 metres OD.

The mean altitude of zones 1 and 2 correspond (±10cm) to the altitude of local mean high water spring tide (MHWST; = 4.7m OD) and highest astronomical tide (HAT; = 5.8m OD) respectively (Zong 1993, 1994 pers. comm.). The altitude of the seawall means that zone 3 is beyond the reach of all but exceptional tides which, combined with a large freshwater input to the estuary and a strong on-shore wind, cause a storm surge component to be added to the altitude reached by the high tide. No such events occurred during the course of this study (Singleton 1994, pers. comm.). Consequently the water inputs to zone 3 can be considered to be unaffected by the tide.

Two key points can be drawn from the altitude of zones 1 and 2. The first is that there is an empirical link between the two zones and the regularity of tidal inundation which may account for the differences in plant habit observed (Chapter 3.5.3). The second is that if it is possible to distinguish *Phragmites* cellulose on the basis of $\delta^{13}C$ and $\delta^{18}O$ values from these two zones, this would be a major help in reconstructing horizons in sedimentary sequences which represent communities at or near these positions in coastal vegetation successions.

In addition to levelling the communities analysed in this study, the seaward limit of *Phragmites* was also levelled at a number of points. The altitude of each of these points was (m OD) 4.71, 4.75, 4.71, 4.71, 4.68, 4.69 and 4.67, which yields an average (±1 standard deviation) of 4.70±0.03m. This is close to the mean figure for zone 1, and also to the average altitude of the seaward limit of *Phragmites* as measured by Adam and Gray (Adam 1976) in 1969 of 4.67m. This suggests that a similar relationship between tidal inundation and *Phragmites* exists today as it did 25 years ago. However, the communities have not stood still over this period. Repeats of surveys carried out in 1969 were made in 1994 along transects L5, L6 and L7 (Adam *op. cit.*). These repeats show that there has been considerable lateral extension of the *Phragmites* communities of 25m, 30m, and 50m. respectively. Further, the altitude of the points along these transects which marks the present limit of *Phragmites* has increased by 49cm, 51cm and 20cm respectively compared to the 1969 altitudes.

This aggradation appears to be the result of the shift of the channel of the River Leven across the estuary to the west (Adam 1994 pers. comm., Singleton 1994 pers. comm., DeLaune *et al.* 1990, Warren & Niering 1993), and is of a very significant (cf. Oenema & DeLaune 1988) order (up to a mean value of 19.8mm *p.a.* of vertical accretion and 2m. *p.a.* of lateral extension of the *Phragmites* communities).

This evidence for lateral extension has two important consequences. Firstly, the relationship between the altitude of the limit of *Phragmites* and the frequency of inundation is the same in 1994 as in 1969. This suggests that there has not been a change in the environmental tolerance of the *Phragmites*, which might suggest that the population at the limit of growth today is related to that at the limit of growth in 1969. This suggests that there has not been an invasion of *Phragmites* of different physio- or geno-typic

structure (Gorenflot 1986), and that concerns of such variations impacting on isotopic ratios are, in this case, probably groundless.

The second consequence relates to the mode of lateral extension adopted by *Phragmites* (Haslam 1972, Kuido & Ito 1988). This is through rhizomes, which invade from a core population. Not only does this mean that a newly-invaded area is potentially being fed from a distant zone (which has implications for measurements of isotopic ratios controlled by gaseous transfer and water uptake), but it also means that the age structure of plants in recently-invaded areas may not reflect the variation inherent in a long-established *Phragmites* stand (Hara *et al.* 1993). As pointed out in Chapter 3.2, age has been shown to impact on the source of C used by *Phragmites*, and this variable is unconstrained in this study. Only detailed examination of the rhizome network would better constrain this variable. Such an examination would also help determine the depth from which soilwater is utilised (Chapter 3.5.5).

In order to analyse the possible role that the rhizome architecture and age structure of *Phragmites* communities has on the isotopic composition of leaf cellulose it would be necessary to establish a controlled experiment utilising transplanted material from the three different populations at Roudsea. By allowing the rhizome to grow in the same substrate and to send up shoots over a period of two years during which both the $\delta^{13}C$ of the atmosphere was held constant and fresh water of known $\delta^{18}O$ was fed to the plants, the impact of age on the isotopic composition of the cellulose metabolised could be assessed, and any differences in biomass production between transplants from the three populations could be measured. Once lateral extension has taken place, the extent to which the plant is able to selectively utilise water and feed water along the rhizome network could be examined by watering one end of the rhizome network with sea water and the other with freshwater. At the same time plants from the three populations without extensive rhizome

networks that link the two growth environments should be grown under the same conditions to determine the success of plants that cannot be fed with water from a non-saline water source. Analysis of the isotopic composition of water in the stem and the rhizome of the plants would determine the extent of selective water use and the importance of water transfer along the rhizome network. A similar type of transplant experiment has been carried out on *Phragmites* plants from genetically different populations to determine the extent to which these different populations can withstand varying salinity conditions (Zong *et al.* 1991), but no isotopic analyses were made, and the possible impact of the age of the plants was not considered.

The raw data showing the number of inundations by altitude for the years 1975-1994 inclusive is given in Appendix 6. Given that MHWST is predicted to be reached on 60 occasions during the year and HAT is a once-a-year event (Admiralty Tide Tables, 1995), the first notable point about these data is that MHWST at Heysham is reached on 100-156 occasions *per annum* over the period of the data. This indicates a significant additional input to the tidal system along this coastline above that caused by astronomical factors alone. This is likely to be a result of short-term variations in freshwater input to Morecambe Bay from local catchments and the impact of strong on-shore winds combining with highs in the tidal cycle to produce higher than predicted tide levels.

At the top end of the high tide spectrum, the altitude of HAT at Heysham is reached between 0 and 9 times per annum between 1975 and 1994. The predicted frequency of tides that reach this level is 1 *per annum*. In all bar 5 years this has been exceeded, although in 1994 the observed matches the expected frequency. The reasons for this excess of observed over expected HAT are the same as those which might explain the unexpectedly high frequency of inundation at 4.5m OD.

The important aspect of these data is that they demonstrate that at Heysham MHWST was reached 132 times and HAT was only reached once in 1994. Translated to Roudsea, and to this project in particular, it shows that the frequency of inundation in the three zones is markedly different, with zone 1 being subject to flooding by an excess of 100 tides, zone 2 by very few tides, and zone 3 never flooded because of protection by the sea defence.

3.5.2 The sediment analyses.

The results of the analyses of LOD%, LOI% and soilwater salinity from zones 1, 2, and 3 plus zone 0 (the area of *Puccinellietum* immediately seaward of zone 1) are given in Appendix 7.

A gap in the data run exists because the samples taken on 6/4/94 from zone 0 leaked during the journey from Roudsea to Durham and consequently they were discarded.

The remaining samples reveal some interesting trends which enable the differences between the three communities to be quantified. Firstly there is very little difference in the characteristics of LOD%, LOI% or salinity between zone 0 and zone 1. With the exception of the value of salinity on 22/4/94, LOI% on 22/6/94 and LOD% on 5/10/94, the mean values (±1 standard deviation) overlap between these zones, and the exceptions all fall within 2 standard deviations. Figures 3.1 and 3.2 clearly demonstrate the similarities between zones 0 and 1 during the sampling period in terms of LOD% and LOI% respectively. They also show the extent of the differences between the 4 zones sampled, and especially those between zones 1, 2 and 3.

Figure 3.1

Roudsea sediment LOD (%)

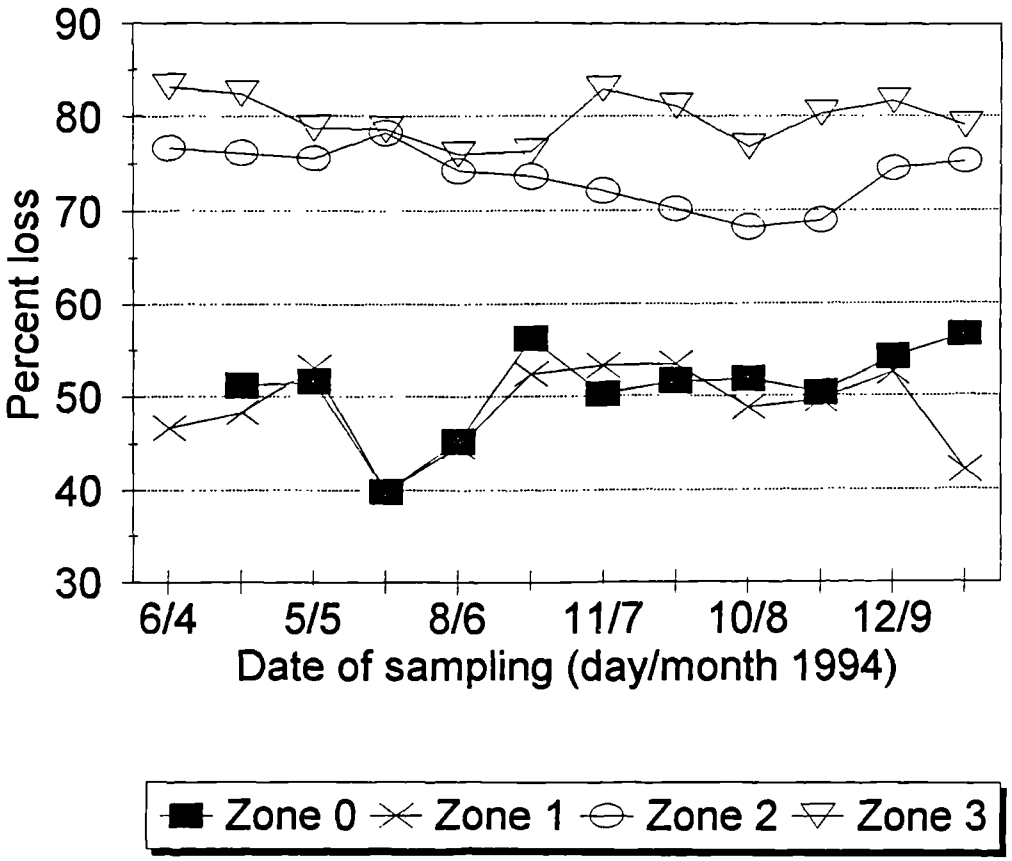
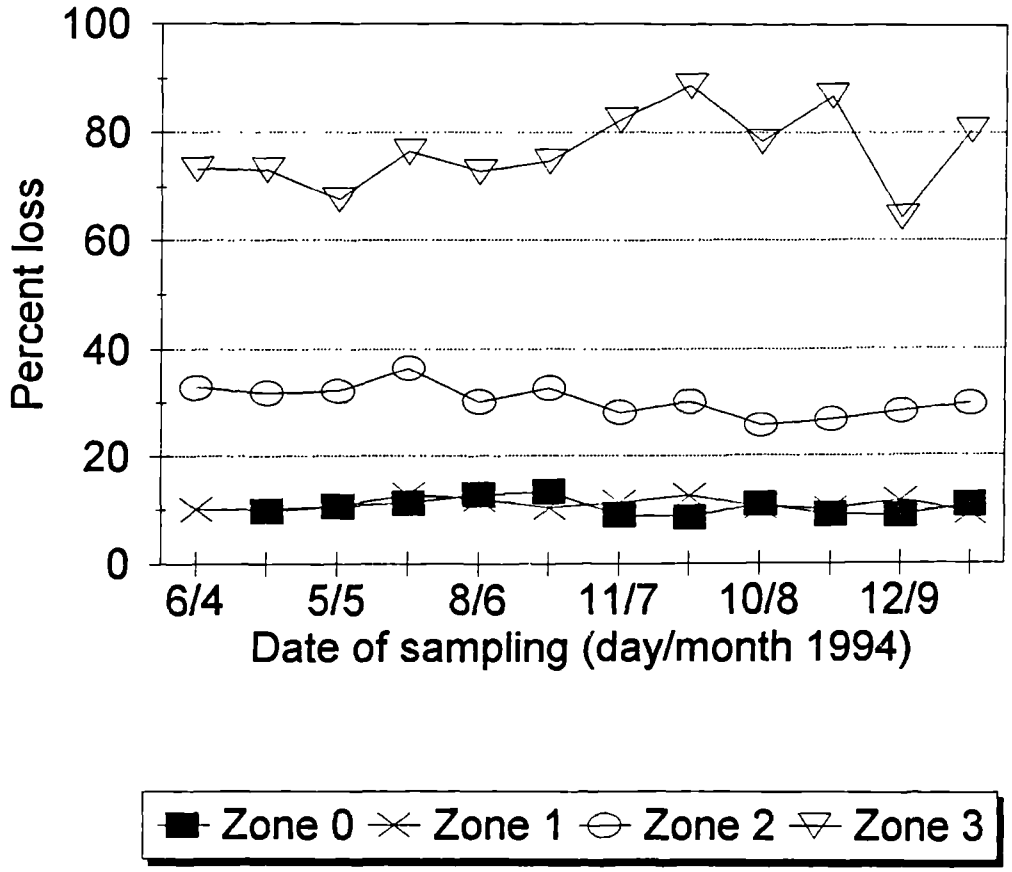


Figure 3.2

Roudsea sediment LOI (%)



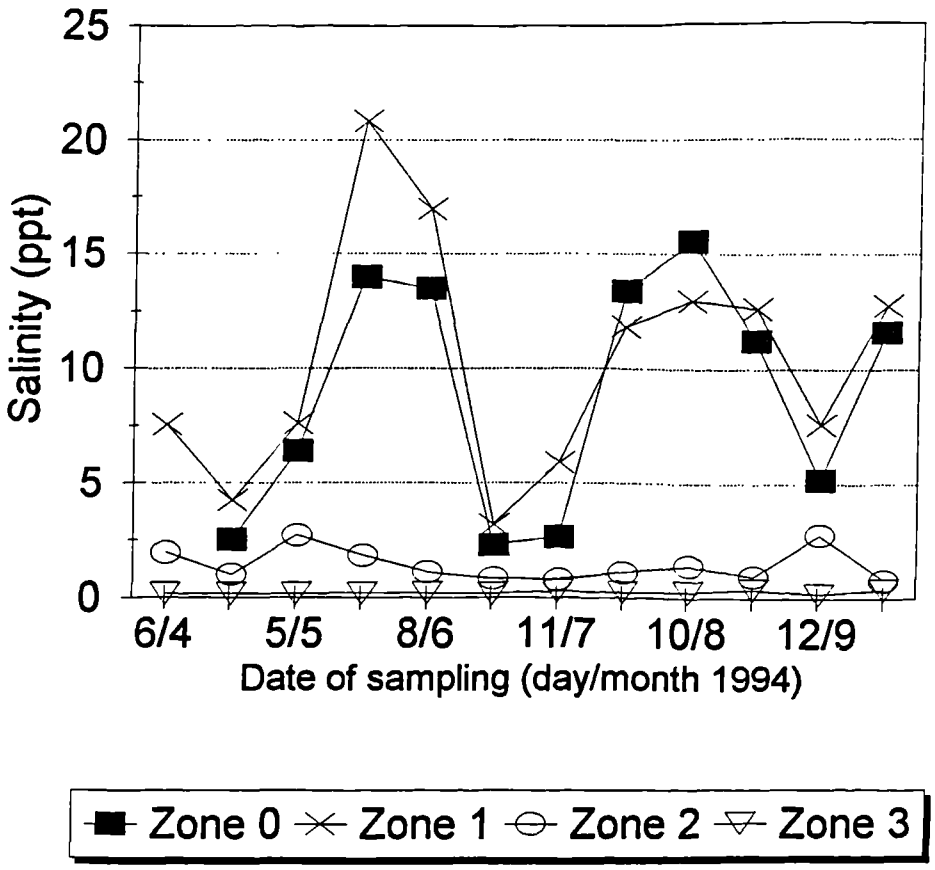
The value of salinity varies considerably through the year in zone 1, from a low of 3.16‰ to a high of 20.84‰ (compared to c.35‰ in sea water, Aston 1978, Price *et al.* 1990). It is notable that this variability is mirrored in zone 0 and that the peak value recorded in zone 0 is less than that in zone 1, as can be seen in Figure 3.3. The fluctuations in these data reflect the changing input of sea water to these zones over the tidal cycle. Since there is no difference between the values for salinity in zones 0 and 1, it seems that it is not salinity which is restricting *Phragmites* from invading this area. The value of 20.84 parts per thousand salinity found as the peak in zone 1 is very close to the value quoted by Haslam (1972) and Ranwell *et al.* 1964 for the salinity limit of *Phragmites*.

The salinity values in zone 2 are markedly lower and vary to a far lesser degree than in zone 1, ranging from a high of 2.75‰ to a low of 0.78‰. This reflects the considerable difference in inundation frequency between the two zones. Salinity in zone 1 reflects the higher incidence of flooding at the position of MHWST, with decreases in salinity caused by sampling at different times of the tidal cycle and by large inputs of freshwater from precipitation and overland flow. Zone 2 is predicted to be reached only once a year by the tide, and although there is a difference between the observed and expected frequency of inundation at this altitude (Chapter 3.5.1), there is still at least one order of magnitude between the number of inundations at these two altitudes per annum.

Given the consistently very low measurements of salinity in zone 3 (0.39 to 0.17‰), it is possible to draw a number of conclusions about these data in terms of potential impacts on isotopic composition. The plants in the community in zone 1 are subject to higher levels of inundation and soilwater salinity than in either zone 2 or zone 3 (Figure 3.3). The level of salinity varies considerably through the growing season, which may lead to variations in stomatal conductance. In addition, the data show the changing

Figure 3.3

Roudsea sediment salinity (ppt)



importance of the different sources of water to the plants. Both these factors may have implications for isotopic ratios. However, there are consistent differences in the moisture content, organic C content and soilwater salinity in each zone, and consequently there are good grounds to believe that $\delta^{13}C$ and / or $\delta^{18}O$ will vary according to zone number.

3.5.3 Plant habit.

The raw data collected on 5/10/94 to assess the variations in plant habit are given in Appendix 8 (density of living stems) and Appendix 9 (height of plant, number of leaves and length of leaf 1). The density measurements show that zone 1 has fewer stems per 50cm×50cm sub-division than zone 2 and zone 3, but that the mean values plus or minus 1 standard deviation overlap. However, significant differences do exist in the number of dead shoots still standing. In zone 1 there are very few upright shoots from previous years of growth compared to zones 2 and 3. This produces apparently denser stands in zones 2 and 3, but because it is not possible to assign an age to the dead shoots they were not included in the survey.

The minimum and maximum heights of these stems also give an indication of the variations in gross physiognomy between zone 1 and zones 2 and 3. These differences in plant height are reinforced in Appendix 9, which shows the length of the 15 shoots sampled in each community, the number of leaves and the length of leaf 1. These data are included in Table 3.2, which summarises the environmental differences and variations in physiognomy between the three communities, and the variations in plant height are graphically shown in Figure 3.4. In both this figure and in Figure 3.5 which shows the variation in the length of leaf 1 in the three zones, the plant numbers are used as symbols. From this a very clear picture emerges of dense, tall *Phragmites* shoots with long leaves in

Figure 3.4 Roudsea *Phragmites*; height of plant by zone.

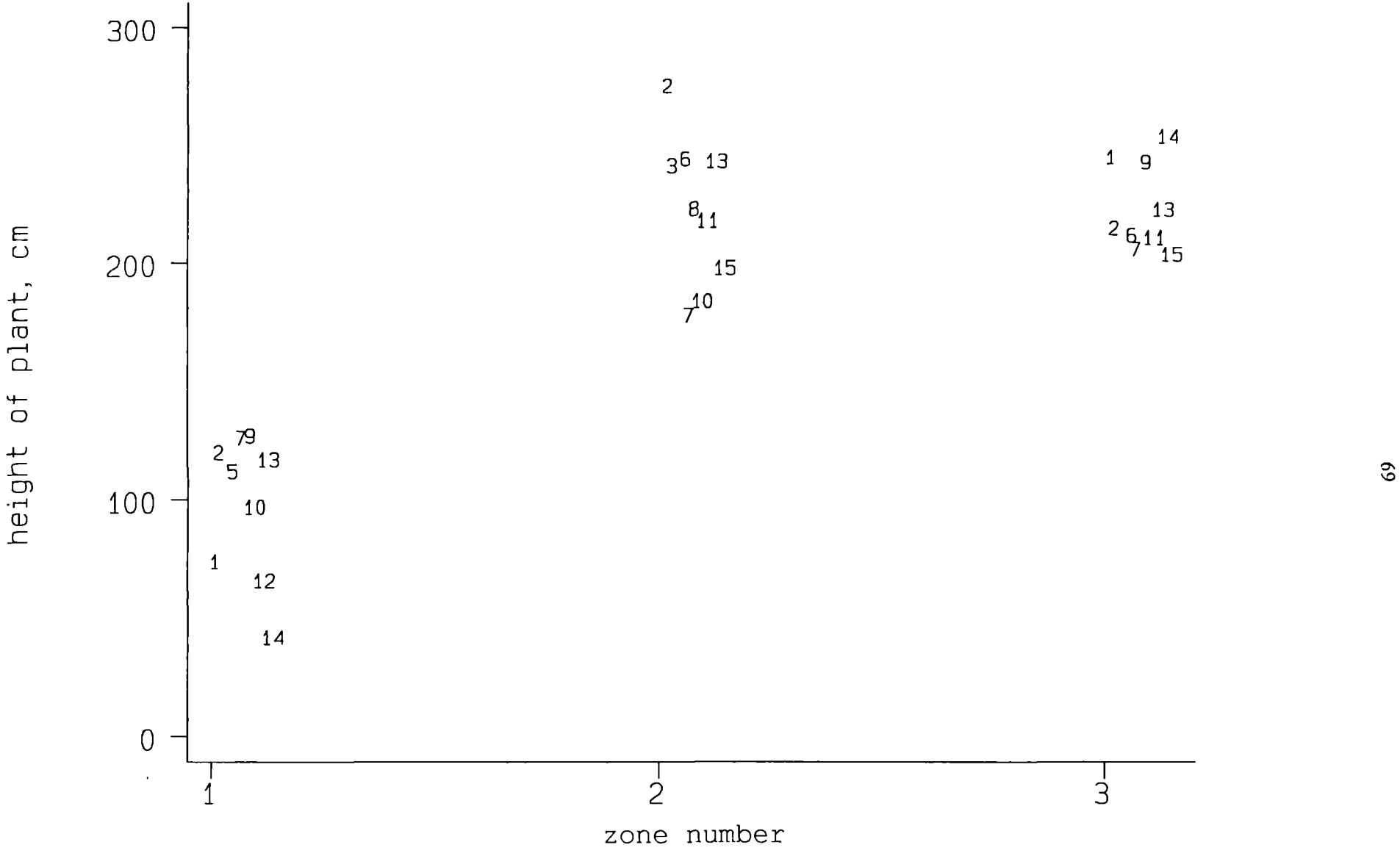
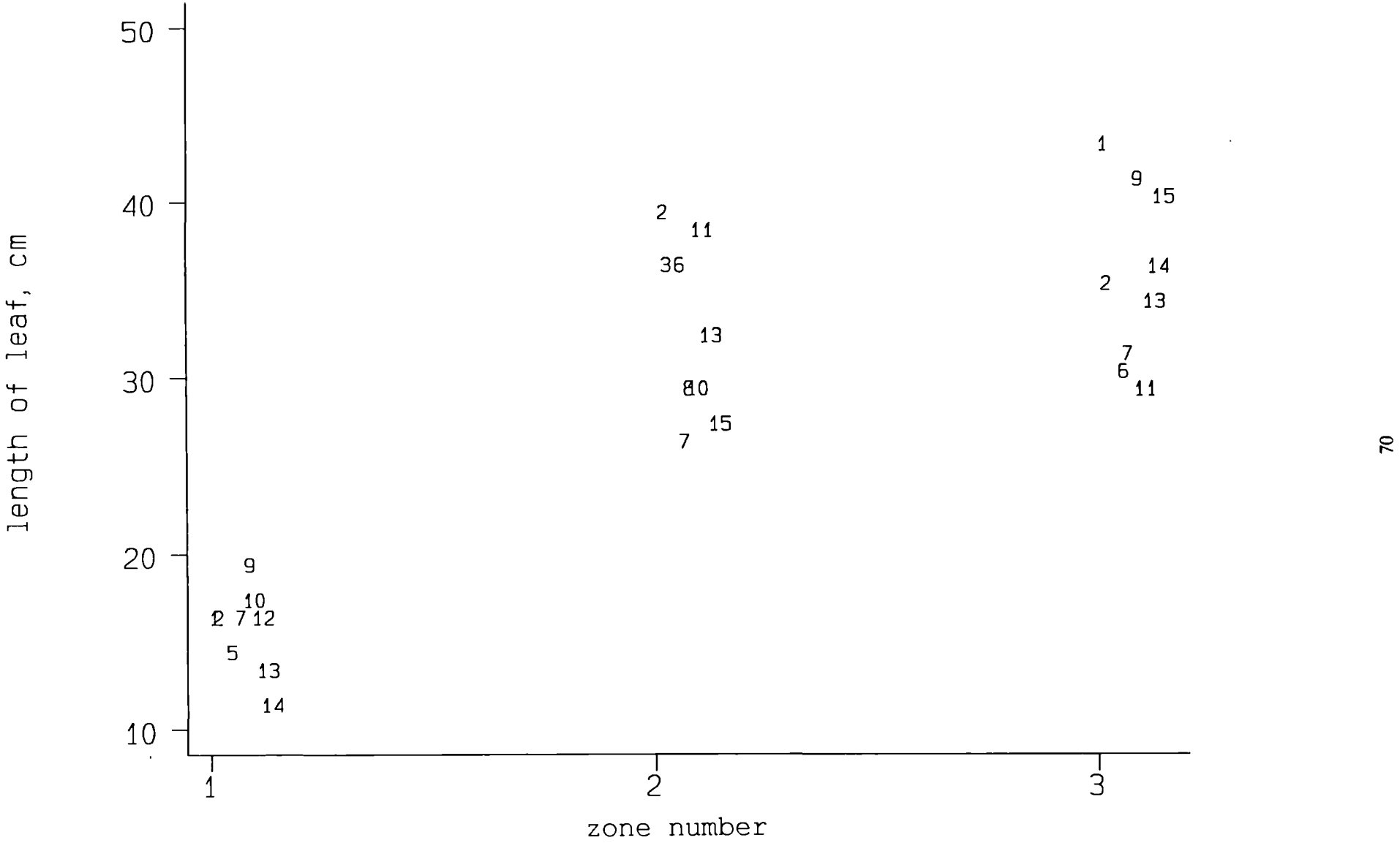


Figure 3.5 Roudsea *Phragmites;* length of leaf by zone.



zones 2 and 3, and considerably shorter shoots and leaves in a less-dense community in zone 1.

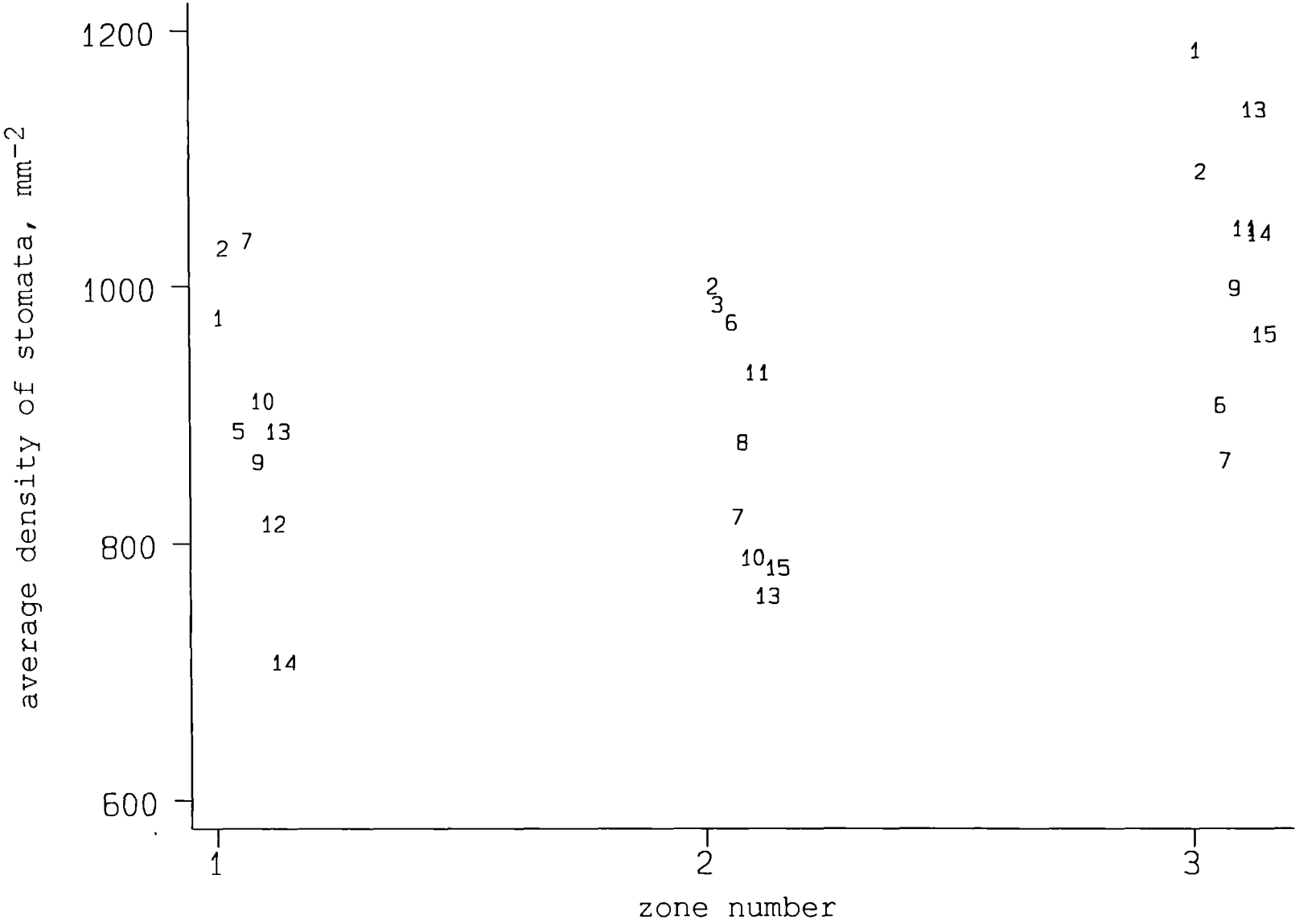
3.5.4 Stomatal density variations.

Appendix 10 contains the raw counts of stomatal density on the 5cm strip on the underside of leaf 1 from all 15 shoots sampled in each community. These raw data were converted into values of stomata per mm^2. This produces a mean value (± 1 standard deviation) on leaf 1 from all 15 shoots of 863±100 stomata per mm^2 in zone 1, 886±93 in zone 2 and 1006±91 in zone 3. The converted data are given in Figure 3.6 which shows the intra- and inter-zone variation in stomatal density in the three zones. There are two important conclusions to be drawn from these data.

Firstly the very large differences in plant height, leaf length and soilwater salinity recorded between the communities in zones 1 and 2 are not mirrored in any difference in stomatal density. This suggests that C uptake is not limited by stomatal density since the obvious differences in plant biomass accumulation between these communities do not appear to be a consequence of limitations on stomatal conductance by varying stomatal density.

The second conclusion is that although there is an apparently large difference between the mean values from zones 1 and 2 and that from zone 3, all three means actually overlap when a single standard deviation either side of the mean is considered. Hence it is not possible to simply assign any particular value of stomatal density to a distinct community.

Figure 3.6 Roudsea *Phragmites*; stomatal density by zone.



The intra-plant stomatal density data and the length of the leaves analysed are given in Appendix 11. The differences in the height and leaf length of the plants from the three communities are apparent. No consistent trend exists between the position of the leaf on the shoot (leaf number) and the length of the leaf either between or within the three communities.

However, the raw data on intra-plant stomatal density variations do show a trend which appears to hold with only a couple of exceptions. This is that the density of the stomata on leaf 7 is significantly lower than that on leaf 1, and often that on leaves 3 and 5 also. This holds in all 5 plants studied from zone 1, where the average value (± 1 s.d.) of stomatal density on leaf 1 is 940±82 per mm^2 and that on leaf 7 is 675±75 per mm^2. In zone 2 the figures are 872±111 and 697±98 respectively, and zone 3 the average of the 5 measurements from leaf 1 is 1030±93 and from leaf 7 the mean is 786±138. Again the mean values plus or minus 1 standard deviation from leaf 1 for each of the three zones overlap, and similarly for leaf 7. However, in zones 1 and 3 there appears to be significant intra-plant variation in stomatal density. Although this is not the case in zone 2, there are some individual plants in this zone that show a striking difference in stomatal density according to the position of the leaf on the shoot.

This has a number of implications. The first is that if stomatal density does have an impact on stomatal conductance, and hence on p_i, then there may be significant intra-plant variations in $\delta^{13}C$ (and possibly $\delta^{18}O$), although no account has been taken of possible changes in stomatal size in this analysis. Since it is impossible to establish the position that a leaf occupied on a plant once it has dropped and entered the depositional record, it may be that the variation inherent in each community is so large that any differences that may exist between the communities are blurred or lose their distinctiveness altogether.

The second implication is that there are obviously quite different processes controlling this aspect of plant physiognomy during the growing season. The timing of the appearance of each leaf was not measured at Roudsea so it is not possible to relate the increase in stomatal density to a particular event. Similarly the lack of physiological monitoring in this project means that it is impossible to draw links with biochemical and metabolic shifts in the three communities. It is notable, however, that this variation is most pronounced between leaf 1 and leaf 7 in plants from all three zones, which seems to suggest that the controlling influence(s) occurred in all three communities. This points either to a common physiological control (perhaps seasonal, Smedley *et al.* 1991) or to a response to an environmental factor other than one measured by the techniques in this project; there is no evidence to suggest a convergence of chemical conditions in the 3 zones at a particular point during the growing season.

3.5.5 Soilwater isotopic composition.

Four samples (5cm, 15cm, 25cm and 35cm depth) taken on the same day (5/10/94) from each of the three communities were analysed for their water content, organic matter content, salinity and soilwater oxygen isotope composition. Although the LOD% results from the cores in zones 2 and 3 overlap (Figure 3.7), the other analyses of sediment characteristics produce graphs which are consistently stratified by zone (Figures 3.8 (LOI%) and 3.9 (soilwater salinity in parts per thousand). The $\delta^{18}O$ data fall remarkably consistently into three groups according to zone number (Figure 3.10), and there are significant differences between the values produced. In zone 1 the mean (±1 s.d.) of the 4 analyses is -3.8±0.2‰, in zone 2 the figure is -5.5±0.1‰ and the mean for zone 3 is -6.5± 0.1‰. The measurement error on each analysis is less than 0.1‰ (Heaton 1995 pers. comm.). The values of $\delta^{18}O$ found by Sternberg *et al.* (1991) in their analyses of the stem water taken from the same day from specimens of *Conocarpus erectus* L. living in

Figure 3.7

Roudsea core LOD (%)

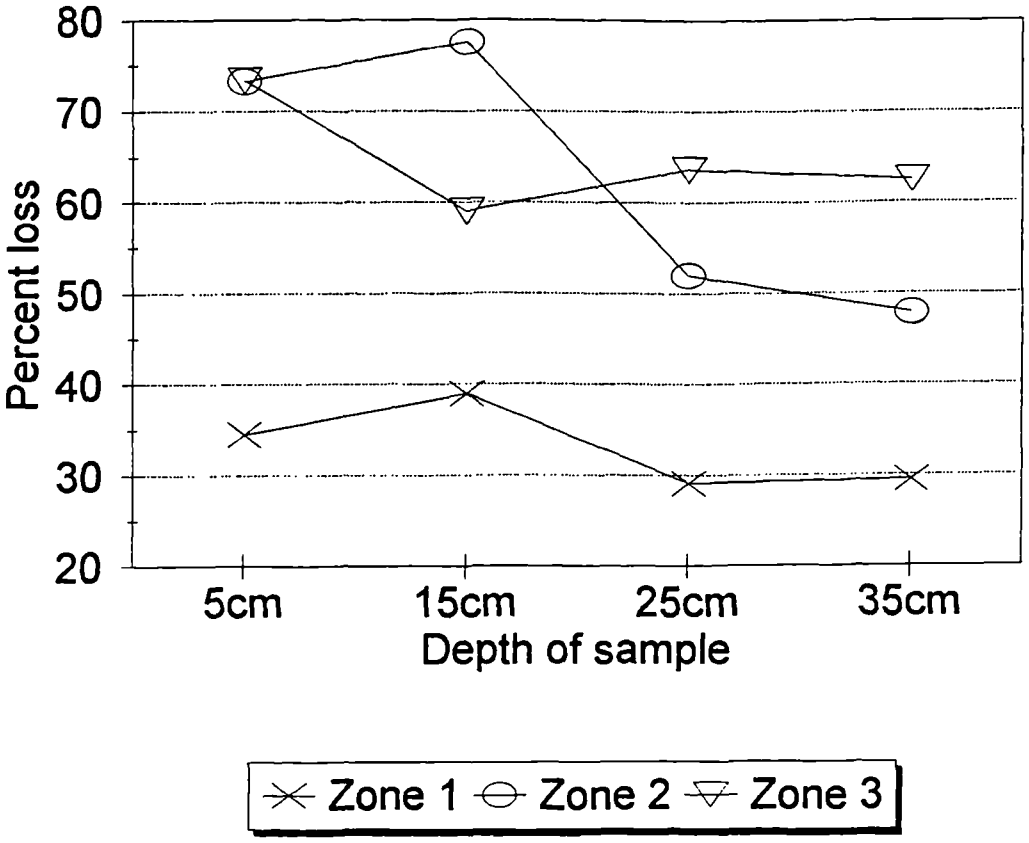


Figure 3.8

Roudsea core LOI (%)

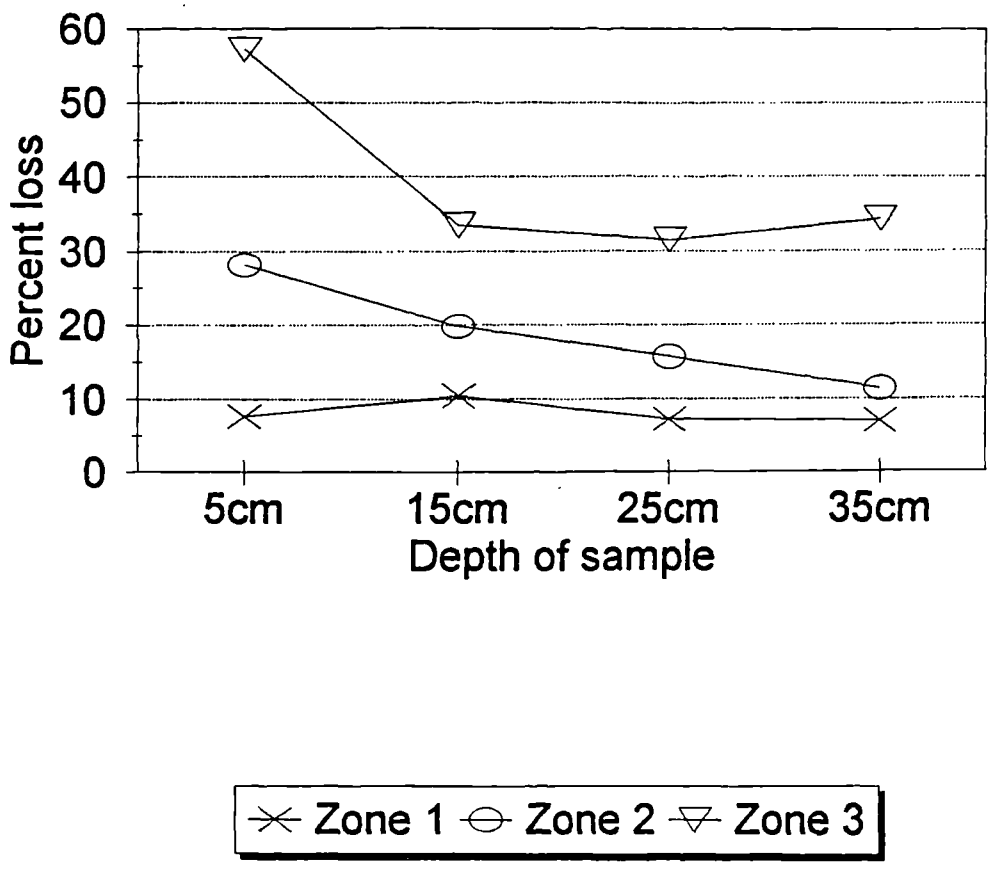
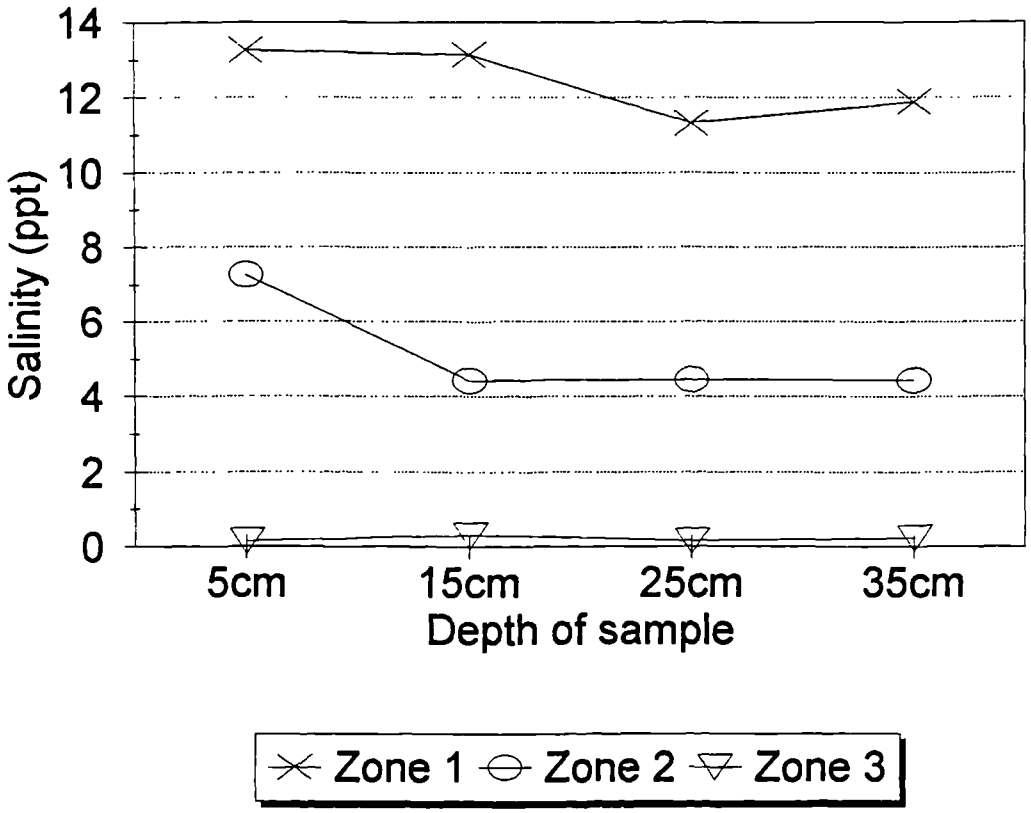


Figure 3.9

Roudsea core salinity



"transitional woodland" and "scrub mangrove" on the Florida coastline at Sugar Loaf Key were -0.8±0.5‰ (n=4) and 0.9±0.2‰ (n=6) respectively. There is therefore a higher range of mean values at Roudsea (2.7‰) compared with the sub-tropical site in the Florida Keys (1.7‰). Since living rhizomes were found at each depth sampled, there is evidence to suggest that the isotopic composition of the water being utilised by *Phragmites* in the three zones at the time of sampling was significantly different. This might be reflected in the $\delta^{18}O$ value of the cellulose if the values from these analyses are representative of the water being utilised by the plants during photosynthesis.

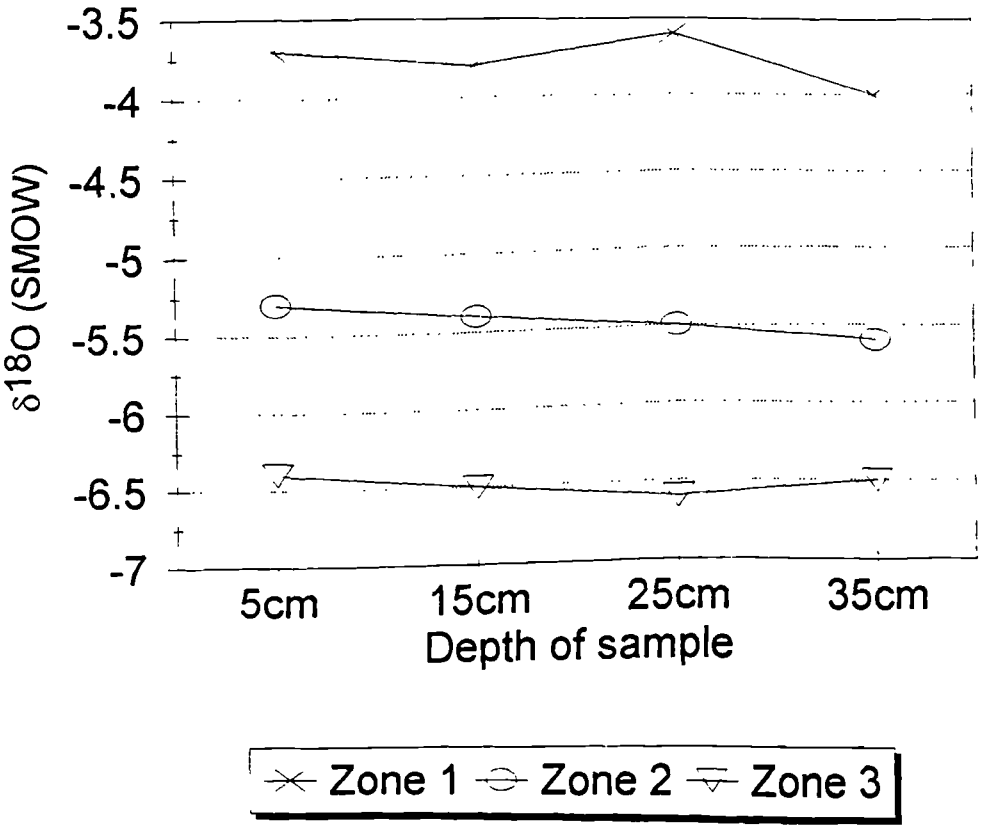
However, leaf water $\delta^{18}O$ values may vary as the $\delta^{18}O$ value of the soil water utilised changes because of variations in the relative importance of fresh water and sea water inputs to the three communities. Further, changes in the extent of ^{18}O enrichment caused by different levels of evaporation from leaf surfaces over time or between the three communities might lead to leaf water $\delta^{18}O$ values (and hence cellulose $\delta^{18}O$ values) which do not reflect the trend found in the $\delta^{18}O$ values of the soilwater samples analysed.

When the mean values for $\delta^{18}O$ from the soil water samples collected at Roudsea are combined with the mean values for δD from the same samples (-24.3±1.0, -37.3±1.0 and -45.8±0.5 from zones 1 to 3 respectively), they fall just below the Meteoric Water Line of Craig (1961a). This suggests an element of mixing of water from a number of sources, which is as expected in a coastal, estuarine site. The most important aspect of these data is that the $\delta^{18}O$ and δD values conceptually agree, and that they become significantly less negative (showing increasing input of marine-derived water) from zone 3 to zone 1.

The raw data from these analyses are given in the second summary table (Table 3.3) below, along with the data on LOD%, LOI% and salinity from the twelve samples. It

Figure 3.10

Roudsea core $\delta^{18}O$ (SMOW)



is interesting to note how moisture content and organic C content drop quite quickly with depth in zones 2 and 3 (Figures 3.7 and 3.8 respectively) - probably as a result of the decomposition of organic matter - and also that there is not a direct relationship between the isotopic composition and the salinity of the water samples.

3.5.6 Summary of these results.

Tables 3.2 and 3.3 summarise the differences between the three zones in the analyses outlined above. Table 3.2 shows the inter-zone variations in all the variables measured. In general terms, zone 1 is shown to be characterised by a relatively high inundation frequency, low soil moisture and organic C content and variable levels of salinity. The *Phragmites* in zone 1 is short, with small leaves, but the stomatal density of leaf 1 from the 15 plants sampled is not significantly different from that in the other 2 zones. The intra-plant measurements of stomatal density show density falling significantly between leaf 1 and leaf 7.

The data from the samples taken in zone 2 indicate much lower levels of salinity, and higher moisture content and organic C content. The plants are very much taller, with longer leaves, but stomatal density is indistinguishable from that in zone 1, and intra-plant comparisons do not reveal a consistent difference between the mean value plus or minus 1 standard deviation of stomatal density from leaf 1 and leaf 7.

Zone 3 was not inundated in 1994, has an organic-rich soil with high moisture content and low salinity. Plant habit is very similar to that in zone 2, but there is the same degree of difference between stomatal density on leaf 1 and leaf 7 as is found in zone 1.

Table 3.2 Variations in environment and *Phragmites* physiognomy at Roudsea.

Variable (* denotes value is mean±1s.d.)	Zone 1	Zone 2	Zone 3
Altitude (metres Ordnance Datum)	4.76	5.85	6.51
Number of inundations (1994)	132	1	0
Loss on drying (%)*	48.7±4.6	73.6±3.2	79.7±2.6
Loss on ignition (%)*	11±1.1	30.3±3	76.5±7.2
Salinity (‰)*	10.3±5.2	1.4±0.2	0.2±0.1
Density (per $0.25m^2$)*	13.5±5.9	18.4±4	19.9±5.4
Plant height (cm.)*	85.1±27.44	225.3±28.74	213.7±22.05
Number of leaves*	9.3±1.35	11±0.65	13.5±1.6
Length of leaf 1 (n=15)*	13.9±2.95	33.1±4.15	34.5±4.73
Stomatal density on leaf 1 (n=15)*	863±100	886±93	1006±91
Length of leaf 1 (n=5)*	16.2±2.2	33.4±4	35±4.8
Length of leaf 3 (n=5)*	19.8±2.2	30.8±6.8	36.2±2.8
Length of leaf 5 (n=5)*	22±3	35.6±5.7	37±1
Length of leaf 7 (n=5)*	21±2.6	32.4±4.6	36.6±2.1
Stomatal density on leaf 1 (n=5)*	940±82	872±111	1030±93
Stomatal density on leaf 3 (n=5)*	871±46	914±90	871±93
Stomatal density on leaf 5 (n=5)*	859±58	900±37	873±70
Stomatal density on leaf 7 (n=5)*	675±75	697±98	786±138

Table 3.3 shows the differences down the 75mm wide core of the near-surface sediments from each zone. The highlights of this data set are the distinct isotopic composition of the soilwater from the 3 zones, the decrease in organic C content down-core, and the higher levels of salinity below the surface found in zone 2. This last point

shows that the link between isotopic composition and salinity of soilwater is not constant, and also demonstrates that there can be quite different conditions down a single core which might impact on the depth below the surface from which plants extract water. Fortunately the isotopic composition of the soilwater appears to be consistent down the core, suggesting that the basis of adopting $\delta^{18}O$ as an analytical tool is well-founded.

Table 3.3 Variations in sediment quality, salinity and soilwater isotopic composition in three cores from zones 1, 2 and 3, Roudsea.

Sample	LOD %	LOI %	Chloride ‰	Salinity ‰	$\delta^{18}O$ (SMOW)	δD (SMOW)
1-5cm	34.56	7.71	7.337	13.255	-3.7	-23
1-15cm	38.91	10.41	7.261	13.117	-3.8	-24
1-25cm	29.13	7.22	6.265	11.318	-3.6	-25
1-35cm	29.47	7.05	6.582	11.891	-4.0	-25
2-5cm	73.3	28.15	4.016	7.255	-5.3	-38
2-15cm	77.51	19.75	2.452	4.43	-5.4	-36
2-25cm	51.98	15.65	2.471	4.464	-5.5	-38
2-35cm	47.78	11.36	2.459	4.442	-5.6	-37
3-5cm	73.4	57.22	0.08	0.145	-6.4	-45
3-15cm	59.06	33.51	0.166	0.3	-6.5	-46
3-25cm	63.69	31.53	0.091	0.164	-6.6	-46
3-35cm	62.52	34.32	0.132	0.238	-6.5	-46

"Sample" shows zone and depth; hence 1-5cm is at 5cm depth in the core from zone 1.

The data summarised in these tables also suggest that if *Phragmites* follows the pattern found in analyses of other species under different salinity and flooding regimes (Chapter 2), there will be significant differences in the $\delta^{13}C$ of cellulose from the 3 zones. However, the stomatal density data produce a more confusing image. There are significant intra-plant differences in this aspect of plant physiognomy, but very little differences in the values from the same leaf (leaf 1) from the plants in the 3 zones. Either there is not a direct link between this factor and $\delta^{13}C$, or there will be significant variations within each

zone due to intra-plant differences, and very little difference between $\delta^{13}C$ values from the 3 zones.

3.6 Results of the $\delta^{13}C$ and $\delta^{18}O$ analyses of *Phragmites* cellulose.

Tables 3.4.1, 3.4.2 and 3.4.3 show the results of both $\delta^{13}C$ and $\delta^{18}O$ analyses on *Phragmites* cellulose combined with data on plant habit and stomatal density from zones 1 to 3 respectively. The $\delta^{13}C$ were done at N.I.G.L., Keyworth, and the measurement error over the three runs of the gas isotope mass-spectrometer varied between 0.06 and 0.12 *per mil.* All values are Craig-corrected relative to PDB, and the inter-plant data all come from analysis of leaf 1. Three intra-laboratory standards with known $\delta^{13}C$ of -30‰ gave results of -29.91‰, -29.97‰ and -30‰ (see Table 3.1).

In zone 1 the range of $\delta^{13}C$ values from leaf 1 is from -25.95‰ to -24.05‰. In zone 2 the corresponding minima and maxima are -26.26‰ and -24.31‰ and in zone 3 there is an even greater range from -26.34‰ to -23.44‰. This shows that even in the zone where the environmental factors such as salinity and the frequency of inundation showed the lowest variation, values of $\delta^{13}C$ vary by nearly 3‰. The range in zone 3 is so large that it extends beyond the upper and lower limits of both zones 1 and 2, making any attempts to fit particular $\delta^{13}C$ values to a particular zone absolutely groundless. This can be clearly seen in Figure 3.11, where the $\delta^{13}C$ values for each plant analysed in the three zones is plotted to show the extent of intra-zone variation. This highlights the extent to which the values from zone 3 encompass the range of values in both zone 1 and zone 2.

The results of the inter-plant data from each community point towards one conclusion in terms of palaeoenvironmental reconstruction. This is that the *Phragmites*

$\delta^{13}C$ values from Roudsea do not record variations in growth conditions in a way that would make it possible to determine the relationship between macrofossil remains and contemporaneous salinity or inundation levels. Consequently, this approach seems to be of no value in such studies. This is reinforced by the intra-plant data, which shows variations within a single plant that are nearly as large as those between the same leaf taken from different plants in the same community.

However, within this intra-plant data there are a couple of interesting trends. These are shown in Figures 3.12 and 3.13 where intra-plant carbon isotope values are plotted against the position of the leaf analysed. Figure 3.12 shows an approximately linear trend of declining $\delta^{13}C$ with position down the plant from plant number 2 in zone 2. A similarly quasi-linear trend in Figure 3.13 shows the results from plant number 2 in zone 3. However, in this case the $\delta^{13}C$ values increase with position down the plant. There would appear to be a relationship between leaf position in these plants and $\delta^{13}C$, but in the other plants examined for intra-plant variations no such pattern is revealed. The fact that the trend observed in plant 2 from zone 2 is reversed in plant 2 from zone 3 also casts doubt on assigning this pattern to a particular mechanism (for example seasonal variations in p_a or *Phragmites* metabolism (Ren & Zhang 1994, Wang *et al.* 1995)), and indeed these patterns may be a random construct.

Figure 3.11 Roudsea *Phragmites* $\delta^{13}C$ (PDB) by zone

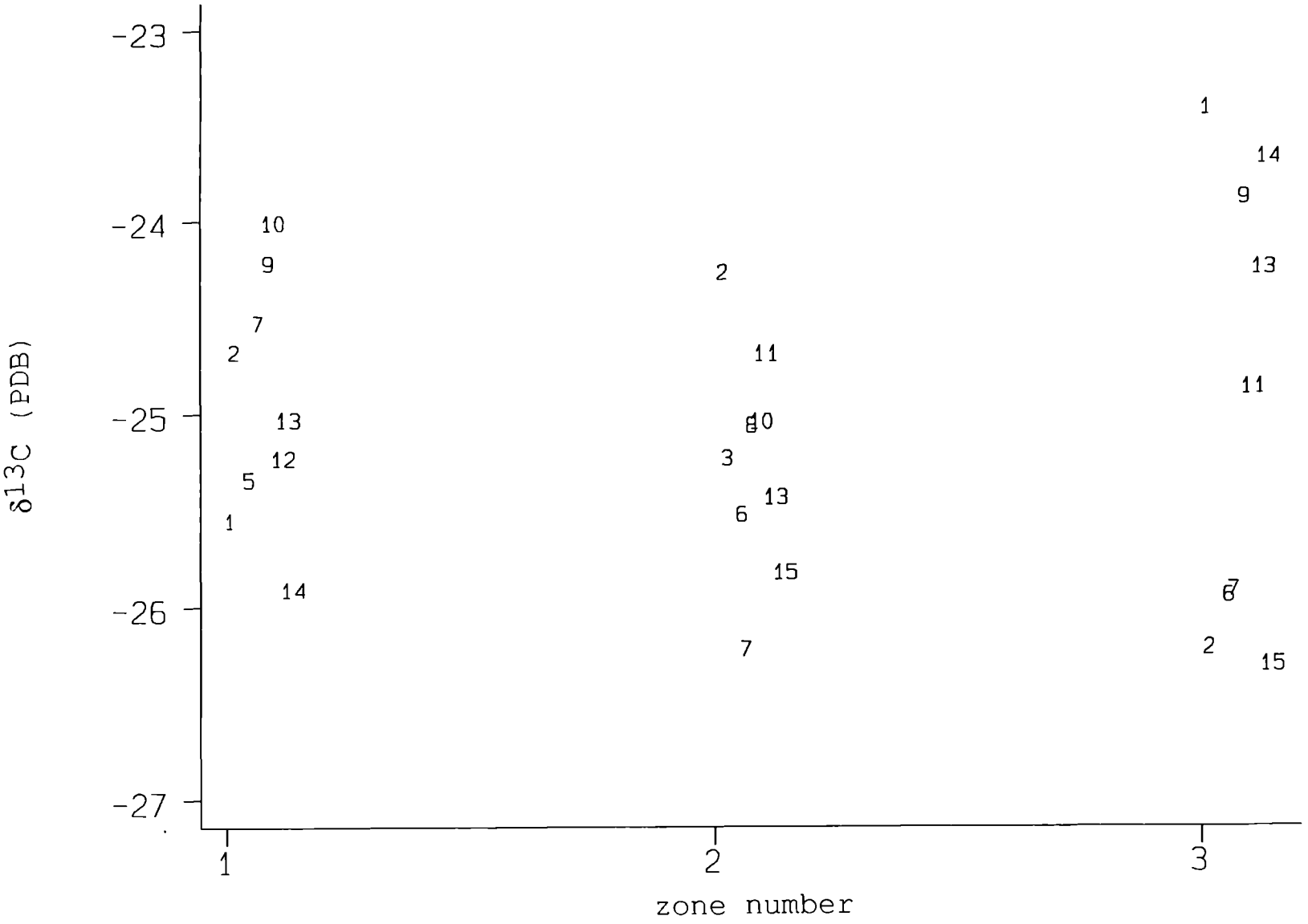


Figure 3.12 Roudsea *Phragmites* intra-plant $\delta^{13}C$ (PDB)
Zone 2, plant number 2.

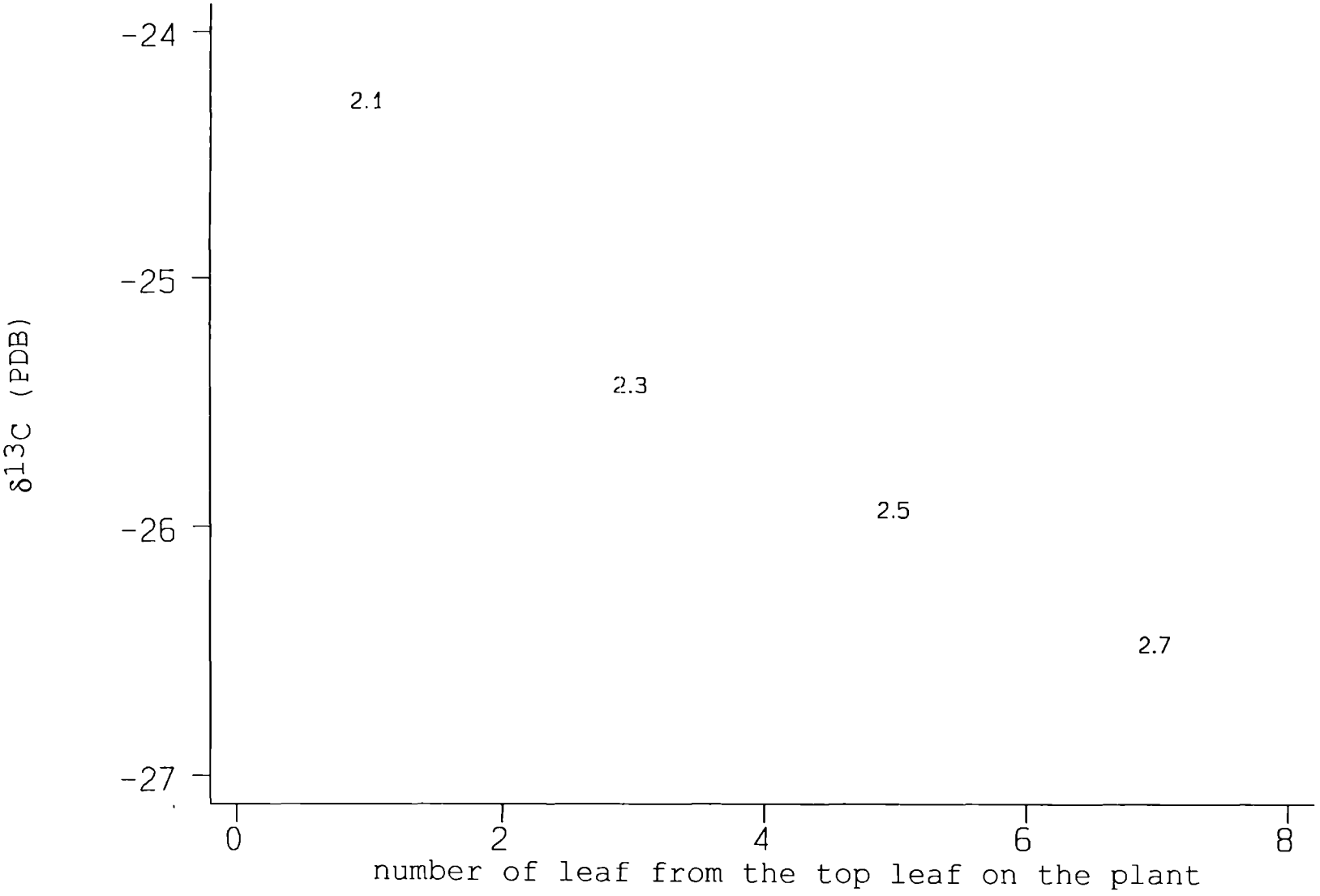


Figure 3.13 Roudsea *Phragmites* intra-plant $\delta^{13}C$ (PDB).
Zone 3, plant number 2.

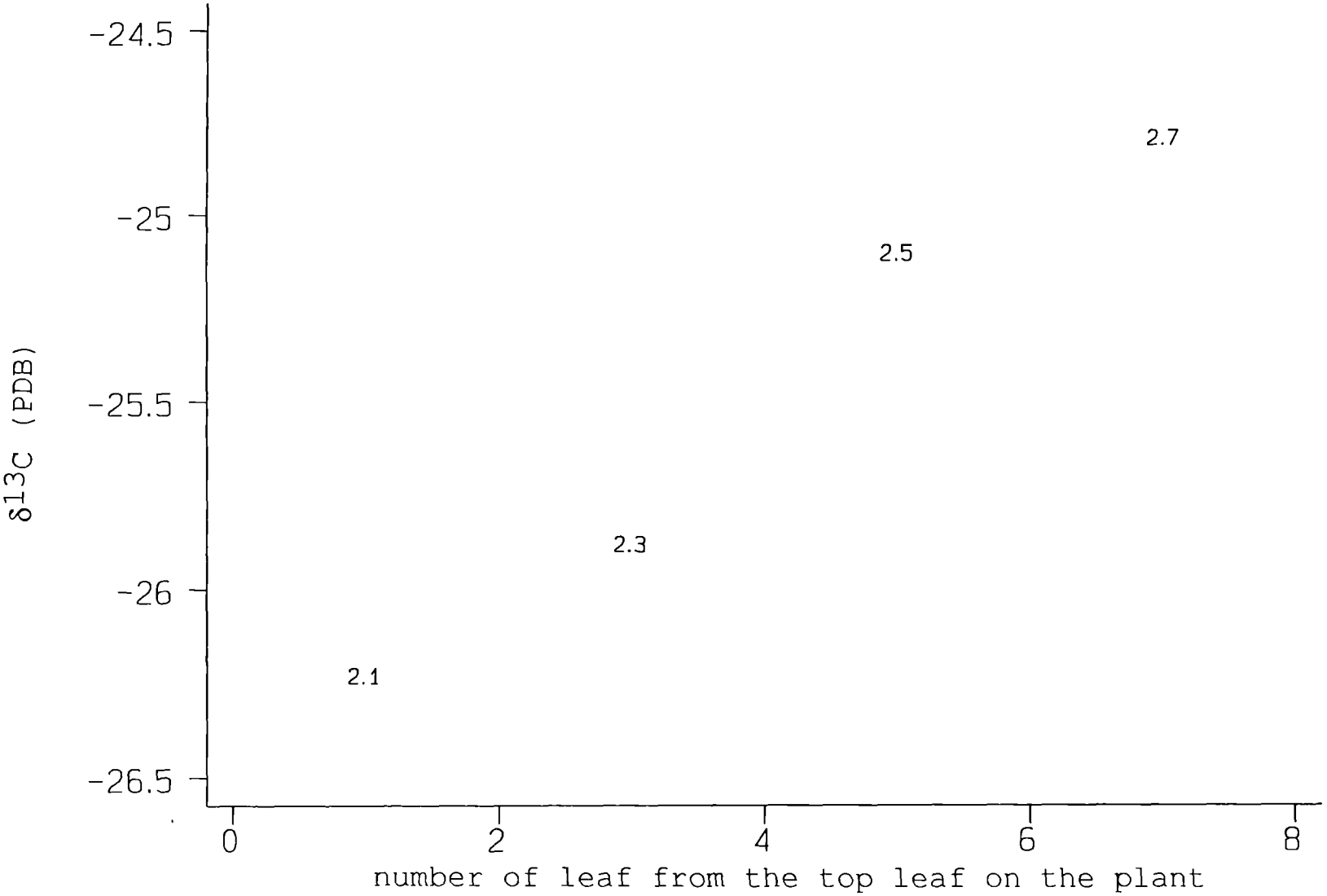


Table 3.4.1 Plant habit and isotopic composition of *Phragmites* in zone 1, Roudsea.

Inter-plant Variations

Plant no.	Height (cm)	Number of leaves	Leaf length (cm)	Stomatal density (mm^{-2})	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
1	71	7	16	970	-25.59	31.03
2	117	10	16	1024	-24.72	------
3	71	10	11	918	--------	------
4	77	10	12	760	--------	------
5	109	11	14	882	-25.38	------
6	58	9	9	770	--------	------
7	123	10	16	1030	-24.57	------
8	94	11	16	876	--------	------
9	124	11	19	858	-24.26	31.33
10	94	9	17	905	-24.05	32.02
11	68	9	12	841	--------	------
12	63	8	16	810	-25.27	30.48
13	114	10	13	882	-25.07	30.88
14	39	7	11	702	-25.95	31.8
15	55	8	10	715	--------	------

Intra-plant Variations

Plant number 2

Number of leaf (top=0)	Length of leaf (cm.)	Stomatal density, mm^{-2}	$\delta^{13}C$ (PDB)
1	16	1024	-24.72
3	19	935	-25.62
5	21	934	-25.81
7	22	554	-25.78

Plant number 13

Number of leaf (top=0)	Length of leaf (cm.)	Stomatal density, mm^{-2}	$\delta^{13}C$ (PDB)
1	13	882	-25.07
3	17	905	-25.78
5	21	896	-26.41
7	20	705	-26.24

Table 3.4.2 Plant habit and isotopic composition of *Phragmites* in zone 2, Roudsea.

Inter-plant Variations

Plant no.	Height (cm)	Number of leaves	Leaf length (cm)	Stomatal density (mm^{-2})	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
1	243	11	36	972	--------	------
2	272	10	39	994	-24.31	31.21
3	238	12	36	980	-25.27	------
4	234	11	35	965	--------	------
5	242	12	36	1002	--------	------
6	241	10	36	996	-25.56	30.06
7	175	11	26	815	-26.26	31.61
8	220	11	29	873	-25.1	33.44
9	187	11	30	897	--------	------
10	181	10	29	783	-25.08	------
11	215	12	38	927	-24.73	------
12	253	11	36	817	--------	------
13	240	11	32	753	-25.47	------
14	243	11	31	769	--------	------
15	195	11	27	775	-25.86	31.49

Intra-plant Variations

Plant number 2

Number of leaf (top=0)	Length of leaf (cm.)	Stomatal density, mm^{-2}	$\delta^{13}C$ (PDB)
1	39	994	-24.31
3	40	975	-25.46
5	41	962	-25.96
7	35	646	-26.5

Plant number 13

Number of leaf (top=0)	Length of leaf (cm.)	Stomatal density, mm^{-2}	$\delta^{13}C$ (PDB)
1	32	753	-25.47
3	24	801	-26.4
5	29	892	-26.0
7	25	585	-26.8

Table 3.4.3 Plant habit and isotopic composition of *Phragmites* in zone 3, Roudsea.

Inter-plant Variations

Plant no.	Height (cm)	Number of leaves	Leaf length (cm)	Stomatal density (mm^{-2})	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
1	242	15	43	1180	-23.44	31.6
2	212	11	35	1084	-26.25	------
3	177	10	38	1013	--------	------
4	216	13	33	1066	--------	------
5	216	15	36	970	--------	------
6	209	13	30	902	-25.98	30.39
7	203	14	31	878	-25.95	30.17
8	183	15	27	859	--------	------
9	240	15	41	993	-23.91	31.66
10	239	13	35	966	--------	------
11	208	15	29	1040	-24.9	------
12	189	14	29	952	--------	------
13	220	15	34	1133	-24.28	33.43
14	251	13	36	1036	-23.7	------
15	201	12	40	957	-26.34	29.47

Intra-plant Variations

Plant number 2

Number of leaf (top=0)	Length of leaf (cm.)	Stomatal density, mm^{-2}	$\delta^{13}C$ (PDB)
1	35	1084	-26.25
3	36	873	-25.9
5	38	789	-25.1
7	35	561	-24.8

Plant number 6

Number of leaf (top=0)	Length of leaf (cm.)	Stomatal density, mm^{-2}	$\delta^{13}C$ (PDB)
1	30	902	-25.98
3	34	800	-25.8
5	37	812	-26.3
7	40	753	-25.4

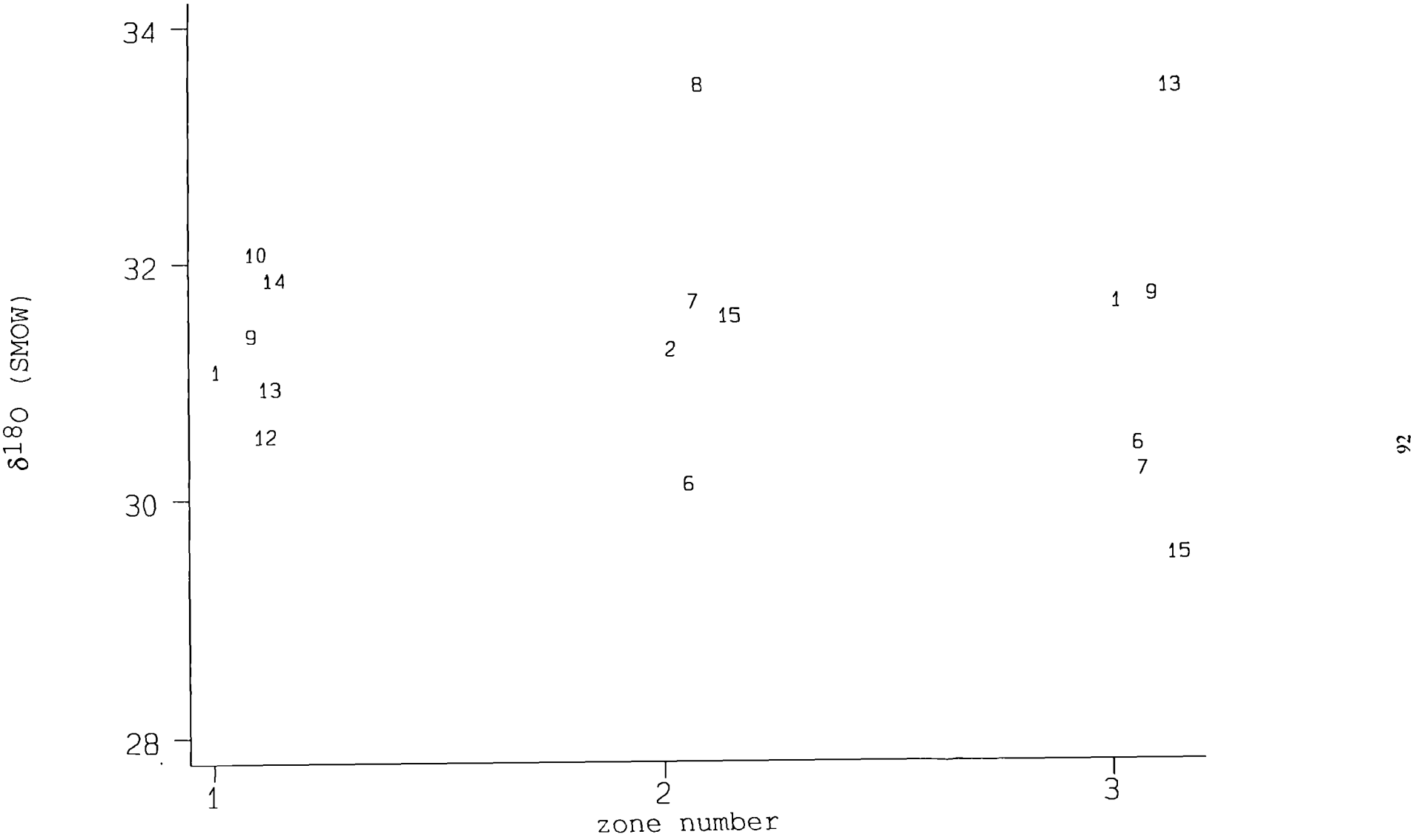
The $\delta^{18}O$ analyses were done at the University of Miami under the supervision of Dr. L. Sternberg. They are related to SMOW and the range of measurement error from the 20 runs of the mass-spectrometer is 0.007 *per mil* to 0.058 *per mil*, with a mean (plus or minus a standard deviation) of 0.023±0.012‰ (standards are given in Table 3.1).

The results of these analyses are very disappointing from a palaeoenvironmental reconstruction perspective since they also show values that are indistinguishable by zone. The range in zone 1 is from 30.48‰ to 32.02‰ which is swallowed by that from zone 2 (30.06‰ to 33.44‰) with zone 3 again having the widest range of values with a minimum of 29.47‰ and a maximum of 33.43‰. As with the $\delta^{13}C$ data shown in Figure 3.11, Figure 3.14 shows the extent to which the $\delta^{18}O$ values from the different zones overlap, and once again points to the large range of values in the apparently stable (re. LOD%, LOI%, salinity) zone 3.

Since there were strict time and money constraints in the visit to Miami, when the inter-plant $\delta^{18}O$ values appeared the intra-plant variation was not measured in the knowledge that $\delta^{18}O$ was not going to produce a useful set of information for the reconstruction of sea-level changes.

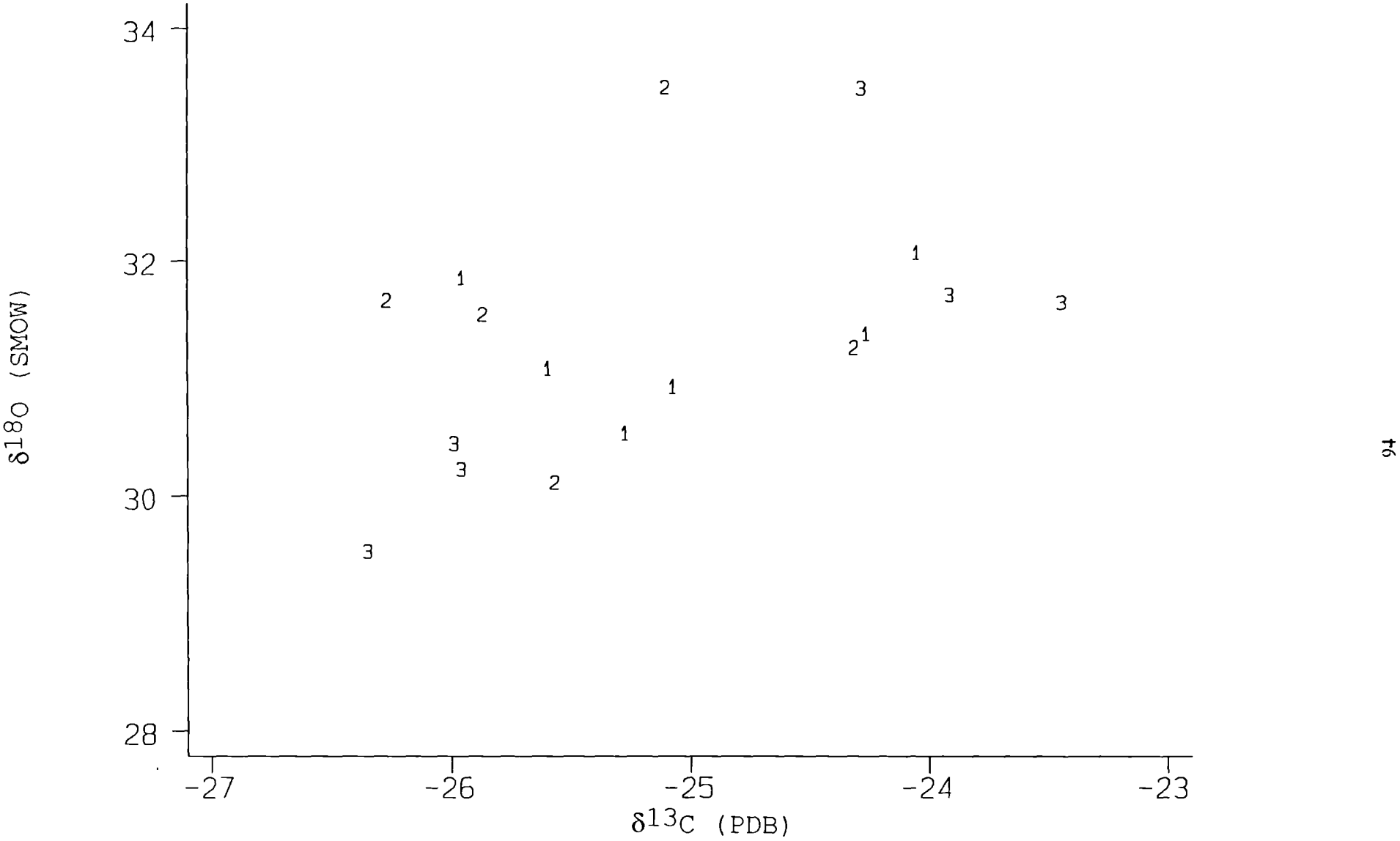
There is no relationship between the cellulose $\delta^{18}O$ values and the soilwater $\delta^{18}O$ values from the sediment core. It is possible that this reflects different depths of water utilisation by rhizomes, but this would only explain the variation in those communities where there is an input from tidal inundation. There is no direct input of seawater into zone 3 , but here the $\delta^{18}O$ values vary more than in either of the other two zones.

Figure 3.14 Roudsea *Phragmites* $\delta^{18}O$ (SMOW) by zone



This suggests that the $\delta^{18}O$ in the cellulose is a function of some factor other than the $\delta^{18}O$ of available soilwater. It has been suggested that $\delta^{18}O$ may be linked to variations in air humidity (Burk & Stuiver 1981) or light incidence in a canopy. High humidity is linked with constant water uptake but low evaporation, and high light incidence with high evaporation and consequently large demands on soilwater. However, both of these factors would also influence gas exchange and stomatal conductance which, in theory, would produce changes in C uptake and hence $\delta^{13}C$. This should mean that $\delta^{18}O$ and $\delta^{13}C$ from the same leaf show co-variance, but no such relationship exists (Figure 3.15): in zone 1 the minimum $\delta^{13}C$ value of -25.95‰ corresponds to $\delta^{18}O$ value of 31.8‰, with the maximum $\delta^{13}C$ value of -24.05‰ corresponding to a $\delta^{18}O$ value of 32.02‰ . The $\delta^{13}C$ analyses falling between these minimum and maximum values have $\delta^{18}O$ values of 30.48 to 31.33‰; this apparently random pattern casts doubt on the application of contemporary theory of C and O isotopic discrimination as outlined in Chapter 2 to analyses of *Phragmites* growing under conditions such as those at Roudsea.

Figure 3.15 Roudsea *Phragmites* $\delta^{18}O$ (SMOW) by $\delta^{13}C$ (PDB)



3.7 Discussion

There are a number of possible reasons why the $\delta^{13}C$ results from the *Phragmites* communities analysed do not fit the predicted pattern of increasingly negative values with increasing tidal inundation and salinity. Firstly, the plants in zone 1 are not subject to a constantly saline environment, and may only be photosynthesising during particular environmental conditions. The variable nature of the growth environment is clear from the data on soilwater salinity presented in Figure 3.3. Although the salinity of the samples taken from this zone was greater than 10 parts per thousand on 6 visits, on the other five days when sampling took place the salinity of the soilwater in this zone was less than 7.5 parts per thousand. This reflects variations of the input of sea water due to changes in the fortnightly tidal cycle and variations in the input of fresh water from precipitation. The plants in zone 1 were shown to accumulate less biomass over the growing season analysed, and this may be because they were only photosynthetically active when the soilwater salinity around these plants was below a critical level. Controlled experiments using transplanted material from the three communities would be necessary to establish this critical level.

If this is the case, it might also explain the greater range of carbon isotope ratios in the cellulose from plants in zone 3. These plants are not subject to either inundation by the tide or soilwater salinity above *circa* 1 part per thousand. Therefore they can operate under a wider range of humidity conditions. The relationship between humidity, stomatal conductance and $\delta^{13}C$ values is outlined in Chapter 2, and in this case the higher range of $\delta^{13}C$ values in the leaf cellulose of plants from zone 3 may be the result of photosynthesis occurring under a wider range of humidity conditions than is possible for plants in the other two zones.

If a combination of environmental factors (salinity, inundation, humidity...) is controlling the duration of the photosynthetically active period in zone 1, it may also explain the difference between the oxygen isotope composition of the soilwater samples and the leaf cellulose analysed. The expected relationship between the isotopic composition of soilwater and leaf cellulose is the function of two processes which lead to enrichment of ^{18}O over ^{16}O in the cellulose relative to the soilwater. The first is the evaporation of water from leaf surfaces and the second is the discrimination during cellulose metabolism (see Chapter 2). The latter is known to result in a 27‰ increase in the $\delta^{18}O$ of cellulose relative to leaf water. However, the $\delta^{18}O$ of leaf water is a function of both the isotopic composition of the soilwater utilised and the extent of enrichment in ^{18}O relative to ^{16}O caused by evaporation from the leaf surface. The plants in zone 1 are subject to a wider range of environmental conditions than those in either zones 2 or 3, but the oxygen isotope composition of the cellulose from the leaves of plants analysed from this zone has the narrowest range of values. This suggests that the plants in zone 1 are only taking up water and utilising this in the formation of cellulose under a narrow range of conditions. If photosynthesis is only occurring when the plants are able to utilise fresh water or when humidity levels are high, the restricted data available on the isotopic composition of the soilwater available to the plants may not be sufficient to explain the pattern of isotopic values found.

Similarly, if the plants in zone 3 are able to photosynthesize under a wide range of humidity or evaporative conditions, it is possible to explain the wide range of oxygen isotope compositions found in the leaf material analysed without there having been a wide range of soilwater $\delta^{18}O$ values in this zone. Although the leaves analysed did occur in equivalent positions on the plants examined, this does not necessarily mean that the leaves formed at the same time or under the same environmental conditions. Equally, the extent of evaporation may have been equal when each of the leaves formed, and the variation be

a product of changes in the isotopic composition of the soilwater due to variations in the temperature at which the water originally fell as precipitation.

In order to establish the extent to which these possible causes can explain the patterns found in the carbon and oxygen isotopic composition of the leaf cellulose of *Phragmites* it would be necessary to carry out intensive experiments on transplanted material under controlled light, moisture, salinity, nutrient and flooding conditions which can be related to conditions in the field.

In addition to these environmentally-determined explanations, any genetic variations which might be related to variations in salinity tolerance (cf. Zong *et al.* 1991) should be investigated. The extent of variations in physiology such as the changes in the proportion of net carboxylation by PEP carboxylase through the year as found by Ren & Zhang (1994) would also have to be established. Since the model to explain the extent of discrimination against ^{13}C in C_3 plants assumes that a constantly low proportion of carboxylation utilises PEP rather than Rubisco, it may be that the variation in the importance of the two carboxylation agents in *Phragmites* causes the intra- and inter-plant variation found by these analyses.

3.8 Summary

The analyses of the sediment samples collected during the 1994 growing season from Roudsea quantify the basic differences between the three *Phragmites* communities. When combined with the inundation data from Heysham a clear picture emerges of the different conditions under which the *Phragmites* plants are growing.

This is most clearly apparent in the differences in plant height between zone 1 and zones 2 and 3, and is reinforced by the pattern of average leaf length. However, stomatal density variations are greater within each plant than between equivalent leaves on plants from different zones. Although this suggests that $\delta^{13}C$ (if it is related to stomatal density) will vary as much within the same plant as between plants in different communities, the consistently different $\delta^{18}O$ values of interstitial water in the three zones suggests that the O isotope composition of the cellulose may prove very useful for palaeoenvironmental reconstructions.

However, the isotopic analyses produced very large spreads of data in each zone. The results of both $\delta^{13}C$ and $\delta^{18}O$ from the three zones overlap, and it is necessary to conclude that these data point to the isotopic composition of *Phragmites* macrofossils being of little or no use in coastal palaeoenvironmental reconstructions.

However, the data are interesting from a physiological point of view. It is notable that the range of both $\delta^{13}C$ and $\delta^{18}O$ is higher in zone 3 than in zone 1. The environmental data suggest that the plants in zone 1 are subject to more variable conditions than in zone 3, but there is no reflection in the isotopic data of either the inputs of saline water to the rhizosphere (cf. Meinzer *et al.* 1994), or the impact of inundation (which is perhaps more important in determining the success of *Phragmites* communities (Phillips 1987, Ostendorp 1991, Orson *et al.* 1987, Gries *et al.* 1990, Coops *et al.* 1994; cf. Guy & Wample 1983).

This may be the result of different age structure (Haslam 1972, Brix 1990), rhizome network (Kuido & Ito 1988), metabolic variations (Chatterton *et al.* 1989, Ren & Zhang 1994, Matsushita & Matoh 1991, 1992, Hellings & Gallagher 1992), genetic structure (Gorenflot 1986, Haslam 1970c, 1972, Gray *et al.* 1979, Siddiqui *et al.* 1994,

Wang *et al.* 1995, Zong *et al.* 1991), or selective water uptake and gas exchange caused by environmentally-determined variations in photorespiration (Yakir & Yechieli 1995, Dawson & Ehleringer 1991, Flanagan *et al.* 1992, Haslam 1970b).

Of these, the last scenario is a relatively attractive explanation. If the plants in zone 1 are only active under favourable conditions it might explain why the $\delta^{13}C$ data from zone 1 is more closely grouped than that from zone 3 where the plants are operating over a wider range of temperature, light and humidity conditions (Gross *et al.* 1991, Leuning 1995). The $\delta^{18}O$ data are more difficult to explain unless there are considerable variations in the isotopic composition of the water utilised by the plants in zone 3 through time (Lawrence & White 1984, Lawrence *et al.* 1982) and / or microhabitat variations (for example aspect, local density of shoots (Yakir & Israeli 1995; see also Ehleringer & Cooper 1988 re. microhabitat and $\delta^{13}C$) which impact on the extent of evaporation from leaf surfaces, resulting in different levels of ^{18}O enrichment in leaf water (see also Armstrong & Armstrong 1990, Armstrong *et al.* 1992).

Therefore, the difference between the expected pattern of both C and O isotope compositions and that revealed by these analyses may be that there are opportunities for the plants in zone 1 to photosynthesize under conditions when salinity is not a restriction and the available soil water is not derived from sea water. Zone 1 is subject to inundation by the tide, but the *Phragmites* plants do not extend down the saltmarsh beyond a point at which fresh water is never available. It may be that the environmental limit of *Phragmites* at Roudsea is not controlled by the frequency of tidal inundation but rather by how often fresh water is available. This would explain the lower biomass accumulation and more constricted range of both C and O isotope compositions in zone 1 than in zone 3 which suggests that *Phragmites* is unsuitable as a source of isotopic data for coastal environmental reconstruction.

Chapter 4

A cross-community investigation of $\delta^{13}C$ values in the flora of a contemporary saltmarsh, and their relationship to $\delta^{13}C$ values from sub-fossil saltmarsh peats.

4.1 Background, aim and potential.

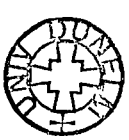
The initiation of organic matter accumulation in a spatial succession from open unvegetated tidal flats to fully terrestrial vegetation is in the lower parts of the saltmarsh communities.This is associated with a stabilisation of the inorganic fraction, often by algae (Adam 1990), and colonisation by higher plants which are able to withstand the high levels of salinity (de Leeuw *et al.* 1991, Price *et al.* 1990) and the effect of flooding by the tide. These specialist colonisers (Brereton 1971) are generally unable to cope with competition (Austin 1990, Bazzaz 1990) from plants which can withstand lower levels of inundation and salinity (Pennings & Calloway 1992), and consequently a marked zonation of vegetation communities occurs at the coast. The breadth of each zone below the highest limit of the tide is positively related to the absolute tidal range (Chapman 1939) and negatively related to the local gradient. As the number of species able to cope with the conditions increases and the input of inorganic matter decreases so the proportion of organic material in the total sediment yield increases. Such a change is represented by an increase in the proportion of peat within a facies change representing temporal shifts from open tidal flats to fully terrestrial / freshwater vegetation communities.

This visible change in facies type is the first indication to a researcher of a temporal environmental shift which might be related to changing sea-level. Subsequent micropalaeontological examination of the sediment will reveal the nature of the

environments represented by this change from inorganic to organic sedimentation. Indicators such as pollen grains, diatom frustules and foraminiferal tests have been very successfully used to elucidate the nature of such changes (for example, Shennan *et al.* 1995, Kosugi 1989, Scott & Medioli 1988).

This project aims to establish whether the contemporary carbon isotope ratios from the plants which occupy 4 adjacent zones of a particular saltmarsh are such that there might be an opportunity to use this technique to identify the equivalent communities in sub-fossil buried units. In this project the approach is much more parochial than the study of *Phragmites* (Chapter 3) since the range of conditions (floristic, tidal, sedimentary, management) at any one saltmarsh are often quite different from those at another (Adam 1978, Long & Masson 1983, Pye 1992), and this consideration becomes even more significant when studying palaeoenvironments. Consequently, the choice of field site is influenced by the desire to hold as many factors as possible constant and to find a marsh that is floristically poor so that the range of plants involved is low.

Kentra Bay National Nature Reserve in north west Scotland (Figure 1.1, Figure 4.1, O.S. grid reference NM646700) fulfils these and other considerations. The area is a large glacial outwash fan with a very low gradient towards the present coast. The contemporary vegetation succession is from tidal sand flats across a species-poor, zoned saltmarsh and into a raised moss community. Due to the interplay between isostatic and eustatic factors, the area has undergone coastal retreat over the last few thousand years. An areally extensive raised moss covers the buried remains of saltmarsh communities that have shifted seawards as the local isostatic rebound outpaced eustatic changes. Consequently the area offers excellent opportunities to track movements of Holocene relative sea-level in the region, and the moss has been crossed by a number of transects of boreholes and detailed examination and dating of sub-fossil material has been done



(Shennan *et al.* 1995). The same group has also examined the contemporary spatial patterns of vegetation communities, pollen grain and diatom assemblages across the sand flat to raised moss succession (Innes *et al.* 1993). Given the existence of this body of data, Kentra offers a good opportunity to establish whether carbon isotope values are linked to inundation and salinity regimes and if so whether they can shed any more light on investigations of sub-fossil deposits than the techniques already in widespread use.

4.2 Limitations

The theory explaining the idea that carbon isotope ratios might be useful in this study is described in Chapter 2, but there are some considerable limitations which are immediately apparent and which might have an impact on data derived from the analyses. These relate primarily to the concept of zonation and the factors which influence it.

The physiological limitations of the halophytic species inhabiting saltmarshes have been tested (Cooper 1982, Bertness 1992, Huiskes *et al.* 1985) and theoretical models which predict the potential extent of various species have been established (Fahrig *et al* 1993, Bertness & Ellison 1987). However, the most significant aspect of this work from the point of view of this project is the suggestion that some form of ecotypic variation exists between plants from the same species inhabiting different zones (for example Rhebergen & Nelissen 1985), even to the extent that a genetically-based difference might exist (Gray 1985, Gray 1987, Gray *et al.* 1979). The significance of this is that the theoretical link between high levels of salinity and inundation and the consequent stomatal and biochemical regulations on the uptake and assimilation of carbon, (which would exist given identical plants operating under increasingly stressful conditions), might not hold if in the field there is genetic differentiation between populations in different zones.

This is also related to the actual process of succession (Roozen & Westhoff 1985), which on saltmarshes is critically linked to the success of plant recruitment. This does not necessarily involve a simple roll-over of species from one zone to another as environments change until species are removed as a result of either competitive or physical and chemical factors (Gray 1992). This study relies on being able to transfer contemporary values of carbon isotope ratios from a series of spatially adjacent vegetation zones to an interpretation of temporally adjacent buried sub-fossil sedimentary units. Even if the floristic composition of the zones has remained identical, this does not establish the genetic integrity of the populations through time.

The project is further complicated by the heavy grazing pressure from sheep. This undoubtedly has a considerable influence on plant physiology and physiognomy, which means that links to pre-agricultural saltmarsh communities may be more difficult to draw. Unfortunately there are no parts of the marsh which are free from grazing, but the benefits of being able to work on a species-poor marsh, backed by a moss overlying extensive sub-fossil saltmarsh remains that have already been investigated, outweigh this problem.

Another limitation is related to the fact that all the higher plants in the saltmarsh zones being examined at Kentra utilise the C_3, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) metabolic pathway. Since each species may respond to environmental stimuli differently, it may be that there is considerable overlap in the carbon isotope ratios of metabolised cellulose from each zone, making it difficult to put a separate figure to each type of community. However, a deterministic relationship between salinity and carbon isotope ratio has been established in both laboratory and field experiments (Farquhar *et al.* 1982, Smith & Epstein 1970, Babikir& Kürschner 1992, Chmura *et al.* 1987). The technique has also been tested by investigators in the U.S.A. (DeLaune 1986, Chmura 1988, Chmura & Aharon 1995) as a tool for palaeoenvironmental reconstruction,

but like work done elsewhere (Schultz & Calder 1976, Bird et al. 1993), a major distinction existed in their field sites between zones dominated by C_4 plants and those dominated by C_3 plants. The significant difference in carbon isotope ratios of cellulose metabolised by plants from these two groups (see Chapter 2) enables identification of the point at which a facies change from one community type to another occurs in sedimentary sequences. This transition from C_4-dominated to C_3-dominated does not occur in British saltmarshes (except in those marshes which have recently been invaded by *Spartina* x *townsendii*), and consequently this very useful marker in buried sequences which has been utilised in some palaeosalinity studies (for example Chmura 1995) will not be present.

Although this C_4 / C_3 shift has been very useful in these studies, there have been problems apparently related to post-depositional alteration of carbon isotope ratios in sub-fossil cellulose away from the value expected from analyses of contemporary material (Canfield 1994, Chmura & Aharon 1995). Earlier archaeological studies showed a similar problem (Deniro & Hastorf 1985), and this may well be an issue in this project.

4.3 Structure of research strategy.

As in Chapter 3, the structure of the research strategy can be reduced to a number of key questions which need to be answered. These are summarised in point form.

1. What altitude is the contemporary marsh, how many floristic zones can be identified, and what is their relationship to the tide?

2. How diverse is the species mix within the saltmarsh flora?

3. Does the density of stomata vary significantly between plants of the same species in the different communities, and are there any other variations in physiognomy that might be related to carbon uptake and assimilation which can be measured?

4. How does carbon isotope discrimination vary between the different species found in the same zone?

5. Do $\delta^{13}C$ values from the same species follow the predicted trend across the saltmarsh?

6. Is there any variation in $\delta^{13}C$ values from the same species occurring in the same zone but sampled at different times of the year? Is it necessary to analyse material from each species throughout the year to establish the range of $\delta^{13}C$ values in cellulose being contributed to the organic sediment fraction in each zone?

7. What is the impact of pooling material from a number of plants to produce a mean figure for each species in each community? How much intra-species variation is there in each community?

8. Do the $\delta^{13}C$ values from the contemporary analyses suggest that there might be a useful signature in the buried sub-fossil material?

9. What evidence is there from established micropalaeontological techniques that the buried sub-fossil material represents similar communities to those studied in the contemporary analyses?

10. Do the $\delta^{13}C$ values from the buried sub-fossil temporal succession reflect those from the contemporary spatial succession? How does this vary between cores of different ages?

11. When compared to the established micropalaeontological techniques used to reconstruct coastal environments - pollen, diatoms, foramininferans - do the data from this project support further use of $\delta^{13}C$ analyses to help elucidate coastal palaeoenvironmental reconstructions?

Questions 1 and 2 aim to establish the basic nature of the saltmarsh at Kentra. Question 3 relates to possible controls on $\delta^{13}C$ by variations in plant physiognomy, whilst 4 to 8 refer to possible complicating factors influencing the $\delta^{13}C$ values. These questions aim to provide an understanding of the limitations on this approach as a result of differences within and between species and communities.

Question 9 asks about the relationship between the contemporary environments being examined and the sub-fossil material which has been proposed as representing an ancient saltmarsh succession. The efficacy of this particular approach is finally established by answering questions 10 and 11.

4.4 Methods adopted to answer these questions.

4.4.1 Establishing the number and altitude of vegetation communities at Kentra Bay.

A survey of the vegetation communities at Kentra Bay was carried out by Innes *et al.* (1993), and a previous study of the flora of Kentra Bay by the Nature Conservancy

Council was supplied by Scottish Natural Heritage. For the purposes of this study a separate survey line was chosen which runs parallel to that sampled by Innes *et al.* (1993) at a distance at a distance of 10 metres to the east. Although a botanical survey was carried out (with the assistance of Kevin Walker of the Institute of Terrestrial Ecology, Monks Wood) across the entire transition from pioneer saltmarsh to the raised bog which backs the marsh, it was decided to concentrate on the communities close to the transition from intertidal flats to saltmarsh. This was done because the ultimate aim of the project is to try to provide additional information for the reconstruction of coastal environments. The techniques presently in use in such projects are able to make the distinction between marine, intertidal and terrestrial environments, and therefore this approach was aimed at establishing whether $\delta^{13}C$ values might help to distinguish particular zones within the intertidal vegetation succession. Consequently, 3 zones across the intertidal sand flats were analysed, and 4 across the lower part of the saltmarsh, and these seven form the basis of this project.

The altitude of these zones relative to Ordnance Datum (Newlyn) was established by levelling from the same benchmark used by Innes *et al.* 1993. Levelling was repeated on two occasions, once when the vegetation survey was done (30/10/93), and again on 8/7/94. The relationship of these communities to the tide was established from data calculated by Innes *et al.* 1993 who determined the altitude of local Mean High Water Spring Tides (MHWST) and Highest Astronomical Tide (HAT) from the Admiralty Tide Tables.

4.4.2 Establishing the diversity of the species mix within the saltmarsh flora.

A simple botanical survey was carried out on 30/10/93 with Mr. K. Walker (ITE Monks Wood) to establish the number and relative abundance of species in each

community. The species were classified as dominant (d), abundant (a), frequent (f), occasional (o) or rare (r) following the approach adopted by Tansley (1939). This scale of classification was adopted because it gives a measure of the relative importance of each species in the contemporary flora (and hence the relative importance of each species in terms of its contribution to the organic sediment fraction) without being prescriptive about the actual extent or biomass production of each species. If an analysis of the $\delta^{13}C$ values of the cellulose in the organic fraction of the sediments being deposited in each of the contemporary saltmarsh zones at Kentra was to be undertaken, it would be necessary to establish the contribution of each species much more accurately. However, due to the limited number of analyses possible at I.T.E. Merlewood, only contemporary plant material and cellulose derived from sediment cores were examined in this study

This highlights the difficulty of calculating the relative importance through time of each species' contribution to the bulk cellulose deposited in each environment. The extent to which this varies with time and as sea level changes (Warren & Niering 1993), and to which different plants contribute to this fraction in different ways may be of vital importance, but it is very difficult to quantify variations in this factor. It was hoped that by establishing the contemporary pattern of $\delta^{13}C$ values in each community it would be possible to determine whether $\delta^{13}C$ values from sub-fossil material might hold a valuable signature. The only measurement taken that relates to this particular aspect of the study is the value of LOI% from the seven zones sampled. The technique for establishing L.O.I.% as outlined in Appendix 1 was used for these measurements of total organic matter in the surface sediments of Kentra Bay.

4.4.3 Establishing the density of stomata and measuring other variations in physiognomy that might be related to $\delta^{13}C$ values.

The method for preparing samples for establishing the density of stomata is given in Appendix 2 (see also Beerling & Chaloner 1993a). This was applied to leaf samples from those higher plant species which occurred in more than one of the communities studied, with the exception of *Puccinellia maritima* Huds. (hereafter *Puccinellia*). Although this was plant found to occur in several zones in the saltmarsh, by the time the leaf material had been returned to Durham it had rolled into very tight cylinders, making it impossible to take stomatal peels.

Whilst counting the impressions of stomata from peels taken of *Armeria maritima* (L.) Mill. (hereafter *Armeria*) leaves a second quite distinct type of pore was noticed. Rather than the typical stomata which are seen as elongated apertures bounded by two guard cells, these "pores" are approximately circular and are divided into quarters. In each quarter there is a small aperture, and after consultation (Pearson 1994 pers. comm.) it was thought that these might be the salt glands of *Armeria* as discussed in Woodell & Dale 1993 (*cf.* Fitzgerald's 1992 work on salt glands in *Avicennia marina* (Forsk.) Vierh.). In order to establish whether this was the case a leaf from a living *Armeria* plant was severed and dipped in a weak solution of silver nitrate. The principle of this experiment was that the silver nitrate will react with any sodium chloride, producing a dark stain on the surface of the leaf where any concentration of NaCl exists. After *circa* 30 minutes of standing in this solution the leaf was examined at 100X magnification and the "pores" had been stained black.

Since the extrusion of salt is a very important part of the physiology of many halophytes, it was decided to establish the density of salt glands on the leaves of *Armeria*

that were being analysed for stomatal density. Since the salt glands are approximately the same dimension as the stomata, exactly the same technique was used to prepare and count samples to study this particular aspect of physiognomy.

4.4.4 Establishing the variations in $\delta^{13}C$ values of the contemporary saltmarsh flora.

Plant material from each species in each zone for $\delta^{13}C$ analysis was sampled on 30/10/93. Samples of *Armeria* were also taken on 7/3/94 and 8/7/94 in order to examine the extent of $\delta^{13}C$ variation in samples of the same species taken at different times of the year. All plant material was refined and only the cellulose fraction was used for $\delta^{13}C$ analysis. The method for extracting cellulose from whole plant material is outlined in Appendix 3.

Two different approaches were taken to the analysis of the $\delta^{13}C$ value of this cellulose. The vast majority of the material was sorted by species and community, and then pooled. This produced a large amount of cellulose from each species in each community, and from this six sub-samples were taken. These were analysed using an automated system for the production, cryogenic separation and isotopic measurement of CO_2 (Anon. 1989, Siegenthaler & Eicher 1986). This facility allows for multiple replicates to be processed much more quickly than the manual system of sample preparation, cryogenic separation and analysis outlined in Appendix 4, but operates under essentially the same principles of pyrolising the samples, splitting the CO_2 from the other gases and then feeding this to a gas isotope ratio mass-spectrometer.

The rapid nature of these analyses allowed each of these subsamples from each pool of cellulose to be analysed. This produced data on inter-species variation within each community (Question 4) and intra-species variation between communities (Question 5).

To test the extent of variation in $\delta^{13}C$ values from samples taken at different times of the year (Question 6) the *Armeria* sampled on 30/10/93, 7/3/94 and 8/7/94 was analysed. *Armeria* was also used to analyse the extent of intra-species variation of $\delta^{13}C$ in plants from the same zone (Question 7). This was done by preparing cellulose from individual plants and analysing it at the Department of Biology, University of Miami using the manual technique described in Appendix 4.

Since the facility at ITE Merlewood had only recently begun to analyse samples for $\delta^{13}C$ there was some doubt about the accuracy of this automated technique. Consequently cellulose standards from a single commercially-produced batch of known isotopic composition (provided by Dr. V. R. Switsur, Godwin Laboratory, University of Cambridge) were put into each batch of cellulose sent there. This provided data about the consistency and accuracy of the analyses done at Merlewood. However, it should be pointed out that when the application for $\delta^{13}C$ analyses was made the committee did point out that the accuracy of the automated facility was likely to be lower than can be achieved using the manual technique. This was one of the reasons why six repeats were made of each pool of material.

The answer to Question 8 had to be positive before the project went ahead and analysed any sub-fossil material. The principle by which this was determined was based on the idea that the contemporary $\delta^{13}C$ values would bear some relationship to those in the sub-fossil buried succession. Therefore, if there was any good reason to think that a contemporary pattern existed which might be reflected in these buried sediments the analyses were to be carried forward to the ancient material.

4.4.5 Establishing that the buried sediment units below Kentra Moss represent similar communities to those presently found in Kentra Bay.

One of the reasons for choosing Kentra as a field site was that members of the Environmental Research Centre, University of Durham had already established that the sediments below Kentra Moss hold a record of sea level change through the late Holocene. These data were kindly made available as were the original cores on which the paper by Shennan *et al.* (1995) is based. Although there are differences in the composition and proportions in the microfossil assemblages between the contemporary analyses of Innes *et al.* (1993) and those from the sub-fossil core material reported in Shennan *et al.* (1995), there is no doubt from these analyses that there are ancient saltmarsh sequences buried below Kentra Moss (see Tables 4.3 and 4.4 for summary of microfossil data from each core). The problem - which cannot be solved - is that it is possible that the species composition, let alone relative abundance of those species, is not the same today as it was when these sediments were deposited.

Without a complete understanding of the local pool of saltmarsh species, sediment dynamics, tidal influence and fresh water inputs in the past it is not possible to assume that the communities on Kentra Marsh today are representative of the communities buried below Kentra Moss (see also discussion in Chapter 4.5.1). The extent of the difference may be small and have no impact on the relationship between present carbon isotope values and those from sub-fossil cellulose derived from equivalent contemporary and ancient material. However, it is necessary to identify the extent of any differences which might exist so that sub-fossil sequences can be investigated which are believed to be derived from similar communities to those analysed from the contemporary saltmarsh.

Fortunately, as Shennan *et al.* (1995) report, the sediment sequences chosen do show a continuous succession from unvegetated intertidal flats through to fully terrestrial peat. Since this study is only interested in the material at the boundary between inorganic and organic deposition, and this is an area where the range of plant species is often restricted by the limiting factors of tide and salinity, it was hoped that the extent of change through time in the species composition of similarly located communities has not been great.

Along a similar vein are the problems which might be associated with the possibility of intra-specific genetic differentiation during plant succession outlined by Gray and others (Gray 1987, Gray *et al.* 1979). It is possible that such genetic changes may have no impact on the relationship between the incidence of tidal flooding and the $\delta^{13}C$ value of cellulose metabolised by plants of the same species. However, in order to establish this it would be necessary to undertake a series of controlled experiments by subjecting plants from both different and identical genetic populations to varying levels of salinity and inundation. Such experiments were not undertaken, but would be necessary to establish the extent to which it might be possible to compare data from one marsh with that collected from plants of the same species under different tidal regimes.

Once the extent to which genetic variations can alter $\delta^{13}C$ values under controlled conditions has been established, the importance of this factor in determining the relationship between contemporary saltmarsh communities and material from sub-fossil saltmarsh peats can be assessed. If there is no evidence that any genetic difference exists between the plants in the different contemporary communities or that any change has occurred between the contemporary population and the plants which contributed to the sub-fossil cellulose being examined, then this factor can be ignored. Similarly, if any genetic differences are found not to impact on the $\delta^{13}C$ values of the species involved

then this factor will not need to be considered. However, until these possiblities are examined, the evidence produced by Kohorn *et al.* (1994) that a 5.1‰ inter-plant range in the $\delta^{13}C$ values from specimens of *Simmondsia chinensis* L. growing in an homogeneous habitat is attributable "at least in part to genetic diversity within the population" (*ibid*, p.1817; see also Chapter 2.6.3) suggests that variations in $\delta^{13}C$ values may have nothing to do with environmental variation.

4.4.6 Establishing the extent to which $\delta^{13}C$ from sub-fossil cellulose can elucidate sea level histories.

Material was taken from two separate cores collected by members of the Environmental Research Centre, University of Durham which are recorded in Shennan *et al.* (1995). The regressive contact in these two cores (KM21C and KM39B) has been dated and there is a considerable difference between these ^{14}C dates (1480±40 BP [SRR 4724] and 3940±45 BP [SRR 4731] respectively, see Tables 4.3 and 4.4). The reason for analysing two cores is not only that it will be a test of the consistency of any pattern in the $\delta^{13}C$ values, but it also gives an opportunity to investigate the possible impact of floristic changes over time on $\delta^{13}C$ values from bulk cellulose.

To achieve this the sampling strategy adopted for taking cellulose samples from the core concentrated on those areas close to lithostratigraphical and biostratigraphical boundaries in the cores as reported by Shennan and co-workers (1995, see also Tables 4.4 and 4.5). Since there is a limited amount of organic matter towards the transition to wholly inorganic sediments at the base of the cores (as recorded by the value for LOI% given in the summary tables (Tables 4.3 and 4.4) below; see also Kosters *et al.* 1987, Phleger & Bradshaw 1966), the amount of cellulose which could be extracted did restrict the number of repeat $\delta^{13}C$ analyses that could be made at certain levels. Where this was

the case the sampling interval was decreased to produce a more intensive analysis of $\delta^{13}C$ variation down the core.

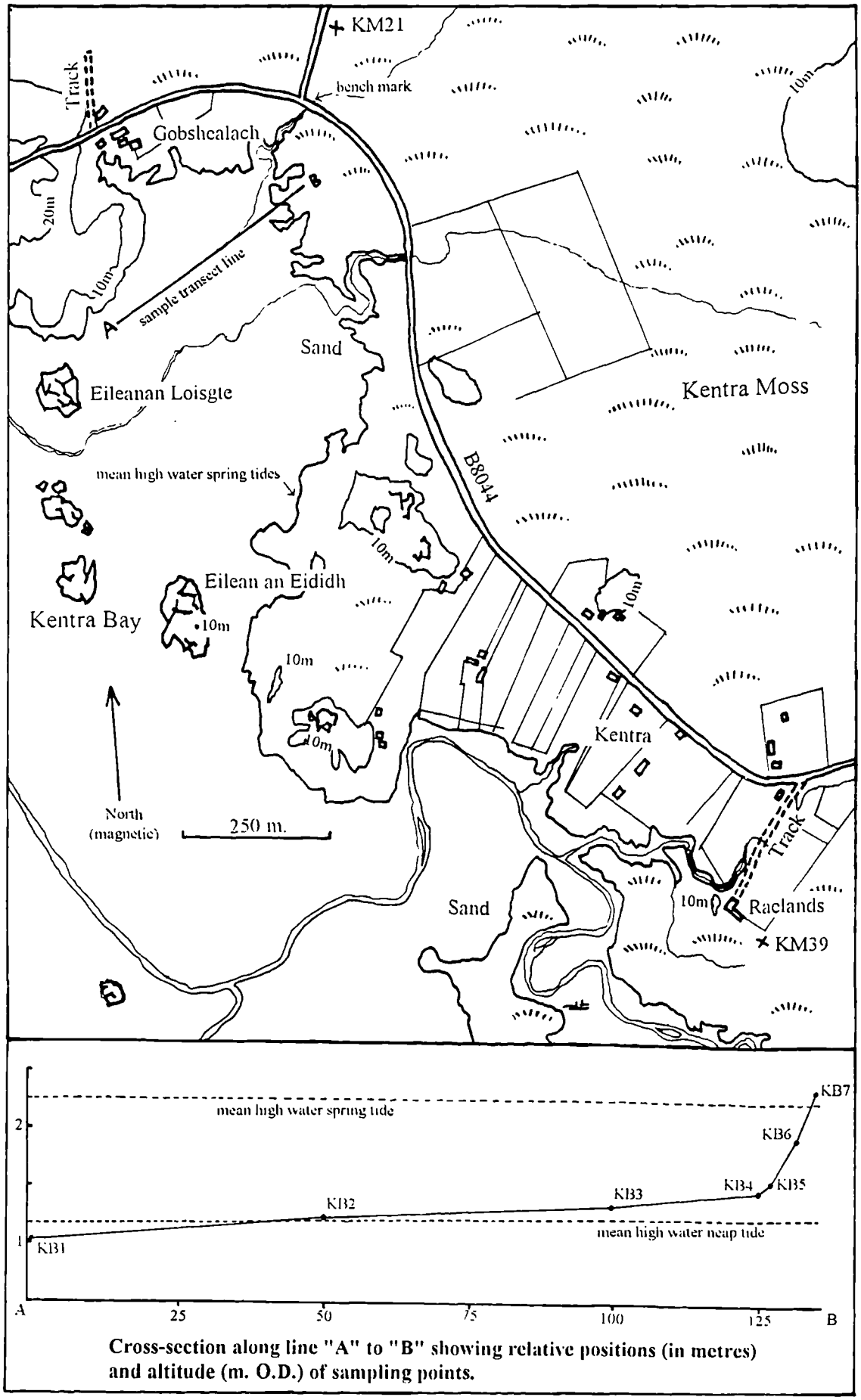
Although these samples will provide the answer to Question 10, and there is a large amount of contemporary and sub-fossil pollen and diatom evidence from both Kentra Bay and the two cores analysed, no foraminiferan assemblage analysis had been undertaken against which to compare $\delta^{13}C$ as a possible technique in studies of coastal sediments (Question 11). In order to fill this gap a detailed analysis of the contemporary foraminiferan fauna in the seven zones identified across the sand flats and salt marsh of Kentra Bay was undertaken. This involved collecting surface sediment on 3 separate occasions (30/10/93, 7/3/94 and 8/7/94) and comparing variations in population composition over this period. The methodology adopted for the collection and preparation of samples to establish the foraminiferan assemblages is given in Appendix 12. Core material was analysed to establish the sub-fossil assemblages (Adams & Haynes 1965, Boomer & Godwin 1993, Culver & Banner 1978), and the results of these and all the other analyses are discussed below.

4.5 Results of these analyses.

4.5.1 The altitude and floristic composition of the zones identified and analysed at Kentra Bay.

The seven contiguous zones identified at Kentra Bay were numbered KB1 - KB7, KB1 being the lowest of the three zones on the sand flats. The position of these zones is shown on Figure 4.1. KB4 - KB7 were determined on the basis of the botanical survey carried out on 30/10/93, and KB1 - KB3 were sited at 50 metre intervals along the same survey line so that the distance between KB3 and KB4 was 25 metres. The altitude of

Figure 4.1. Location of sampling transect across Kentra Bay and cores KM21 and KM39.



Cross-section along line "A" to "B" showing relative positions (in metres) and altitude (m. O.D.) of sampling points.

each zone relative to Ordnance Datum (Newlyn) was established on two occasions, and the results of these measurements are given in Appendix 13.

Surface sediment samples were taken on three occasions between October 1993 and July 1994 to establish the proportion of organic material in the sediment in each zone. The results (given in Appendix 14) clearly show the increasingly organic nature of the sediments in zones KB1 to KB7. These data not only provide a quantifiable estimate of the importance of organically-derived material to the sediment yield in each zone, but may also prove useful for comparisons with L.O.I.% data from the sub-fossil core material analysed.

The data from Appendix 13 and Appendix 14 are summarised in Table 4.1 which also shows the species composition of the vegetated zones at Kentra Bay. The lowest of the vegetated zones (KB4) is a very narrow pioneer marsh zone containing sparse clumps of *Armeria*, discrete plants of *Puccinellia maritima* Huds. (hereafter "*Puccinellia*") and, amongst the clumps of *Armeria*, a brown alga. This alga occurs in a number of other zones (below), but has proved difficult to identify with absolute certainty because it is cropped heavily by grazing sheep in each zone. Specimens of the alga sent to Dr. B. Whitton (Department of Biological Sciences, University of Durham) were forwarded to the phycology unit in the British Museum (Natural History), and the consensus from there was that it is a miniature of *Pelvetia canaliculata* Dcne & Thur., hereafter "*Pelvetia*" (Fletcher 1987).

This has been recorded as a "turf fucoid" (Adam 1990) in a number of saltmarshes in the west of Scotland (Polderman & Polderman-Hall 1980) and also in Wales (de Oliveira & Fletcher 1980). It is of particular interest because algae assimilate C in a completely different way to higher plants. Their use of dissolved HCO_3^- as a source of C

and the different metabolic pathways involved in carboxylation in algae results in $\delta^{13}C$ values which are considerably less negative than those of higher plants (cf. Benedict *et al.* 1980). Algae have also been shown to demonstrate a relationship between $\delta^{13}C$ of cellulose and inundation frequency (Swift & Raven 1989). The discovery that this alga is present at Kentra adds to the hope that $\delta^{13}C$ values of bulk plant cellulose from the sediments laid down under different inundation and salinity regimes will be distinct.

It is interesting to note that although the transect line adopted by Innes *et al.* (1993) is only 10 metres to the west of the line used in this project, they recorded bryophytes in the lower zones of the saltmarsh along their transect. No bryophytes were found during this survey and it is possible that they misidentified the brown alga recorded in this study.

Zone KB5 is characterised by a more extensive vegetational cover than in KB4 (recorded by the increase in LOI%), and the introduction of *Glaux maritima* L. (hereafter "*Glaux*") in to the flora. *Pelvetia* remains part of the assemblage and is in fact dominant in this zone. However, in KB6 the proportion of *Pelvetia* in the flora decreases, and to the *Glaux*, *Puccinellia* and omnipresent *Armeria* is added *Plantago maritima* L. (hereafter "*Plantago*"). *Plantago* has been studied by Flanagan & Jeffries (1989) and shown to respond to salinity in a manner which would produce the theoretically-predicted relationship between $\delta^{13}C$ and zone position in a saltmarsh. It is also the focus of Jerling's (1985) study on population dynamics in which he discusses the extent of environmental control on plant distributions in Baltic saltmarshes, and Maathius and Prins' (1990) paper comparing salt tolerant and salt sensitive *Plantago* spp. The variability in physiognomy, physiology and phytosociology shown by *Plantago maritima* suggests that there may be differences in assemblage composition over time which are not predictable. Both the work of McManus & Alizai's (1987) on variations in marsh levels and Reed's (1991) work on

marsh erosion show the plasticity of saltmarsh plants (see also Rhebergen & Nelissen 1985 on *Festuca rubra* L.). This suggests that the underlying principle that contemporary spatial plant successions can be related to ancient buried temporal successions may be open to question (see Chapter 4.4.5).

However, this succession is not atypical of marshes in north west Scotland (Adam 1978), although the diversity of species recorded is lower than in many other sites (Adam 1981), and consequently offers a representative analogue of the general form of saltmarsh assemblages in this area.

The most important aspect about zone KB6 is that it corresponds to a point just at or below local MHWST according to the calculations of Innes *et al.* (1993) (see Table 4.1). The significance of this is that *Pelvetia* is still part of the flora up to this point, but disappears from the zones above. If *Pelvetia* is contributing to the bulk organic matter yielded to the sediments in each of the zones in which it occurs, then its considerably less negative $\delta^{13}C$ value may mean that it is possible to distinguish sediments laid down in saltmarsh communities below local MHWST from those above MHWST. *Puccinellia* also leaves the flora after KB6, and zone KB7 contains *Armeria*, *Glaux*, *Plantago* and *Juncus gerardii* Loisel., hereafter "*Juncus*" (Rozema & Bloom 1977). This apparent shift away from obligate halophytes demonstrates the decreasing influence of tidal inundation (Brereton 1971, Fahrig *et al.* 1993) and soil salinity (Bertness 1992, Price *et al.* 1990) in determining the flora of the upper marsh communities (Adam 1976, Bertness 1988, Long & Masson 1983).

The relative importance of physical factors (Bertness & Ellison 1987), competition (Austin 1990, Bazazz 1990) and chemical variation (Shumway & Bertness 1992, Singer & Havill 1993) in saltmarsh successions (Pennings & Calloway 1992, Roozen & Westhoff

1985) is an important aspect of saltmarsh ecology from the point of view of this, and other, palaeoenvironmental reconstructions of coastal vegetation communities (see Gray 1992 for review). Not only does this have an impact on the nature of succession itself in an area of retreating marine influence such as Kentra (cf. Healy 1991), but the possibility that successions may be quite different if the pool of species in an area varies (Russell *et al.* 1985) has a significant impact on the potential to draw conclusions about sub-fossil temporal successions from contemporary spatial successions. Although microfossil evidence should give some indication of the type of environment a particular sediment was derived from, any analysis of bulk plant material must accept that it is highly unlikely that exact analogues of ancient vegetation communities exist (Schiegl 1972, Van Smeerdijk 1989), especially in an area such as the United Kingdom where the influence of man on the vegetation has been so significant.

The exact contribution of each species to the organic fraction of the sediment is therefore very difficult to assess. However, the important aspect of this particular part of the project is to outline the differences that exist in the contemporary succession, and to acknowledge that these only act as a guideline when interpreting palaeoenvironmental data. To this end the results of the botanical survey, the LOI% tests, the levelling and also the relative position of local MHWST are summarised in Table 4.1 below.

Table 4.1 Summary data by zone relating to the contemporary succession analysed at Kentra Bay.

Variable	KB1	KB2	KB3	KB4	KB5	KB6	KB7
Altitude 30/10/93	1.01m O.D.	1.23m O.D.	1.29m O.D.	1.46m O.D.	1.57m O.D.	1.88m O.D.	2.30m O.D.
Altitude 8/7/94	0.98m O.D.	1.18m O.D.	1.32m O.D.	1.48m O.D.	1.63m O.D.	1.85m O.D.	2.25m O.D.
L.O.I.% 30/10/93	1.25	2.44	1.05	1.06	13.87	13.57	35.28
L.O.I.% 7/3/94	0.47	0.64	0.52	1.82	8.84	23.99	35.03
L.O.I.% 8/7/94	1.41	1.34	1.20	3.10	13.32	17.61	41.75
Species present	Classified as:	dominant 'd'	abundant 'a'	occassional 'o'	after Tansley	(1939)	
Armeria				a	d	a	o
Puccinellia				a	a	d	
Pelvetia				o	a	o	
Glaux					o	o	o
Plantago						a	d
Juncus							a
Tide levels (m. O.D.)		MHWNT 1.18m					MHWST 2.25m

The important conclusions to be drawn from these data are that there are very few species in the four vegetated zones, that the proportion of vegetation cover increases from KB4 to KB7 (see LOI% data) and that the limit of *Pelvetia* corresponds approximately to the altitude of local MHWST. Although the LOI% figures from each zone do vary between the three sampling dates, the changes probably reflect the variations in seasonal biomass production in the seven zones. Most notably, the values in zones KB1 - KB5 from the sampling in early March are low. However, the sequence of figures from KB1 - KB7 on each date shows the increasing organic matter content of the surface sediment.

None of the C_4 species, such as *Spartina* x *townsendii* (Long *et al.* 1975) which are the basis of other palaeosalinity reconstructions using $\delta^{13}C$ in other parts of the world are present in the marsh at Kentra. The relatively recent appearance of this species in British saltmarshes may enable accurate reconstructions of saltmarsh communities from the past 30 years using $\delta^{13}C$, but this is not relevant to the timescales involved in this project.

Acknowledging all the potential problems regarding succession and genetic variations within and between communities (especially in the light of Gray's (1985) work on heritable variation in *Puccinellia* and his 1979 paper with Parsell & Scott), these data do, however, point towards Kentra being a suitable site for work on the efficacy of $\delta^{13}C$ as a palaeoenvironmental technique. The work of Guy and co-workers (Guy & Reid 1986a, b, Guy et al. 1980, 1986, 1989) and Haines (1976) also supports the theory that the higher plants present should show some relationship between salinity and $\delta^{13}C$.

4.5.2 The contemporary foraminiferan assemblages.

The raw counts from these analyses are given in Appendix 15. The count base adopted was 300 tests or 10g of dried sediment, whichever was achieved sooner. Each sample taken was counted twice under the microscope, removing any tests found on either count, to establish the contemporary fauna. All the tests which were found still contained the protoplast of the foraminiferan, and consequently these counts show the living fauna (Scott & Medioli 1980). The only exception to this was 6 tests of *Elphidium williamsonii* Haynes found in the sample from KB5 taken on 7/3/94. These were badly corroded and of approximately equal size, suggesting they may have been imported during on-shore movement of sediment (Murray 1979).

The assemblages are shown from each of the three dates as raw counts and also in percentage forms respectively in Figures 4.2.1 and 4.2.2 for 30/10/93, Figures 4.3.1 and 4.3.2 for 7/3/94, and Figures 4.4.1 and 4.4.2 for 8/7/94. (The abbreviation 'foram' is used in the figures.) There are a number of conclusions to be drawn from these figures and the data in Appendix 15. Firstly the number of foraminifera per 10 g. of sediment not only varies by zone in each set of samples, but there are consistently fewer foraminifera in the samples taken from all the zones on 8/7/94 than on the previous dates. However, before drawing any conclusion about the seasonality of foraminifera (cf. Murray 1980), it should be noted that they are not homogeneously dispersed across the surface of sand flats or saltmarshes. Consequently this variation may be purely spatial rather than temporal.

Figure 4.2.1

Kentra foram counts, 30/10/93.

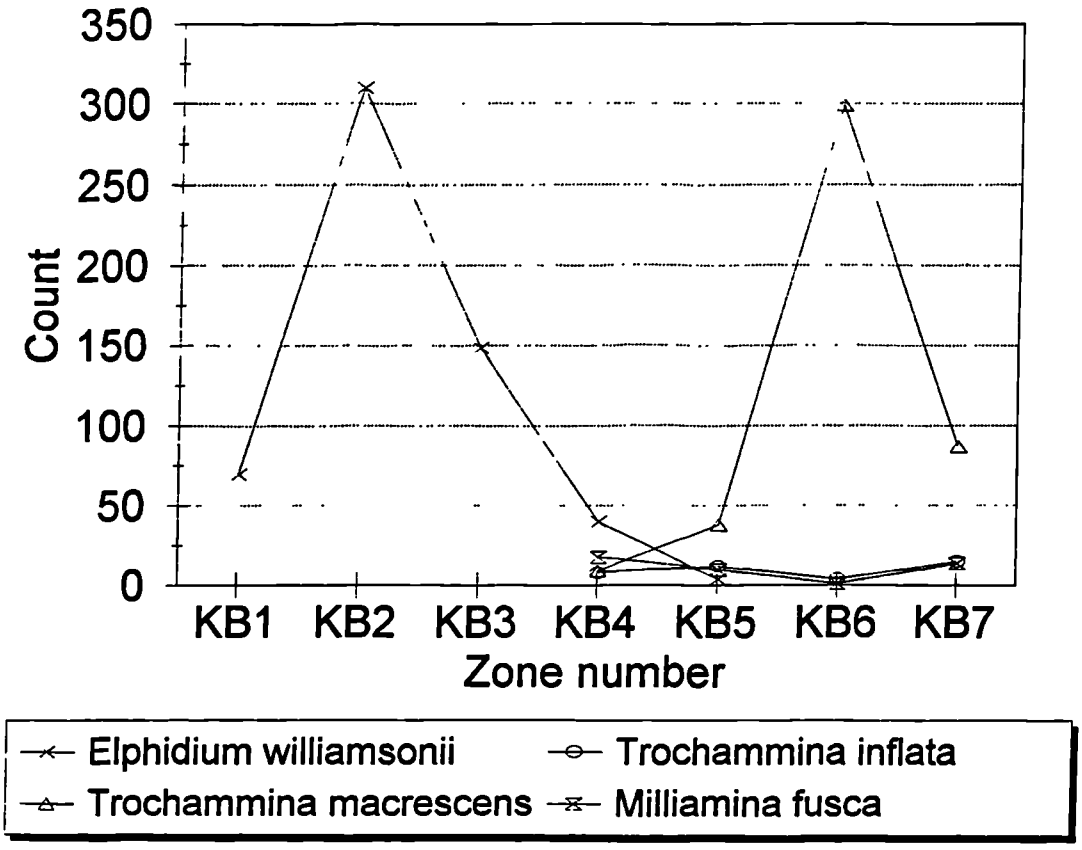
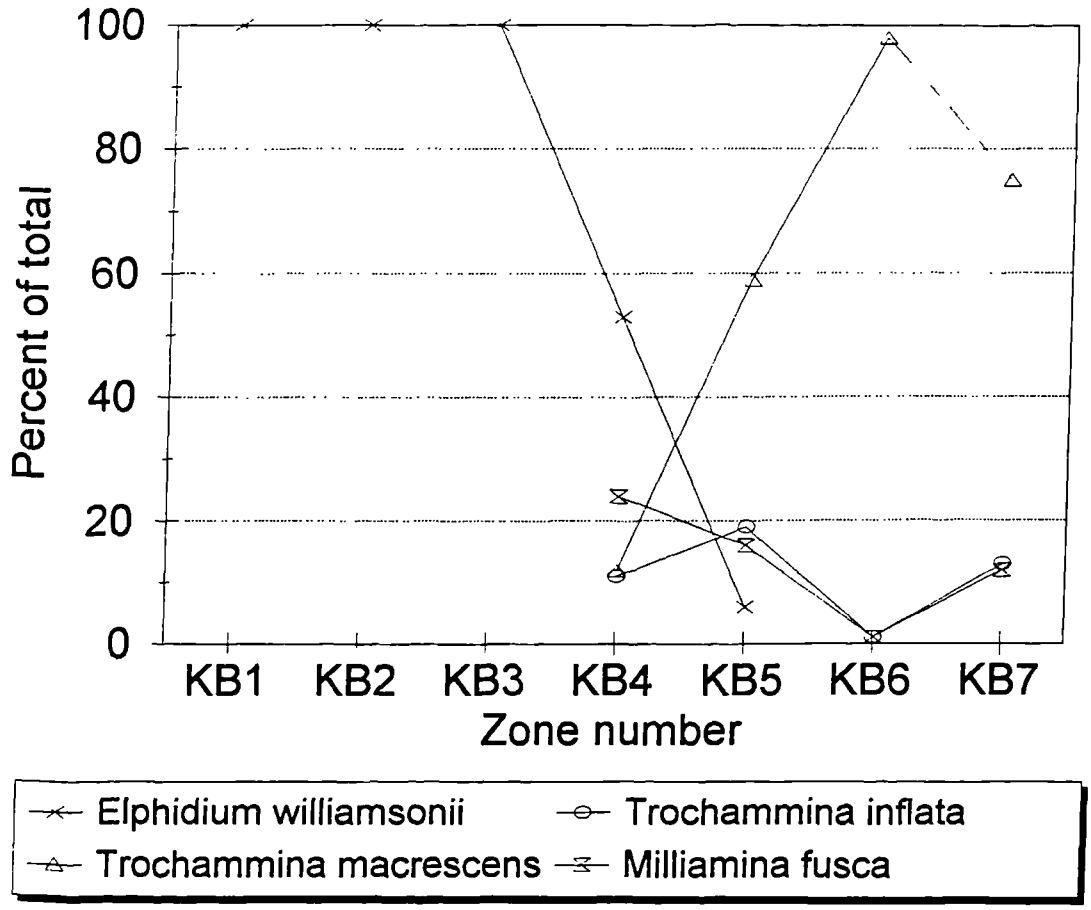


Figure 4.2.2

Kentra foram assemblages, 30/10/93.



Although this variation is interesting, the more important aspect of these data is the overall consistency of the proportions in the assemblages from each sampling date. With the exception of zone KB1 on 7/3/94, where the number of foraminifera found was just 2 and one was the agglutinated foraminiferan *Trochammina macrescens* Brady, the sand flat proportional assemblages are dominated by *Elphidium williamsonii* Haynes. This is a robust calcareous foraminiferan, typical of intertidal flats. The domination of this form ends in KB4, which appears to be pivotal in the faunal succession on all three occasions. At this point the agglutinated foraminifera typical of vegetated saltmarshes begin to make up a significant part of the assemblage. The agglutinated forms found at Kentra are *Milliamina fusca* Brady, *Trochammina macrescens* and *Trochammina inflata* Montagu, and their presence (and that of *Elphidium williamsonii*) fits the contemporary zonations found elsewhere in Britain. *Haynesina germanica* (= *Protolophidium germanicum*) Ehrenberg, a smaller calcareous form than *Elphidium williamsonii*, occurs in very small quantities in zone KB4 on 7/3/94 and 8/7/94, but its importance is minimal.

It is especially important to note the difference between the assemblages found in the vegetated and non-vegetated zones along the transect line chosen at Kentra. Calcareous forms dominate the sand flats, and agglutinated forms occur - often very densely - on the saltmarsh. Zone KB4 appears to be the point at which these overlap and the varied composition of the assemblage in this narrow zone may be an excellent marker if the sub-fossil assemblages mirror the contemporary ones. Jennings *et al.* 1995 came to a similar conclusion in their comparison of contemporary foraminiferal assemblages and vascular plant communities on the Chilean coast.

Another interesting, and useful, aspect of these data is that, just like the flora on the saltmarsh, the foraminiferan fauna is species poor. This may mean that it is possible to identify the environment represented by sub-fossil assemblages relatively accurately. The

Figure 4.3.1

Kentra foram counts, 7/3/94.

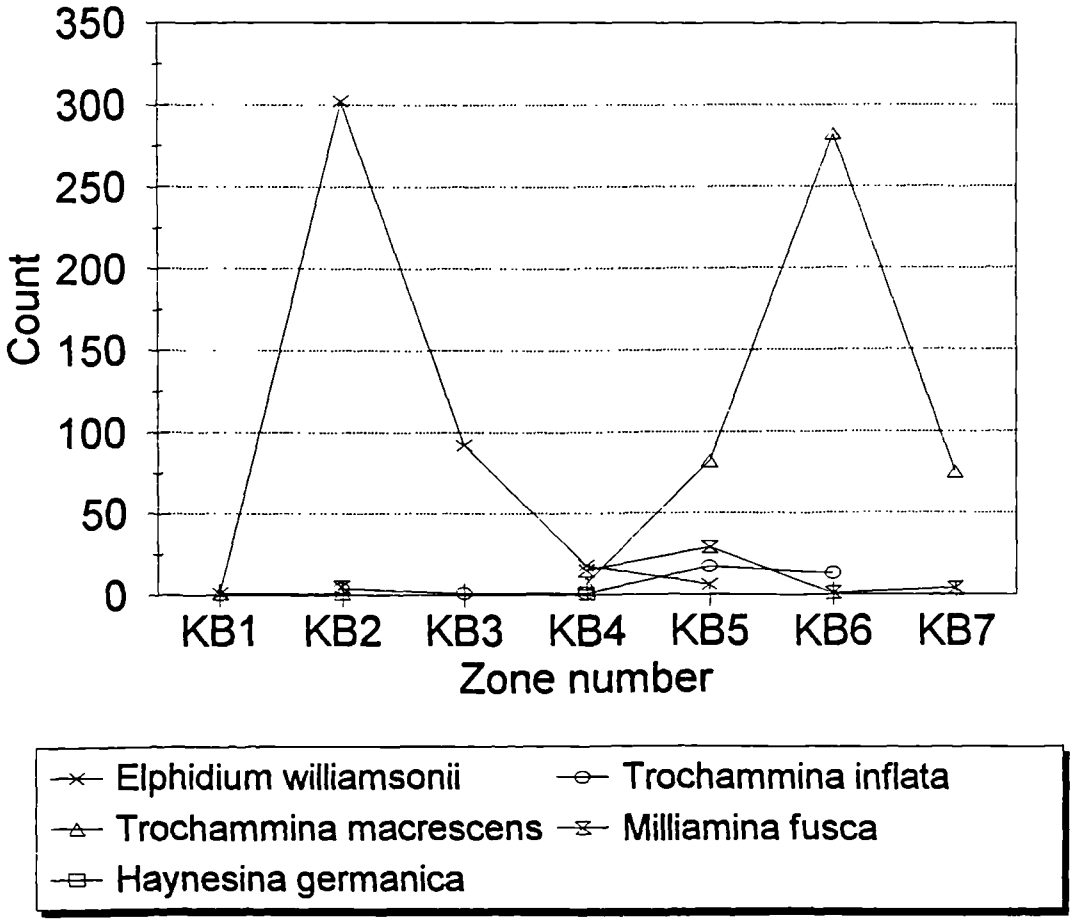


Figure 4.3.2

Kentra foram assemblages, 7/3/94.

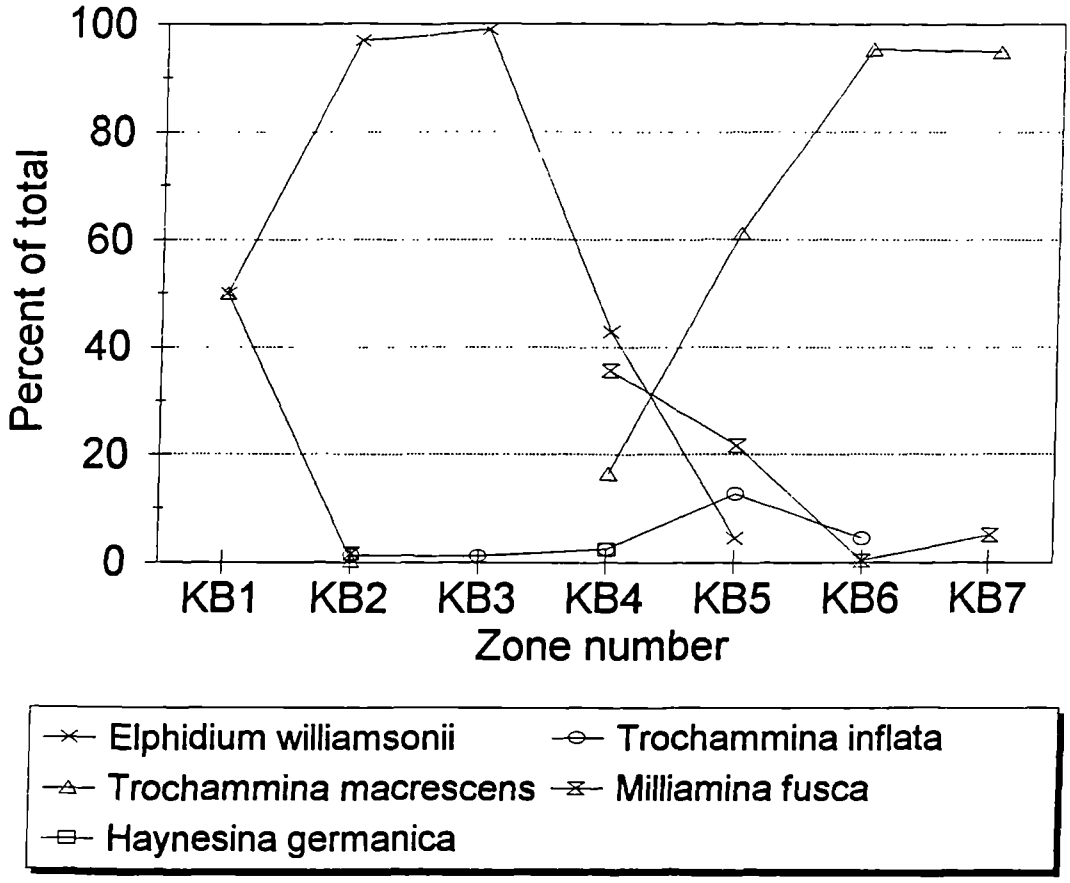


Figure 4.4.1

Kentra foram counts, 8/7/94.

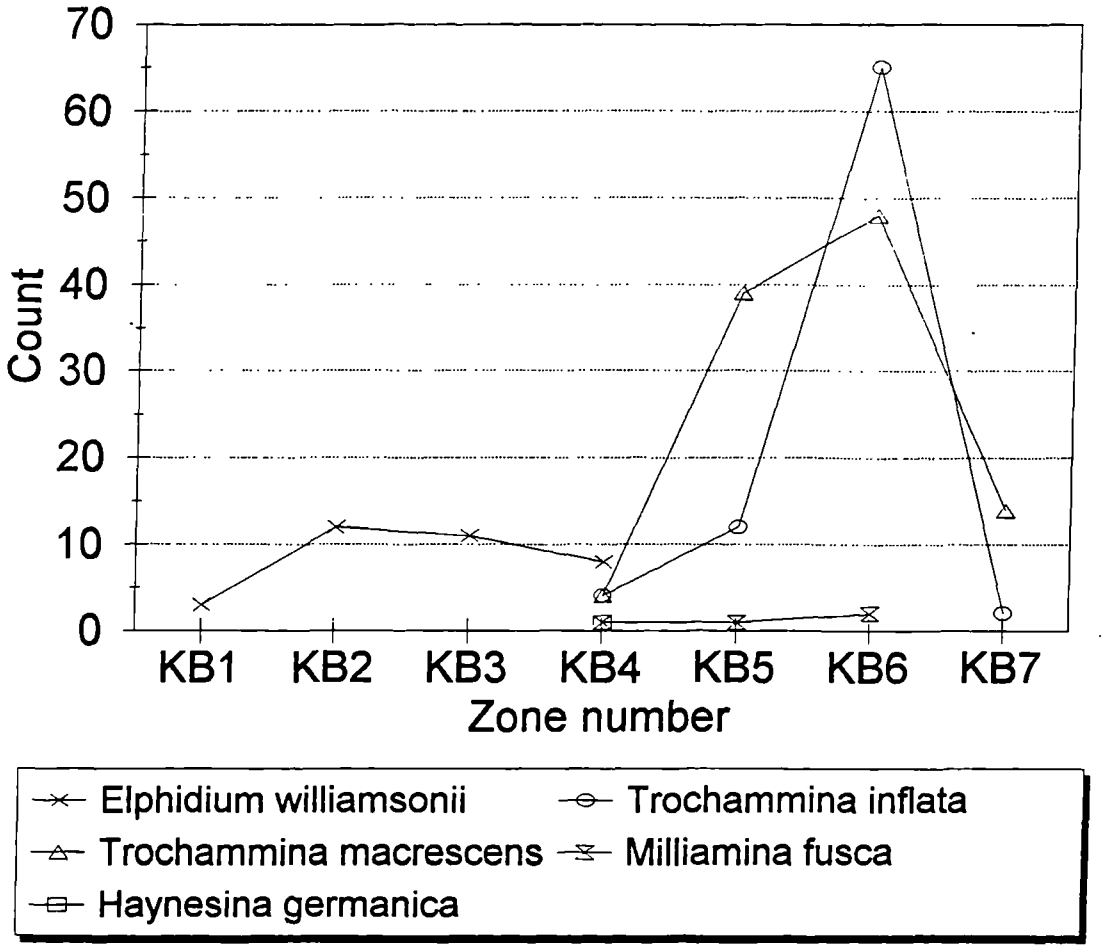
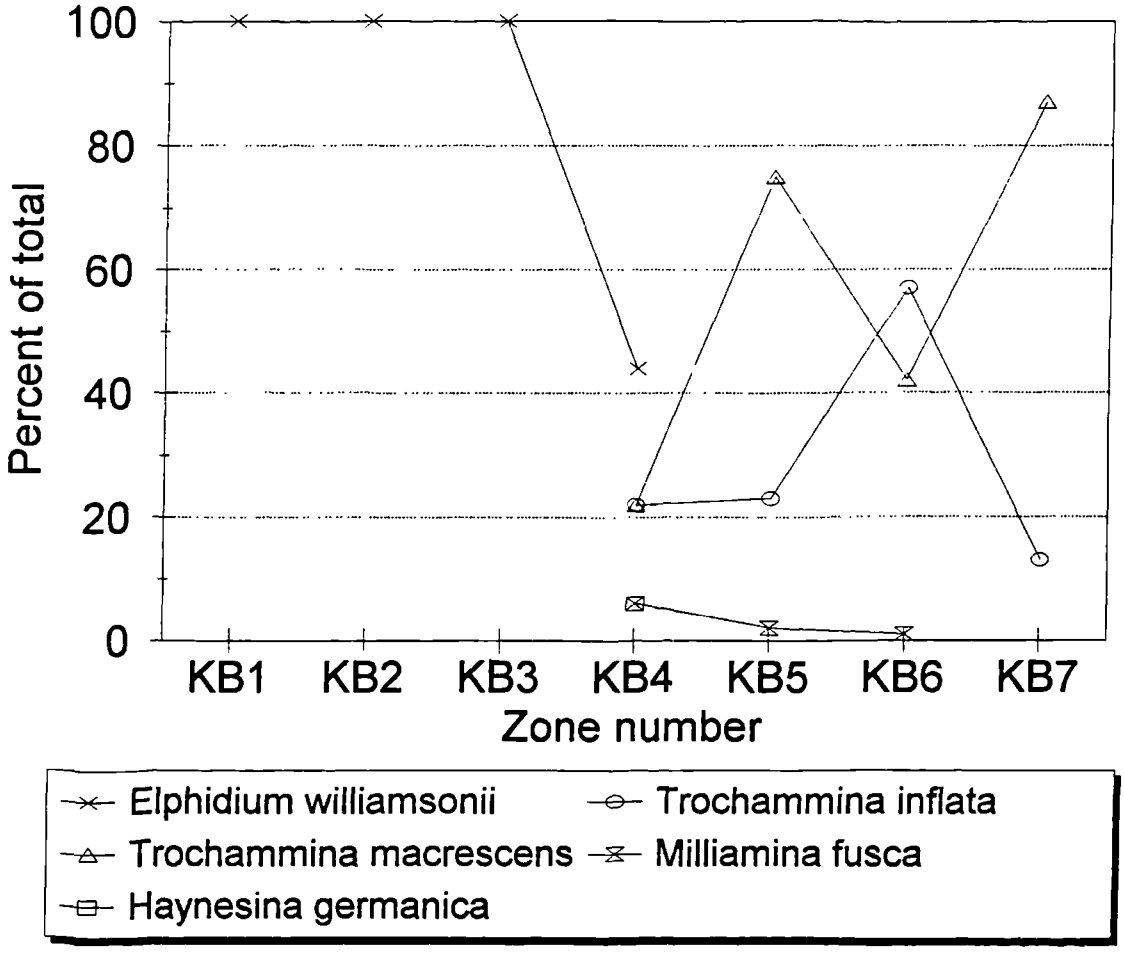


Figure 4.4.2

Kentra foram assemblages, 8/7/94.



conclusion must therefore be that the contemporary foraminferan assemblages appear to offer a very good tool for palaeoenvironmental reconstruction (Scott & Medioli 1980, 1988) if the sub-fossil record mirrors the contemporary pattern (cf. Kelley *et al.* 1995).

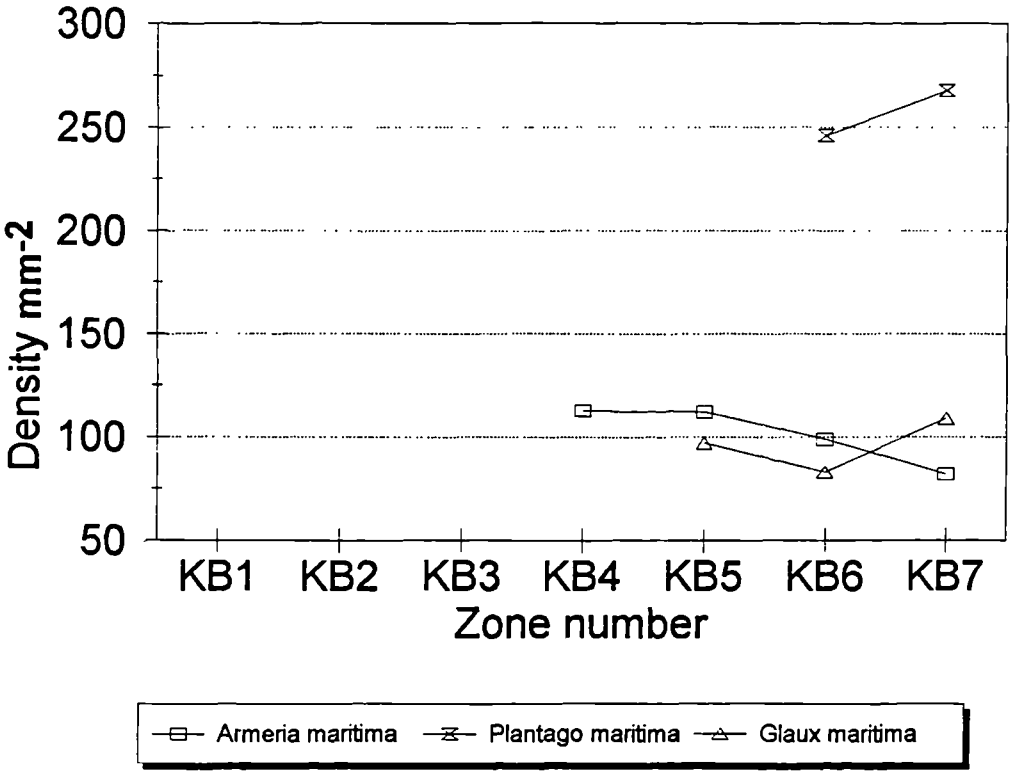
However, although these are interesting data, and provide the first analysis of contemporary faunal successions in western Scotland, they are only a part of the project so that the efficacy of the $\delta^{13}C$ analyses can be tested against reconstructions based on microfossil assemblages.

4.5.3 Variations in leaf physiognomy across the saltmarsh.

As mentioned in Chapter 4.4.3, only those species occurring in more than one zone on the saltmarsh were analysed to establish the density of stomata on their leaves. In each case a peel taken from the underside of the leaf was examined, and five randomly spaced views were made of 10 leaves from different plants in each zone. Conversion of the raw counts taken from *Armeria*, *Glaux* and *Plantago* to density per mm^2 is by the same method as described in Chapter 3.5.4. The raw data are given in Appendix 16, and the converted data in stomata mm^{-2} are plotted in Figure 4.5. Although only a small amount of data is represented, this Figure does show how different species respond to the same environmental changes in very different ways (cf. Beerling *et al.* 1993). The density of stomata on *Armeria* appears to fall from KB4 to KB7, whilst on *Glaux* there is no simple trend in either direction. However, the density of stomata on the leaves of *Plantago* rises from KB6 to KB7. Each of these species appears to be reacting quite differently to the variations in inundation and salinity across the zones sampled. However, the data given in Appendix 17 show that there is no statistical difference between the mean values shown in Figure 4.5 for any of the species once one standard deviation either side of the mean is considered. Therefore, although the variations in stomatal density may be

Figure 4.5

Kentra contemporary stomatal density



real, it is not possible to assign a particular density to a particular zone for any of the three species analysed. Consequently this particular aspect of plant physiognomy cannot be considered useful for palaeoenvironmental reconstruction, although it may have an impact on the extent of discrimination against ^{13}C by altering the gas exchange characteristics of these species in the different zones at Kentra.

The other aspect of leaf physiognomy mentioned in Chapter 4.4.3 is the salt glands on the leaves of *Armeria*. The count base is identical to that for establishing stomatal density, but once again there is no straightforward link between the counts and the position of the plants on the transect, as the data in Appendix 18 show. Converted to density per mm^2 of leaf surface area, the mean of the 10 means from zones KB4 to KB7 is 19.9±10.9, 28.6±10.4, 22.2±6.4 and 21.5±3.6 respectively. There is no link between these data and those for stomatal density on the same *Armeria* plants, and the extent of variation in the zones with the highest and lowest average figures (KB4 and KB5 respectively) means that the values from KB6 and KB7 are swamped, and it appears that neither stomatal density nor the density of salt glands on *Armeria* varies consistently according to zone.

An important aside relating to the microscopic analysis of prepared slides for establishing stomatal density is that the power of magnification - and consequently the area of view - can alter the density of stomata counted. This was found by counting the same slide using two different microscopes. In one case the size of the view was calculated as 0.125664 mm^2, and the other (used for all the data presented here) was 0.084496 mm^2. Counting samples of *Armeria* from KB7 using the same count base under the first microscope an average of 97.1±36.5 stomata per mm^2 was established, but using the microscope with the smaller field of view (higher magnification eyepieces) a density of 82.4±17 stomata per mm^2 was observed. Although they are statistically identical, a similar

pattern of lower densities at higher magnification was found on samples from KB6 (136.1 ±29.1 and 99.4±18.6 stomata per mm^2 respectively). The reasons for this difference seem to be that at higher magnification a lot more of the stomata are only partially seen, and there is therefore a degree of subjectivity about the actual number of stomata in each view. To overcome this it should be stated whether the count includes only whole stomata (as in this thesis) or partially visible stomata as well. Another reason may be that the stomata on *Armeria* are not equally spaced over the area of the slide. This intra-leaf difference in stomatal density is not restricted to *Armeria*, and was also noted on *Plantago* leaves.

4.5.4 The contemporary $\delta^{13}C$ values.

The possibility that the automated cryogenic separation facility at ITE Merlewood might not be as accurate or consistent as careful manual separation was mentioned above. Although it would not have been possible to perform the relatively large number of $\delta^{13}C$ analyses in this project (155) without this facility, the variability inherent in this system can be seen in the $\delta^{13}C$ values from the cellulose standards introduced to each batch of samples. This standard was kindly provided by Dr. V. R. Switsur, Godwin Laboratory, University of Cambridge, and is produced by Sigma. The $\delta^{13}C$ value of this cellulose is known to be -23.4‰. The $\delta^{13}C$ values of this standard calculated as part of the three batches of samples sent to the automated line at ITE Merlewood are

-23.6, -23.44, -23.37, -23.22, -23.19, -23.7, -23.22 and -23.55‰.

This yields a mean of -23.41±0.19‰. The mean value is therefore accurate, but the standard deviation is relatively large for stable isotope analyses; for example the $\delta^{13}C$ analyses performed manually in Miami (below) have a typical standard deviation of 0.02‰. Consequently any differences in average $\delta^{13}C$ of less than 0.4‰ from plants of the same species occurring in different zones at Kentra should not necessarily be

considered to show a significant difference in carbon isotope composition. This is an important consideration, and reinforces the use of multiple replicates from each batch of cellulose produced. However, variation in the $\delta^{13}C$ values derived from the same batch of cellulose should not necessarily be assigned to measurement error since intra-zone variability in C uptake and assimilation may mean that there is natural variability within the pooled cellulose. This will be tested by comparing $\delta^{13}C$ values from individual *Armeria* plants with those from pooled material.

4.5.4.1 Results from Kentra Bay.

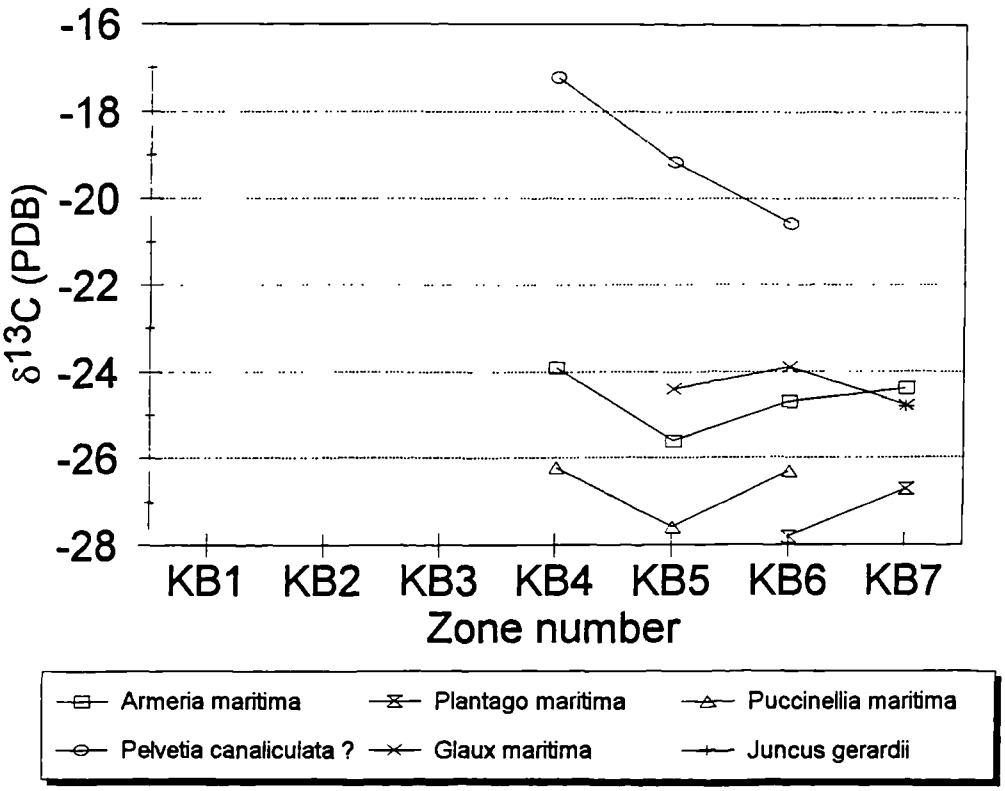
The results of the analyses of the contemporary C isotope composition of the flora in zones KB4 to KB7 is given in Appendix 19, and shown in Figure 4.6. The first amd most obvious conclusion to be drawn from these data is that the $\delta^{13}C$ values from the alga are considerably less negative than those from the higher plants. In addition, the pattern of the $\delta^{13}C$ values from *Pelvetia* follows the predicted pattern (observed in other coastal algae by Surif and Raven 1990) of becoming increasingly negative as the influence of the tide decreases.

The second conclusion to be drawn from these data is that a significant species effect is apparent amongst the results from the higher plants. A range of nearly 4‰ is present between the mean values from the higher plants in zone KB6. This is very significant for this project since it is combined with intra-specific patterns of $\delta^{13}C$ variation between the zones which do not fit the simple predicted relationship of increasing discrimination against ^{13}C with decreasing inundation and salinity.

This may be a reflection of the relatively high direct fresh water input to these communities from precipitation. The higher plants on the saltmarsh may be able to restrict

Figure 4.6

Kentra contemporary mean $\delta^{13}C$ (PDB)



photosynthesis to periods when local soilwater is low in marine-derived salts. If this is the case, the extent of carbon isotope discrimination is not a product of the incidence of tidal inundation and therefore the $\delta^{13}C$ values of the higher plants will not fall in to an increasingly negative pattern with decreasing marine influence. From this point of view it would perhaps be more appropriate to analyse plants from a saltmarsh where precipitation is lower, perhaps on the Norfolk or Lincolnshire coast.

If the $\delta^{13}C$ values of the cellulose from the sediment cores from Kentra Moss are derived solely from higher plants as represented in zones KB4 to KB7, it will be impossible to distinguish one zone from another using this technique. However, the presence of the fucoid casts a different light on the data. If it made a contribution to the bulk organic material deposited in each zone that it inhabited, and if this species has been consistently present in the communities at Kentra, then the $\delta^{13}C$ values of the lower 2 or 3 sedimentary units containing organic material in the sequence below Kentra Moss should be considerably less negative than those further up the organic sequence.

It is important to note that Figure 4.6 shows only the mean value of the six sub-samples from each batch of cellulose. The extent of the variation within these samples is clearly shown in Appendix 19, and, of the species analysed, the results from *Armeria* have particularly high standard deviations about the mean (up to ±0.73‰). To establish whether this is a product of natural variability in the sample or analytical inconsistency, samples of individual plants of *Armeria* were analysed in Miami using the manual separation technique outlined in Appendix 4. Three plants from each zone were analysed from material collected at two different dates, making a total of 24 analyses. All of the data given in Appendix 19 and Figure 4.5 are from material collected on the same day (30/10/93). In Miami, *Armeria* plants collected on this day and 8/7/94 were analysed. The

results are given in Table 4.2. The average standard deviation from these results is 0.018‰.

Table 4.2 $\delta^{13}C$ values from individual *Armeria* plants sampled on two separate dates; seasonal comparisons and intra-zone variations.

Zone	$\delta^{13}C$ values, 30/10/93	$\delta^{13}C$ values, 8/7/94
KB4	-24.51, -24.00, -24.00	-24.35, -24.68, -24.78
KB5	-25.65, -25.47, -25.40	-27.15, -26.94, -26.91
KB6	-24.56, -24.97, -25.02	-27.17, -26.88, -27.25
KB7	-25.66, -25.81, -25.53	-25.52, -25.71, -26.02

These data point to two conclusions. Firstly, *Armeria* can demonstrate considerable intra-community variation in $\delta^{13}C$ (up to 0.51‰), although the extent of this variation is generally in the range 0.2 to 0.3‰. Secondly, there is a considerable difference between the $\delta^{13}C$ of the samples from KB5 and KB6 taken on 30/10/93 and 8/7/94. In both cases the $\delta^{13}C$ values of 8/7/94 show significantly greater discrimination against ^{13}C . Seasonal variation in $\delta^{13}C$ has been noted before (Smedley *et al.* 1991), and this has serious implications for this project since it suggests that sampling on only one occasion during the year does not produce a representative $\delta^{13}C$ value which can be used as a guideline for the $\delta^{13}C$ of organic material being contributed to the organic fraction of net sediment yield for each community.

Therefore, it is necessary to conclude that although some of the variability in the data presented in Appendix 19 may be a result of the analytical method adopted (as highlighted by the variation in the results from the standards), there is also some natural

intra-community variation in $\delta^{13}C$ values. Combined with the very different $\delta^{13}C$ values from the species analysed, the apparent lack of a consistent link between $\delta^{13}C$ and environment, and the seasonal variations in $\delta^{13}C$ recorded in *Armeria*, the probability that cellulose produced by the higher plants in adjacent saltmarsh communities will have discrete $\delta^{13}C$ values appeared to be low. However, the hope that *Pelvetia* will be increasing the bulk cellulose $\delta^{13}C$ values in the lower saltmarsh zones, and especially the link between the limit of *Pelvetia* and MHWST in the contemporary saltmarsh, provided a sufficient stimulus to analyse the core material from Kentra Moss.

4.5.5 The $\delta^{13}C$ values of bulk cellulose taken from the two cores on Kentra Moss.

The location of cores Kentra Moss 21 (KM21) and Kentra Moss 39 (KM39) are given in Figure 4.1. The stratigraphy, microfossil, LOI% and ^{14}C data from these cores as recorded by Shennan *et al.* (1995) is summarised in Table 4.4 and Table 4.5 respectively. Also in these tables are the results of the $\delta^{13}C$ analyses of the cellulose extracted from the bulk organic material through the cores.

The stratigraphy follows the methodology of Troels-Smith (1955) and clearly shows the shift down the cores from organic-rich herbaceous peats (Th) into silts (Ag) and sands (Ga). This is reflected in the LOI% data (some of which is missing from KM39), which shows 95%+ organic material in the sediments at the top of the sequences analysed, but only 2-3% at the base of the cores.

In both cases the pollen and diatom microfossil evidence is consistent with an increasing marine influence down the cores, although the assemblages are not identical and do not reflect the contemporary assemblages found across the saltmarsh at Kentra. In core 21, *Plantago* is the dominant saltmarsh species represented in the pollen assemblages,

although *Armeria* is present in small quantities. These species are both present in the contemporary marsh flora. Pollen from *Juncus* , and other members of the Juncaceae, does not fossilize, and that from *Puccinellia* would be grouped with Gramineae, and therefore neither would be distinguished in the assemblage. Similarly, the fucoid would not appear in a pollen assemblage diagram. Therefore although it is difficult to draw any direct links between the pollen data presented from this core and the contemporary saltmarsh assemblage analysed, there is at least no evidence of "rogue" species present in the microfossil record but not on the contemporary marsh.

The diatom assemblage in the two cores is typical of a shift from vegetated saltmarsh to sand flat (Kosugi 1989), showing an increase in halophytes down the core. However, there is no foraminiferan assemblage presented for either KM21 or KM39 to compare with the contemporary assemblages recorded above. This is because no foraminifera tests are preserved in the sediments in either of the two cores (Haslett 1994). This is likely to be a result of post-depositional dissolution of the calcite (possibly caused by oxidation of the sediments at some stage, Hemond & Chen 1990) which is the basis of these tests, and this absence means that a potentially very useful indicator for testing the efficacy of $\delta^{13}C$ - and also establishing the nature of the environment of deposition of the sediments being investigated - is not available.

However, the pollen evidence in KM39 also supports the general picture of a transition from a fully terrestrial assemblage, typified by *Calluna vulgaris* and *Corylus avellana* to a saltmarsh flora of *Plantago* and *Armeria* . In the diatom assemblage, the appearance of large numbers of *Paralia sulcata* below 256cm depth demonstrates the increasingly marine-dominated nature of the lower sediment units.

Table 4.3 Kentra Moss core 21 data; Troels-Smith stratigraphy, loss on ignition (%), summary of microfossil evidence and radiocarbon dates (Shennan *et al.*, 1995 and unpublished data) with stable carbon isotope values of sub-fossil cellulose.

depth(cm.)	stratigraphy	L.O.I. (%)	microfossil evidence	^{14}C dates	$\delta^{13}C$ values
88		97.2	Consistently high Gramineae		
89			with increasing Cyperaceae		
90		97.3	& decreasing *Calluna*		
91	$Th^3 2$		*vulgaris* with depth.		
92	$Th(Er)^2 1$	97.2			
93	Sh1				
94		97.4	*Plantago maritima*		
95			appearing at base of unit.		-27.42, -27.31, -27.57
96		87.6			
97					
98				1370±45 BP	
99		49.3	Pollen count dominated by	SRR 4723	-28.37, -28.30
100		38.4	*Plantago maritima* with		
101			*Calluna vulgaris* at		-28.59,-28.44
102	$Th^3 2$	56.6	negligible levels and		
103	Sh2		Cyperaceae falling with		-28.65, -29.06
104		60.6	increasing depth.		
105			Brackish diatoms		-29.02, -28.51
106			dominating assemblage.	1480±40 BP	-28.87, -28.92
107			*P.maritima* still dominant,	SRR 4724	
108	Ag2		with small amounts of		-28.95, -28.84
109	$Th^3 1$		*Armeria maritima*.With		-28.73, -29.06
110	Sh1		depth marine-brackish		
111			species of diatoms are		-27.98
112		25.6	increasingly important.		
113			Very similar pollen		-27.48
114		17.7	assemblage to unit above;		
115			*P.maritima* peaking at 60%		-27.76
116		11.7	total land pollen at 119cm.		
117	Ag3				-27.61
118	Ga1	8.0	*Diploneis didyma* replacing		
119	Sh+		*D.interrupta* as dominant		-27.99
120		6.7	diatom frustule. Increasing		
121			proportion of halophytic		-28.09
122		6.5	species in total with depth.		
123					-27.76
124		5.2			
125					-27.64
126	Ga3	4.5	Diatom assemblage made		
127	Ag1		up of circa 40%		-27.13, -26.90
128	Sh+	3.4	halophytic ecotype forms.		
129					
130		2.9			
131					
132	Ag4		No microfossil evidence.		

Table 4.4 Kentra Moss core 39 data; Troels-Smith stratigraphy, loss on ignition (%), summary of microfossil evidence and radiocarbon dates (Shennan *et al.*, 1995 and unpublished data) with stable carbon isotope values of sub-fossil cellulose.

depth(cm.)	stratigraphy	L.O.I. (%)	microfossil evidence	^{14}C dates	$\delta^{13}C$ values
219		95.9			
220		97.7			
221			Gramineae, *Calluna vulgaris*		
222		98.2	and *Corylus avellana*		
223			dominate the pollen		
224		98.5	assemblage, each comprising		
225			>20% of total land pollen.		
226	Th(Er)^{3}4	98.0			-27.95, -27.48, -27.87
227					-27.84, -28.33, -28.21
228		98.2			
229				3435±45 BP	
230		97.9		SRR 4730	
231					
232		97.3			
233					
234		?			-28.07
235					
236		?	*Calluna vulgaris* and		
237			*Corylus avellana* fall away		
238		?	with increasing depth.		-29.47, -29.39, -29.23
239			Initially replaced by		-29.12, -29.24, -29.19
240		?	Cyperaceae and then by		
241	Sh3		*Plantago maritima* with		
242	Ag1	?	some *Aster*-type.		-28.07
243					
244		?	Diatom assemblage		
245			dominated (>80%) by		
246		?	frustules of brackish		-28.55, -28.04
247			water ecotypes.		
248		?		3940±45 BP	-26.91
249				SRR 4731	

continued overleaf...

depth(cm.)	stratigraphy	L.O.I. (%)	microfossil evidence	^{14}C dates	$\delta^{13}C$ values
250		?	*P.maritima* remains		
251			dominant, with *Armeria*		
252	As2	?	*maritima* inceasing.		-27.85, -27.93, -27.98
253	Ag2		Marine-brackish diatom		
254		?	species rising to 60%		-26.02
255			of total valves at 258 cm.		
256		21.9	*Diploneis didyma, D.smithii*		
257	Ga3		and *Paralia sulcata* each		
258	Ag1	19.7	accounting for >20% of the		-27.94, -27.83
259			diatom assemblage.		
260		9.9			
261					
262		5.7			-27.81
263			*Paralia sulcata* peaking		
264		9.6	at circa 40% of total valves		
265			at 262 cm. 80% of diatom		
266	Ga4	2.9	assemblage is brackish-		-27.73
267			marine, marine-brackish		
268		3.1	or marine.		
269					
270		3.9			
271					

The age difference between the two cores is significant. The two cores were deliberately chosen so that it would be possible to compare results from two different ages. It could equally be argued that two cores of the same age should have been chosen to establish the degree of consistency of this technique when atmospheric $\delta^{13}C$ is constant. However, limitations on the number of $\delta^{13}C$ analyses available restricted this, and such analyses are perhaps more appropriate if the technique shows any signs of being able to be applied in this field. The same problem of limitations on the number of $\delta^{13}C$ analyses that could be undertaken also meant that it was not possible to analyse the organic material in the contemporary saltmarsh sediments. This would obviously have been an ideal link between the $\delta^{13}C$ of the contemporary flora and the investigation of the sub-fossil units.

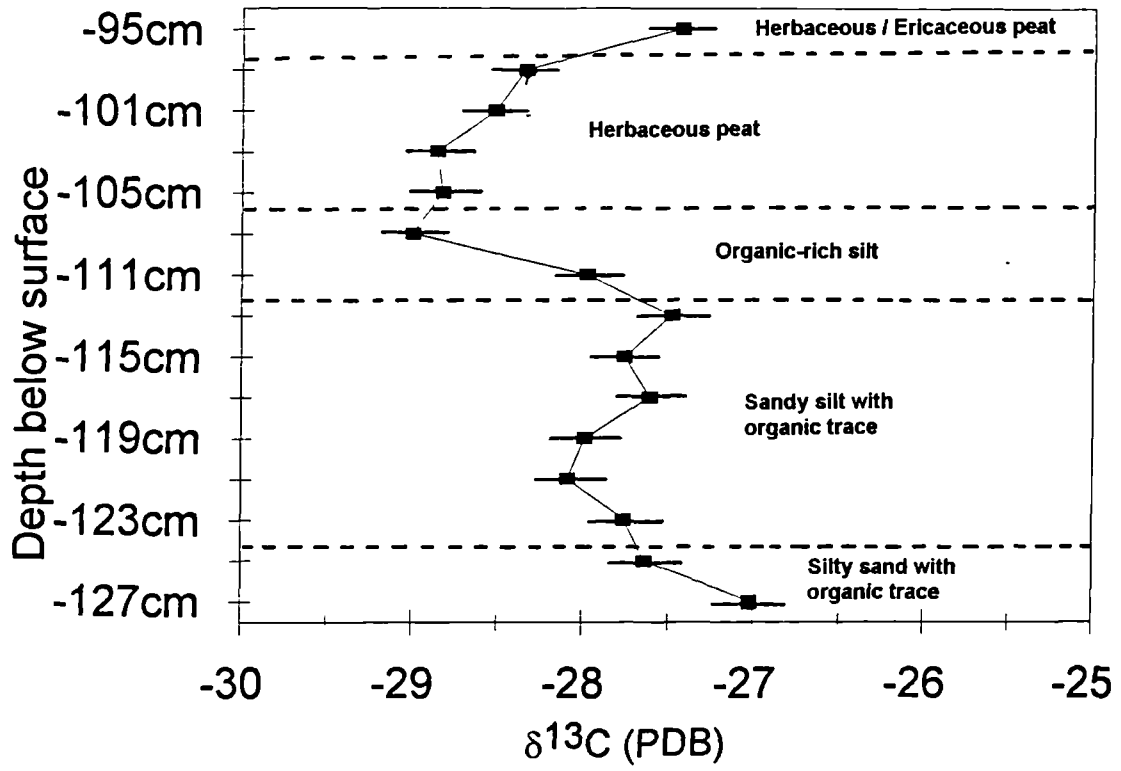
This decision is discussed because of the nature of the $\delta^{13}C$ data from the bulk cellulose which was analysed. Each result is shown at the depth of the sample analysed from the cores in Tables 4.3 and 4.4, and these data are shown in Figures 4.7 for KM21 and 4.8 for KM39. The error bar shown in Figures 4.7 and 4.8 is equal to the standard deviation of the analyses on the cellulose standard (0.19‰, Chapter 4.5.4). It is immediately apparent that the $\delta^{13}C$ value of the bulk cellulose in both cases does not reflect the values from the contemporary analyses. The $\delta^{13}C$ values in the cores are generally more negative than those that would be expected from looking at the contemporary data. This suggests that the contribution to the sediments analysed from the fucoid found in the contemporary succession is either nil or negligible. It was hoped that the $\delta^{13}C$ values from the sediments formed below contemporaneous MHWST would reflect the relatively high $\delta^{13}C$ values of the fucoid, but this does not seem to occur.

Further, it is not possible to assign particular $\delta^{13}C$ values to particular types of deposit. For example, in both cases the $\delta^{13}C$ values at the top of the sequence analysed are statistically identical to the values at the bottom of the sequence. The sediments analysed in both cases are herbaceous and ericaceous peats with over 95% organic matter content at the top of the sequence, and silts and sands with less than 10% organic matter content at the base. These lithostratigraphic changes are mirrored by the biostratigraphic changes from terrestrial to intertidal vegetation communities revealed by the microfossil analyses of Shennan *et al.* (1995). This suggests that microfossil assemblages can reveal more about the environment of deposition of coastal sediment sequences than carbon isotope analyses.

However, there are some variations in the $\delta^{13}C$ values from the cores which are statistically significant, most notably the c.2.5‰ variation between 238cm and 248cm depth in core 39B. The trend towards less negative values with depth through this unit is

Figure 4.7

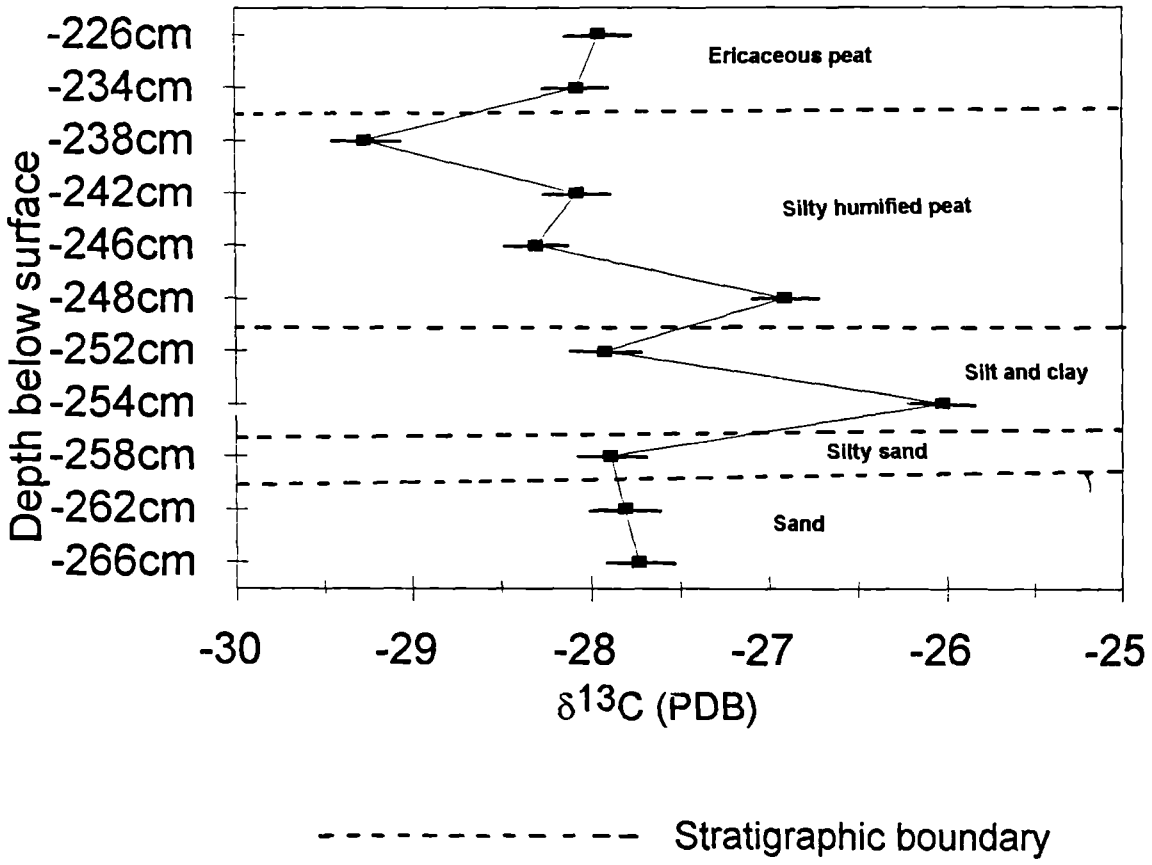
Kentra Moss core 21C mean $\delta^{13}C$ (PDB) and stratigraphy



The composition of the stratigraphic units is qualitatively described. See Table 4.3 for description using the Troels-Smith (1955) classification scheme.

Figure 4.8

Kentra Moss core 39B mean $\delta^{13}C$ (PDB) and stratigraphy



The composition of the stratigraphic units is qualitatively described. See Table 4.4 for description using the Troels-Smith (1955) classification scheme.

matched with a change in the pollen assemblage from fully terrestrial species to saltmarsh species (Table 4.4). The lithostratigraphy does not record this change, but it is noted in the microfossil assemblages. Such a change might be related to increasing marine influence if the rise in $\delta^{13}C$ is a function of either increased water use efficiency (and hence less discrimination against ^{13}C) by the plants contributing to the organic matter, or a small contribution of organic material from algae with a much higher $\delta^{13}C$ value. However, it may also represent the change in the species composition of the communities contributing to the organic matter content of the sediments analysed. It is not possible to distinguish between these possible causes on the basis of the information available.

The only comparable change in the $\delta^{13}C$ values from core 21C takes place between 108cm and 113cm depth. This increase in $\delta^{13}C$ is of lower magnitude (c.1.5‰) and occurs more than 10cm below the equivalent change in the pollen assemblage which is associated with the increase in $\delta^{13}C$ core 39B. Further, the lithostratigraphy is different in the two cores at the point at which the $\delta^{13}C$ values become more negative (in core 21C there is 50-75% silt, in core 39B this figure is only 25%). However, it may be that the same process caused the increase in $\delta^{13}C$ in both cores, and that this process is not related to either the proportion of inorganic material in the sediment or the pollen assemblage at this depth.

If the same process is influencing the carbon isotope composition of the two cores at the depths discussed above, it is not clear that the $\delta^{13}C$ values in both cores below these changes are controlled by the same processes. The highest $\delta^{13}C$ values in both sequences are found towards the bottom of the cores, but these values are nearly 1‰ different and are found in different lithostratigraphic units. Further, the drop in LOI% in core 39B from c.20% to c.3% (the range of values from the contemporary zones analysed) between 256 and 266 cm coincides with no statistical change in the $\delta^{13}C$ values,

but in core 21C the same change in the organic matter content of the sediment (between 112cm - 126cm depth) is associated with a c.1‰ increase in $\delta^{13}C$.

It is possible that the variations in the $\delta^{13}C$ data are a function of some process which is not idenifiable from the other litho- and bio-stratigraphic markers in these cores. However, if the reason for these changes can not be determined, it is impossible to assign a meaning to the changes seen in the cores. Alternative applications for the technique adopted in this study are discussed in Chapter 5.2. Possible reasons why it does not seem possible to consistently associate the changes in carbon isotope composition noted in these cores with any of the other information derived from the cores, or from the $\delta^{13}C$ values from the contemporary flora, are discussed below.

4.6 Discussion

There are a number of possible reasons for the apparent lack of consistent information from the carbon isotope analyses of the bulk organic matter in the cores investigated. Firstly the cellulose analysed from the cores may have been derived from different species than were investigated in the contemporary analyses (see Brenninkmeijer *et al.* 1982). The pollen assemblages from both cores do not contain evidence of any species which are not present in the contemporary saltmarsh vegetation communities. However, the pollen assemblage cannot be used to determine whether the fucoid was present in either of the vegetation successions which contributed to the sediment in the cores analysed. Further, the different distributional ranges of pollen from the various species in the assemblages may mean that although pollen from a particular species (for example *Plantago*) is present in an horizon of the sediment core, this species may not necessarily have contributed to the organic sediment fraction at that same level.

It is very difficult, from the data available, to determine the composition of the vegetation communities which contributed to the core material analysed. Even if every species present could be identified, it is not possible to determine the relative importance of the contribution of each species to the bulk organic fraction of the sediment. Although there is no evidence that species other than those found on the saltmarsh today were present in the vegetation successions in either core, there is not sufficient evidence to prove that every species which is present today was also present in the past. If this is the case, and most notably if the relative importance of the fucoid in the communities below contemporaneous MHWST was lower than it is today, it might explain the difference between the contemporary and the sub-fossil data.

In addition, there is the possibilty that although plants of the same species which are present in the contemporary vegetation succession contributed to the organic fraction of the sediment recovered in the two cores, these plants were genetically distinct from those examined in the contemporary flora. The extent to which genetic variation can impact on carbon isotope ratios is discussed in Chapter 4.4.5. The circa 2‰ difference between the contemporary and the ancient cellulose in this study (assuming that the ancient cellulose was produced by C_3 plants) is less than half of the variation noted by Kohorn *et al.* (1994) which they assign to genetic factors.

Another possibility is that there has been post-depositional modification of the organic material which has altered the $\delta^{13}C$ record (Aller 1994, Canfield 1994, DeNiro & Hastorf 1985, Macko *et al.* 1994, Meyers 1994). There is some evidence for this in coastal sequences, notably highlighted by the work of Chmura and others (Chmura 1988, Chmura & Aharon 1995, Chmura *et al.* 1987, Bird *et al.* 1993, DeLaune 1986, Pulich *et al.* 1985). The mechanism by which the $\delta^{13}C$ value of cellulose changes over time has yet to be explained, but in situations where the sediments are buried under anaerobic

conditions it is likely that any changes occur before burial when microbial action is high. Changes in the carbon isotope composition of the cellulose may be a function of intra-molecular variations in $\delta^{13}C$ values (as found by Schmidt and co-workers, see Chapter 2) and the preferential denudation of particular parts of the cellulose molecule. Alternatively the different nature of the locations in which cellulose is found in the different plant species may make some cellulose more prone to degradation. For example, cellulose from non-woody plants where no lignin is present may be more subject to decay, and cellulose from algae may be the most vulnerable.

These suggestions require further analysis using cellulose from a range of plant species and a variety of parts of the plants in order to establish the extent to which post-depositional transformation of $\delta^{13}C$ values takes place. If it is found that certain parts of the cellulose molecule are subject to decay or that cellulose from certain species or from particular parts of the same plant is under-represented in sub-fossil deposits, studies which utilise bulk organic matter from sediment sequences will have to become much more focused on utilising particular identifiable remains. This might take the form of only analysing material from identified macrofossils (see for example the approach adopted by White *et al.* 1994) if the problem relates to species-specific differences in preservation, or attempting to isolate particular parts of the cellulose molecule if intra-molecular variations are the basis of differences between present and ancient $\delta^{13}C$ values. The rationale behind examining particular parts of the molecule is that certain carbon atoms (notably carbon 1) in the molecule are known to follow a particular route through the Calvin cycle (Pearson 1995, pers. comm.). If the isotopic composition of the atoms at this site in the molecule can be determined in isolation, the extent of intra-molecular variations in preservation can be nullified.

The $\delta^{13}C$ values from ancient cellulose and contemporary cellulose produced by plants living under the same conditions will also vary because of the changes in the proportion and isotopic composition of CO_2 in the atmosphere caused by the burning of fossil fuels over the last 150-200 years. Since *circa* 1750 to 1988 the CO_2 content of the atmosphere increased from 280 μmol mol^{-1} to 348 μmol mol^{-1} and the isotopic composition of the CO_2 in the atmosphere changed from -6.4‰ to -7.9‰ (Penuelas & Azcon-Bieto 1992). On the basis of an examination of contemporary and herbarium material from 12 Mediterranean C_3 species, these authors state:

"In conclusion, carbon isotope discrimination has decreased in the last few decades. This implies that, according to models, stomatal conductance has declined and/or assimilation rates have increased" (*ibid*, p.488).

Penuelas & Azcon-Bieto found that the average $\delta^{13}C$ from these plants changed by c. 1.1‰ over the period 1750-1988. Similar changes may have occurred in all environments, but it is not possible to assign the data found in this study purely to changes in either p_a or the isotopic composition of the atmosphere. It may be that relative humidity or the amount of water available to the plants has changed and that water use efficiency has increased. This would also cause a decrease in the extent of carbon isotope discrimination. Although it is not possible to determine the actual impact of these factors on the plants analysed at Kentra, it is important to acknowledge the possibility that the isotopic differences between the contemporary and the ancient cellulose examined in this study may reflect natural or anthropogenic variations in climate or atmosphere over the last few thousand years.

4.7 Summary

The $\delta^{13}C$ data from the contemporary saltmarsh presented in this chapter show that the extent of intra- and inter-zone variability is such that differences in sub-fossil $\delta^{13}C$ from buried saltmarsh successions are only likely if particular (non-C_3) indicator species are present. This variability suggests that the links between salinity and inundation and those aspects of plant physiology which control carbon isotope discrimination (Pearcy & Ustin 1984, Farquhar *et al.* 1982, DeJong *et al.* 1982, Cooper 1982), which are exemplified - albeit in a C_4 plant by the work of Bowman & Strain (1988a, b) - are not the controlling influence on $\delta^{13}C$ values at Kentra. This suggests that the mechanisms by which plants at Kentra cope with the conditions on the saltmarsh are not necessarily linked to the processes influencing discrimination against ^{13}C during C uptake and assimilation described in other studies of coastal vegetation (Smith & Epstein 1970, Pezeshki *et al.* 1987b, Tiku 1976, Vernon *et al.* 1993, Winter 1974, Siddiqui *et al.* 1994). Alternatively, the plants may be able to take advantage of periods when the input of fresh water from precipitation is high, and they do not have to cope with high soilwater salinity levels. This may explain why the pattern of $\delta^{13}C$ values from certain higher plant species does not become less negative with increasing tidal influence, but the values from the fucoid (which relies on sea water for its survival) do follow this theoretically-predicted trend.

There may also be genetic variations within the populations examined which cause significant intra and inter-zone differences in $\delta^{13}C$ (Gray 1987; see also Glenn's (1987) analysis of *Atriplex* spp. and Huiskes *et al.*s 1985 analysis of variation in *Aster tripolium* L. populations). Without a separate examination of the behaviour of material transplanted from each zone under controlled conditions it is not possible to isolate any one or a combination of these factors as the principal control on the $\delta^{13}C$ values from the contemporary saltmarsh.

The $\delta^{13}C$ values from the higher plants are not related to the values of stomatal density, and neither of these two data sets suggested that it would be possible to utilise them as part of an environmental reconstruction from the organic material in the sediment cores. However, the presence of *Pelvetia* in zones KB4 - KB6 did offer a chance of defining those units at or below MHWST if the higher $\delta^{13}C$ of this alga was recorded in the subfossil bulk cellulose $\delta^{13}C$. Unfortunately this proved not to be the case, and, as with other analyses of bulk plant cellulose where only C_3 plants have contributed to the organic sediment fraction, it has been shown that this technique is often severely affected by the lack of control on past plant assemblages.

Comparisons between the microfossil record and the carbon isotope ratios from the two cores analysed suggests that the $\delta^{13}C$ analyses can not elucidate any more palaeoenvironmental information than the microfossil analyses. Further, the carbon isotope ratios of the organic material at opposite ends of the two sequences analysed were indistinguishable, and it therefore appears that this technique should not be used in isolation.

Chapter 5.

Conclusions and Implications.

5.1 Conclusions from the $\delta^{13}C$ and $\delta^{18}O$ data.

5.1.1 Data from the *Phragmites* project at Roudsea.

The isotope data, both $\delta^{13}C$ and $\delta^{18}O$, from the *Phragmites* communities at Roudsea do not fall into consistently different groups according to the environment of growth. The $\delta^{13}C$ values do not follow the theoretically predicted pattern that discrimination against ^{13}C will decrease as stress - such as inundation and salinity - causes water efficiency to rise and stomatal conductance to fall. In addition, the range of carbon isotope ratios in the leaf cellulose was greatest in zone 3 (lowest measured variation in salinity), and smallest in zone 1 (highest measured variation in salinity).

The $\delta^{18}O$ values of soilwater taken from the rhizosphere suggested that the water available to the plants in each zone on the day of sampling was isotopically distinct. However, the range of $\delta^{18}O$ values from the cellulose of the leaves of plants in zone 3, where no inundation by the tide took place, was greater than that from plants living in zone 1, which is subject to direct inundation by the tide.

It is possible to explain both of these data sets if the factor controlling photosynthesis is the availability of fresh water rather than the number of times each zone is inundated by sea water. If the plants are only photosynthesizing when they are able to utilise fresh water, the range of humidity and light conditions (which can influence both the extent of stomatal conductance and the level of evaporation from the leaf surface - and hence $\delta^{13}C$ and $\delta^{18}O$ values) under which plants in zone 3 can operate will be larger than

for plants in zone 1 which have to rely on inputs of precipitation when the incidence of tidal flooding is low (during neap tides). The level of soilwater salinity in zone 1 was shown to vary over the growing season, and the plants in zone 1 are considerably smaller than those in zones 2 and 3 where the incidence of flooding and the level of salinity is lower. This suggests that in this case the *Phragmites* plants are not having to utilise sea water at all, but can rely on supplies of fresh water because they do not extend beyond the point at which soilwater is solely influenced by sea water inputs.

There are a number of other possible explanations for the pattern of isotope ratios in the leaves analysed. The plants may be inter-connected via an extended rhizome network to plants in different areas, and water and metabolites may be moving from one area to another. Analysis of stem and root water would be necessary to establish the rate and direction of any of these flows.

Genetic variations between *Phragmites* populations inhabiting different environments have been noted (Zong *et al.* 1991), and if such differences are present at Roudsea they may have had an impact on the extent of carbon and oxygen isotope discrimination. Genetic sequencing would be necessary to establish whether this exists, and if so a series of controlled experiments growing transplanted material from each population under different salinity and inundation conditions would be able to determine whether the genetic factor is important in carbon and oxygen isotope discrimination.

5.1.2 Data from the study of contemporary and ancient material at Kentra.

In the project at Kentra the contemporary $\delta^{13}C$ patterns vary according to species. In some species the $\delta^{13}C$ values follow the theoretically-predicted trend of less negative values as salinity and inundation frequency increase, most notably in the turf fucoid

Pelvetia. However, other species do not seem to fit with the theory, and the $\delta^{13}C$ values appear to be controlled by other factors. Similar patterns are apparent in the stomatal density data, where some species show a response to inundation frequency in this aspect of their physiognomy. It is possible that, as with the *Phragmites* in zone 1 at Roudsea, there is sufficient input of freshwater to the saltmarsh for the higher plants to be able to exploit this as a water source. If this is the case, the variation in $\delta^{13}C$ values may be a function of factors other than the incidence of tidal inundation, for example local light and humidity conditions or the impact of grazing on the marsh.

The most significant aspect of the carbon isotope ratios from the bulk organic material in the two cores analysed is that in both cases they are more negative than would be expected if they simply reflected the values from the contemporary analyses. This might be because of differences in floristic composition, or might be the result of post-depositional modification of the $\delta^{13}C$ of cellulose. There is some evidence for the latter in other studies which have compared contemporary and sub-fossil cellulose from what are believed to be analogous communities and buried units respectively. Again, there does not seem to be a complete explanation of why this might happen, and Gleixner (1995, pers. comm.) has found no significant variations in cellulose of different ages that he has examined.

The variations which do exist in the values from the cores are not associated with either a particular biostratigraphic or lithostratigraphic change. Although the reason for examining the $\delta^{13}C$ values of the core material was to determine whether any new information was revealed by these analyses, these data do not appear to distinguish between basic changes in the sediment type being examined. It is possible that the change which does occur from more negative to less negative values in the centre of the core sections analysed is caused by some factor which has previously gone unnoticed.

However, the differences in sediment type and pollen assemblage at the levels where this takes place suggests that the controlling influence is not related to either a botanical or sedimentological change.

5.2 Implications of these results.

The possible rôle of genotypic variation in determining these variations should be assessed, but given the level of detail that can be routinely re-created using methods presently in widespread use in investigations of sea-level (notably microfossil analysis) it is difficult to see this type of analysis having a viable part to play in such studies. Such an investigation would involve an analysis of vegetation succession and the variations in genetic structure that are associated with changing saltmarsh vegetation communities. The information that such a project could produce would be very useful in trying to understand the data from Kentra and would be invaluable to research on the processes of colonisation and succession on saltmarshes.

The water and carbon balance of *Phragmites* is also a field that seems to be inadequately understood in terms of the implications for $\delta^{13}C$ and $\delta^{18}O$. There appears to be little or no relationship between the $\delta^{13}C$ and the $\delta^{18}O$ data from the *Phragmites* communities at Roudsea. If an environmental factor was controlling stomatal conductance - and hence altering p_i and therefore $\delta^{13}C$ if the Farquhar *et al.* (1989) model is correct - this would also alter the value of $\delta^{18}O$ by impacting on the extent of isotopic enrichment of ^{18}O in the leaf water through a change in evaporation. This link between the two isotopic ratios through water use efficiency does not appear to exist in the *Phragmites* data, which seems to imply that stomatal conductance is not the only factor controlling $\delta^{13}C$.

By varying each possible factor (age, genotype, salinity, water $\delta^{18}O$, inundation frequency, depth of rhizome, humidity and incident light) whilst maintaining the others in a series of controlled experiments it might be possible to establish the mechanism(s) controlling cellulose $\delta^{13}C$ and $\delta^{18}O$. This would reveal a great deal about the factors which control isotopic discrimination in *Phragmites*, and would determine whether there is any chance of utilising macrofossils from this species for environmental reconstruction in other environments, for example from lacustrine deposits.

Further investigation is also necessary to determine the exact relationship between stomatal density and isotopic discrimination. The stomatal density variations in the species examined in these projects suggest that there is not necessarily a link between environment and this particular aspect of plant physiognomy. This is presently being examined in greater depth (Metcalf 1995 pers. comm.), as are variations in stomatal size and index.

Post-depositional modification of $\delta^{13}C$ values requires systematic examination to determine the importance of this factor in comparisons of contemporary and ancient isotopic ratios. Analysis under a range of different microbial and atmospheric conditions involving different plant species and different parts of the same plants would be necessary to determine the differences between pristine and sub-fossil cellulose. Related to this is the extent of intra-molecular variation in $\delta^{13}C$ and $\delta^{18}O$ in cellulose. This is presently being examined at the Technical University in Munich by Schmidt, Gleixner and co-workers (1995 pers. comm.) and Sternberg (1995 pers. comm.) is about to start work in Miami on isolating particular C and O atoms in the cellulose molecule. Similarly, Switsur (1995 pers. comm.) has mentioned an interest in investigating this with the eventual aim of incorporating it into the important contemporary analyses of C, H and O isotopes in pine and oak tree-rings that he and his co-workers are undertaking.

Changes in the molecular abundance and isotopic composition of atmospheric CO_2 over the last two centuries has changed the $\delta^{13}C$ values of some plants growing in the same place over this period, and this must be taken in to account when comparing contemporary and ancient material. It is also important to acknowledge that there is considerable intra-community variation in $\delta^{13}C$ in many species, and that any analyses of ancient material should also include an analysis of the variation found in apparently homogeneous contemporary populations.

All of the points discussed above are applicable to other studies wishing to adopt isotopic analysis as a tool for environmental reconstruction. However, the choice of field sites in these projects may have been an important factor in determining the patterns of both $\delta^{13}C$ and $\delta^{18}O$. At both sites it may be that there is sufficient fresh water available for the plants to be able to avoid utilising sea water. In the case of the $\delta^{18}O$ of *Phragmites* in zone 1 at Roudsea this means that it does not utilise the isotopically distinct sea water. Similarly, in both sets of contemporary $\delta^{13}C$ analyses the extent of discrimination against ^{13}C is not a function of stomatal conductance being restricted by constantly saline soilwater since the input of fresh water from precipitation is relatively high.

Consequently, it might be that the pattern of isotopic values which these studies was hoping to find will only exist on saltmarshes in areas with less precipitation than the west coast of Scotland or in those species which extend further down the intertidal vegetation succession than *Phragmites*.

All of the higher plant species analysed in these projects utilise the C_3 metabolic pathway, but *Spartina* x *townsendii* is a C_4 plant which is increasing its range around the saltmarshes of the U.K. The much higher $\delta^{13}C$ values associated with plants which dominantly use PEP carboxylase rather than Rubisco was discussed in Chapter 2, and it

may be possible to utilise this information to trace recent changes in saltmarshes. If this plant is to prove useful in such studies it is necessary to determine the relationship between it and local tidal levels. At Roudsea this species occurs in sparse groups on the mudflat beyond the limit of other saltmarsh plants, and also close to the *Phragmites* community in zone 1. Consequently it may prove difficult to relate organic material identified as coming from *Spartina* x *townsendii* by its high $\delta^{13}C$ value to a particular tidal level. However, if the date at which this species invaded the marsh is known, the reconstruction of the recent history of the marsh may be possible if the lowest horizon containing material with a $\delta^{13}C$ value which resembles that of a C_4 plant can be found. If this is combined with an accurate method of dating recent saltmarsh sediments, perhaps utilising radioactive isotopes from nuclear fall-out or pollution events, or even ^{210}Pb dating if the sources of sediment to the marsh are known (Oldfield 1991, pers. comm.), it could prove a useful tool in understanding the history of some U.K. saltmarshes over the past 30-40 years.

The $\delta^{13}C$ values from the alga in the study at Kentra were not reflected in the data from the buried sediment sequences. This might be because there was no fucoid present at the time of deposition, or it might be that cellulose from algae is more susceptible to microbial degradation. However, the higher carbon isotope values from algae may be useful in other studies. The initial stage of vegetation colonisation on mudflats is often in the form of algal mats (Adam 1990). Such mats can cover significant areas of intertidal flats in areas such as The Wash (Reid Thomas 1994, pers.comm.), and it may be possible to identify sediments from such areas by the $\delta^{13}C$ value of cellulose (or perhaps a different plant fraction if an appropriate one can be found) extracted from core material. If the extent of carbon isotope discrimination by the algae which form these mats can be related to the frequency of tidal inundation (as with *Pelvetia* in this study), it might also be possible to determine whether the core sequence represents a period of changing relative

sea level. This may reflect changes in sediment dynamics within the marsh, but it might also be possible to relate these sequences to regional patterns of isostatically or eustatically driven sea level change. If the sediment dynamics of intertidal mudflats could be determined for periods of known sea level fall and rise on the basis of the algal remains contained within them, this would prove a very useful tool for both coastal palaeoenvironmental reconstructions and predictions of future change under different relative sea level projections.

In conclusion, although neither of these projects have produced information that can be immediately applied to studies of coastal environmental change, both highlight different limitations to isotopic analysis which must be taken into account if this technique is going to be successfully applied in other palaeoenvironmental studies, and have also yielded some pointers towards possible future research.

References

Adam, P. 1976. Plant sociology and habitat factors in British saltmarshes. Unpublished Ph.D. Thesis, University of Cambridge.

Adam, P. 1978. Geographical variation in British saltmarsh vegetation. *Journal of Ecology*, **66**, 339-366.

Adam, P. 1981. The vegetation of British saltmarshes. *New Phytologist*, **88**:143-196.

Adam, P. 1990. Saltmarsh Ecology. Cambridge University Press, Cambridge.

Adams, T.D. and J. Haynes. 1965. Foraminifera in Holocene marsh cycle at Borth, Cardiganshire. *Paleontology*, **8**:27-38.

Aller, R.C. 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. *Chemical Geology*, **114**:331-345.

Allison, G.B., J.R. Gat and F.W.J. Leaney. 1985. The relationship between deuterium and oxygen-18 delta values in leaf water. *Chemical Geology*, **58**:145-156.

Anon. 1989. VG Isotech User Manual - Prism Series II Isotope Ratio Mass Spectrometer.

Aphalo, P.J. and P.G. Jarvis. 1993. The boundary layer and the apparent responses of stomatal conductance to wind speed and to the mole fractions of CO_2 and water vapour in the air. *Plant, Cell and Environment*, **16**:771-783.

Armstrong, J. and W. Armstrong. 1988. *Phragmites australis*: A preliminary study of soil-oxidizing and internal gas transport pathways. *New Phytologist*, **108**:373-382.

Armstrong, J. and W. Armstrong. 1990. Light-enhanced convective throughflow increases oxygenation in rhizomes and rhizosphere of *Phragmites australis* (Cav.) Trin. ex Steudal. *New Phytologist*, **114**:121-128.

Armstrong, J., W. Armstrong and P.M. Beckett. 1992. *Phragmites australis*: Venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizome oxidation. *New Phytologist*, **120**:192-207.

Aston, S.R. 1978. Estuarine Chemistry. **In:** R. Chester (ed.). *Chemical Oceanography*, Second edition. Academic Press, London.

Austin, M.P. 1990. Community theory and competition in vegetation. **In:** J.B. Grace and D. Tilman (eds.). *Perspectives on plant competition*. Academic Press, San Diego.

Babikir, A.A.A. and H. Kurschner. 1992. Vegetational patterns within a coastal saline of NE-Qatar. *Arabian Gulf Journal of Scientific Research*, **10**:61-75.

Bariac, T., E. Deleens, A. Gerbaud, M. Andre and A. Mariotti. 1991. La composition isotopique (^{18}O, ^{2}H) de la vapour d'eau transpiree: Etude en conditions asservies. *Geochimica et Cosmochimica Acta*, **55**:3391-3402.

Barnes, F.A. and C.A.M. King. 1953. The Lincolnshire coastline and the 1953 storm flood. *Geography*, **38**:141-160.

Baron, W.M.M. 1967. Physiological aspects of water and plant life. Heinemann Educational, London.

Bauer, W.D. 1977. Plant cell walls. **In:** H. Smith (ed.). *The molecular biology of plant cells*. Blackwell, Oxford.

Bazzaz, F.A. 1990. Plant-plant interactions in successional environments. **In:** J.B. Grace and D. Tilman (eds.). *Perspectives on plant competition*. Academic Press, San Diego.

Beerling, D.J. and W.G. Chaloner. 1993,a. The impact of atmospheric CO_2 and temperature change on stomatal density: Observations from *Quercus robur* lammas leaves. *Annals of Botany*, **71**:231-235.

Beerling, D.J. and W.G. Chaloner. 1993,b. Stomatal density responses of Egyptian *Olea europaea* L. leaves to CO_2 change since 1327BC. *Annals of Botany*, **71**:431-435.

Beerling, D.J., W.G. Chaloner, B. Huntley, A. Pearson and M.J. Tooley. 1991. Tracking stomatal densities through a glacial cycle: their significance for predicting the response of plants to changing atmospheric CO_2 concentrations. *Global Ecology and Biogeography Letters*, **1**:136-142.

Beerling, D.J., W.G. Chaloner, B. Huntley, J.A. Pearson and M.J. Tooley. 1993. Stomatal density responds to the glacial cycle of environmental change. *Proceedings of the Royal Society of London, B*, **25**:133-138.

Beerling, D.J., D.P. Mattey and W.G. Chaloner. 1993. Shifts in the $\delta^{13}C$ composition of *Salix herbacea* L. leaves in response to spatial and temporal gradients of atmospheric CO_2 concentration. *Proceedings of the Royal Society of London, B*, **253**:53-60.

Benedict, C.R., W.W.L. Wong and J.H.H. Wong. 1980. Fractionation of the stable isotopes of inorganic carbon by seagrasses. *Plant Physiology*, **65**:512-517.

Bernstein, L. and H.E. Hayward. 1958. Physiology of salt tolerance. *Annual Review of Plant Physiology*, **9**:25-46.

Berry, J.A. 1989. Studies of mechanisms affecting the fractionation of carbon isotopes in photosynthesis. **In:** P.W. Rundel, J.R. Ehleringer and K.A. Nagy (eds.). *Stable isotopes in ecological research*. Springer Verlag, Berlin.

Bertness, M.D. 1988. Peat accumulation and the success of marsh plants. *Ecology*, **69**:703-713.

Bertness, M.D. 1992. Salt tolerances and the distribution of fugitive saltmarsh plants. *Ecology*, **73**:1842-1851.

Bertness, M.D. and A.M. Ellison. 1987. Determinants of pattern in a New England saltmarsh plant community. *Ecological Monographs*, **57**:129-147.

Bird, M.I., P. Giresse and A.R. Chivas. 1993. $\delta^{13}C$ composition of sediments from the Sanaga River, Cameroon. *Chemical Geology*, **107**:211.

Boar, R.R., C.E. Crook and B. Moss. 1989. Regression of *Phragmites australis* reed swamps and recent changes of water chemistry in the Norfolk Broadland, England. *Aquatic Botany*, **35**:41-56.

Boomer, I. and M. Godwin. 1993. Palaeoenvironmental reconstruction of the Breydon Formation, Holocene of East Anglia. *Journal of Micropalaeontology*, **12**:35-46.

Bowman, S.D. and B.R. Strain. 1988,a. Response to long- and short-term salinity in populations of the C_4 nonhalophyte *Andropogon glomeratus* Walter BSP. *Oecologia*, **75**:73-77.

Bowman, S.D. and B.R. Strain. 1988,b. Physiological responses in two populations of *Andropogon glomeratus* Walter BSP to short term salinity. *Oecologia*, **75**:78-82.

Boyer, J.S. 1974. Water transport in plants: Mechanism of apparent changes in resistance during absorption. *Planta*, **117**:187-207.

Brenninkmeijer, C.A.M., B. Van Geel and W.G. Mook. 1982. Variations in the D/H and $^{18}O/^{16}O$ ratios in cellulose from a peat bog core. *Earth and Planetary Science Letters*, **61**:283-290.

Brereton, A.J. 1971. The structure of the species populations in the initial stages of salt marsh succession. *Journal of Ecology*, **59**:321-338.

Brix, H. 1988. Light-dependent variations in the composition of the internal atmosphere of *Phragmites australis* Trin. ex Steudal. *Aquatic Botany*, **30**:319-330.

Brix, H. 1989. Gas-exchange through the soil atmosphere interface and through dead culms of reed *Phragmites australis* (Cav.) Trin. ex Steudal. *Aquatic Botany*, **35**:81-98.

Brix, H. 1990. Uptake and photosynthetic utilization of sediment-derived carbon by *Phragmites australis* (Cav.) Trin. ex Steudal. *Aquatic Botany*, **38**:377-390.

Broadmeadow, M.S.J., H. Grifftihs, C. Maxwell and A.M. Borland. 1992. The carbon isotope ratio of plant organic material reflects temporal and spatial variations in CO_2 within tropical forest formations in Trinidad. *Oecologia*, **89**:435-441.

Brugnoli, E. and O. Bjorkman. 1992. Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. *Planta*, **187**:335-347.

Burk, R.L. and M. Stuiver. 1981. Oxygen isotope ratios in trees reflect mean annual temperature and humidity. *Science*, **211**:1417-1419.

Canfield, D.E. 1994. Factors influencing organic preservation in marine sediments. *Chemical Geology*, **114**:315-329.

Castonguay, Y., P. Nadeau and R.R. Simard. 1993. Effects of flooding on carbohydrate and ABA levels in roots and shoots of alfalfa. *Plant, Cell and Environment*, **16**:695-702.

Chapman, V.J. 1939. Studies in salt marsh ecology. Sections IV and V. *Journal of Ecology*, **27**:160-201.

Chatterton, N.J., P.A. Harrison, J.H. Bennett and K.H. Asay. 1989. Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. *Journal of Plant Physiology*, **134**:169-179.

Cheeseman, J.M. 1988. Mechanisms of salinity tolerance in plants. *Plant Physiology*, **87**:547-550.

Chmura, G.L. 1988. The use of stable carbon isotopes for identification of paleosalinities in buried coastal wetlands. American Quaternary Association programs and abstracts of the tenth biennial meeting 1988, University of Massachusetts, Amherst. .

Chmura, G.L. and P. Aharon. 1995. Stable carbon isotope signatures of sedimentary carbon in coastal wetlands as indicators of salinity regime. *Journal of Coastal Research*, **11**:124-135.

Chmura, G.L., P. Aharon, R.A. Socki and R. Abernethy. 1987. An inventory of ^{13}C abundances in coastal wetlands of Louisiana, USA: vegetation and sediments. *Oecologia*, **74**:264-271.

Cizkova-Koncalova, H., J. Kvet and K. Thompson. 1992. Carbon starvation: A key to reed decline in eutrophic lakes. *Aquatic Botany*, **43**:105-113.

Clarke, L.D. and N.J. Hannon. 1970. The mangrove swamp and saltmarsh communities of the Sydney district III. Plant growth in relation to salinity and waterlogging. *Journal of Ecology*, **58**:351-369.

Clough, B.F. and R.G. Sim. 1989. Changes in gas exchange characteristics and water-use efficiency of mangroves in response to salinity and vapour pressure deficit. *Oecologia*, **79**:38-44.

Collatz, G.J., J.A. Berry, G.D. Farquhar and J. Pierce. 1990. The relationship between the Rubisco reaction mechanism and models of photosynthesis. *Plant, Cell and Environment*, **13**:219-225.

Cooper, A. 1982. The effects of salinity and waterlogging on the growth and cation uptake of saltmarsh plants. *New Phytologist*, **90**:263-275.

Cooper, L.W. and M.J. Deniro. 1989,a. O-18 content of atmospheric oxygen does not affect the oxygen isotope relationship between environmental water and cellulose in a submerged aquatic plant, *Egeria densa* Planch. *Plant Physiology*, **91**:536-541.

Cooper, L.W. and M.J. DeNiro. 1989,b. Covariance of oxygen and hydrogen isotopic compositions in plant water: species effects. *Ecology*, **70**:1619-1628.

Cooper, L.W., M.J. Deniro and J.E. Keeley. 1991. The relationship between stable oxygen and hydrogen isotope ratios of water in astomatal plants. *Stable Isotope Geochemistry: A tribute to Samuel Epstein. Special Publication no.3*:247-255.

Coops, H., N. Geilen and G. Van der Velde. 1994. Distribution and growth of the halophytic species *Phragmites australis* and *Scirpus lacustris* in water depth gradients in relation to wave exposure. *Aquatic Botany*, **48**:273-284.

Coplen, T.B. 1995. Discontinuance of SMOW and PDB. *Nature*, **375**:285.

Coplen, T.B., I.J. Winograd, J.M. Landwehr and A.C. Riggs. 1994. 500,000-year stable carbon isotopic record from Devil's Hole, Nevada. *Science*, **263**:361-364.

Cowie, N.R., W.J. Sutherland, M.K.M. Dithlogo and R. James. 1992. The effects of conservation management of reed beds. II. The flora and litter disappearance. *Journal of Applied Ecology*, **29**:272-284.

Craig, H. 1953. The geochemistry of the stable isotopes. *Geochimica et Cosmochimica Acta*, **3**:53-92.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectromectric analysis of CO_2. *Geochimica et Cosmochimica Acta*, **12**:133-149.

Craig, H. 1961,a. Isotopic variations in meteoric waters. *Science*, **133**:1702-1703.

Craig, H. 1961,b. Standard for reporting concentrations of deuterium and oxygen-18 in natural waters. *Science*, **133**:1833-1834.

Culver, S.J. and F.T. Banner. 1978. Foraminiferal assemblages as Flandrian palaeoenvironmental indicators. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **24**:53-72.

Daniels, R.E. 1991. Variation in performance of *Phragmites australis* in experimental culture. *Aquatic Botany*, **42**:41-48.

Dansgaard, W. 1953. The abundance of ^{18}O in atmospheric water and water vapour. *Tellus*, **5**:461-469.

Dawson, T.E. and J.R. Ehleringer. 1991. Streamside trees that do not use stream water. *Nature*, **350**:335-337.

DeJong, T.M., B.G. Drake and R.W. Pearcy. 1982. Gas exchange responses of Chesapeake Bay tidal marsh species under field and laboratory conditions. *Oecologia*, **52**:5-11.

DeLaune, R.D. 1986. The use of $\delta^{13}C$ signature of C_3 and C_4 plants in determining past depositional environments in rapidly accreting marshes of the Mississippi River deltaic plain, Louisiana, USA. *Chemical Geology*, **59**:315-320.

DeLaune, R.D. and C.W. Lindau. 1987. $\delta^{13}C$ signature of organic carbon in estuarine bottom sediment as an indicator of carbon export from adjacent marshes. *Biogeochemistry*, **4**:225-230.

DeLaune, R.D., S.R. Pezeshki and J.H. Pardue. 1990. An oxidation-reduction buffer for evaluating the physiological resonse of plants to root oxygen stress. *Environmental and Experimental Botany*, **30**:243-248.

DeLaune, R.D., S.R. Pezeshki, J.H. Pardue, J.H. Whitcomb and W.H. Patrick. 1990. Some influences of sediment addition to a deteriorating saltmarsh in the Mississippi River deltaic plain: A pilot study. *Journal of Coastal Research*, **6**:181-188.

DeLaune, R.D., S.R. Pezeshki and W.H. Patrick. 1987. Response of coastal plants to an increase in submergence and salinity. *Journal of Coastal Research*, **3**:535-546.

DeNiro, M.J. 1981. The effects of different methods of preparing cellulose nitrate on the determination of D/H ratios of non-exchangeable hydrogen of cellulose. *Earth and Planetary Science Letters*, **54**:177-185.

DeNiro, M.J. and L.W. Cooper. 1989. Post-photosynthetic modification of oxygen isotope ratios of carbohydrates in the potato: Implications for palaeoclimatic reconstructions based upon isotopic ratios. *Geochimica et Cosmochimica Acta*, **53**:2573-2580.

DeNiro, M.J. and L.W. Cooper. 1990. Water is lost from leaves and trunks of trees by fundamentally different mechanisms. *Geochimica et Cosmochimica Acta*, **54**:1845-1846.

Deniro, M.J. and S. Epstein. 1979. Relationship between the oxygen isotope ratios of terrestrial plant cellulose, carbon dioxide and water. *Science*, **204**:51-53.

DeNiro, M.J. and N. Hastorf. 1985. Alteration of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of plant matter during the initial stages of diagenesis: Studies utilizing archaeological specimens from Peru. *Geochimica et Cosmochimica Acta*, **49**:97-115.

DeNiro, M.J., L.d.S.L. Sternberg, B.D. Marino and J.R. Druzik. 1988. Relation between D/H and $^{18}O/^{16}O$ ratios in cellulose from linen and maize - implications for paleoclimatology and sindonology. *Geochimica et Cosmochimica Acta*, **52**:2189-2196.

DeNiro, M.J., L.d.S.L. Sternberg, B.D. Marino and J.R. Druzik. 1990. Corrigendum to article by same authors in *Geochemica et Cosmochimica Acta* 52, 2189-2196. *Geochimica et Cosmochimica Acta*, **54**:1847.

Dewar, R.C. 1995. Integration of an empirical model for stomatal conductance in terms of guard cell function. *Plant, Cell and Environment*, **18**:365-372.

Dithlogo, M.K.M., R. James, B.R. Laurence and W.J. Sutherland. 1992. The effects of conservation management of reed beds I. The invertebrates. *Journal of Applied Ecology*, **29**:265-276.

Dreesman, D.C., C. Harn and J. Daie. 1994. Expression of genes encoding Rubisco in sugarbeet (*Beta vulgaris* L.) plants subjected to gradual dessication. *Plant and Cell Physiology*, **35**:645-653.

Duponey, J.-L., S. Leavitt, E. Choisnel and S. Jourdain. 1993. Modelling carbon isotope fractionation in tree rings based on effective evapotranspiration and soil water status. *Plant, Cell and Environment*, **16**:939-947.

Dzurec, R.S., T.W. Boutton, M.M. Caldwell and B.N. Smith. 1985. Carbon isotope ratios of soil organic matter and their use in assessing community composition changes in Curlew Valley, Utah. *Oecologia*, **66**:17-24.

Edwards, G. and D. Walker. 1983. C_3,C_4: mechanisms, and cellular and environmental regulation of photosynthesis. Blackwell, Oxford.

Edwards, T.W.D. 1990. New contributions to isotope dendroclimatology. Comment on DeNiro and Cooper (1989) *Geochimica et Cosmochimica Acta* 53, 2573-2580. *Geochimica et Cosmochimica Acta*, **54**:1843-1844.

Edwards, T.W.D., R.O. Aravena, P. Fritz and A.V. Morgan. 1985. Interpreting palaeoclimate from ^{18}O and ^{2}H in plant cellulose: comparison with evidence from fossil insects and relict permafrost. *Canadian Journal of Earth Science*, **22**:1720-1726.

Edwards, T.W.D., W.M. Buhay, R.J. Elgood and H.B. Jiang. 1994. An improved nickel-tube pyrolosis method for oxygen isotope analysis of organic matter and water. *Chemical Geology*, **114**:179-183.

Ehleringer, J.R. and T.A. Cooper. 1988. Correlations between carbon isotope ratio and microhabitat in desert plants. *Oecologia*, **76**:562-566.

Ehleringer, J.R. and T.E. Dawson. 1992. Water uptake by plants; perspectives from stable isotope composition. *Plant, Cell and Environment*, **15**:1073-1082.

Ehleringer, J.R., C.B. Field, Z.-F. Lin and C.-Y. Kuo. 1986. Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia*, **70**:520-526.

Eleuterius, L.N. 1989. Taximetric analysis of female and hermaphroditic plants among populations of *Juncus roemerianus* under different salinty regimes. *Journal of Coastal Research*, **5**:29-35.

Ellison, R.L. 1984. Foraminifera and meiofauna on an intertidal mudflat, Cornwall, England. *Hydrobiologia*, **109**:131-147.

Englefield, G.J.H., M.J. Tooley and Y. Zong. 1990. *An assessment of the Clwyd coastal floods of February 1990*. Durham, Environmental Research Centre.

Epstein, S., P. Thompson and C.J. Yapp. 1977. Oxygen and hydrogen isotopic ratios in plant cellulose. *Science*, **198**:1209-1215.

Epstein, S. and C.J. Yapp. 1976. Climatic implications of the D/H ratio of hydrogen in C-H groups in tree cellulose. *Earth and Planetary Science Letters*, **30**:252-261.

Epstein, S. and C.J. Yapp. 1977. Isotope tree thermometers; comment on Libby, L.M. *et al.*, *Nature* 261, 284-288. *Nature*, **266**:477-478.

Epstein, S., C.J. Yapp and J.H. Hall. 1976. The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth and Planetary Science Letters*, **30**:241-251.

Everard, J.H., R. Gucci, S.C. Kann, J.A. Flore and W.H. Loescher. 1994. Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. *Plant Physiology*, **106**:281-292.

Fahrig, L., B. Hayden and R. Dolan. 1993. Distribution of barrier island plants in relation to overwash disturbance: A test of life history theory. *Journal of Coastal Research*, **9**:403-412.

Farquhar, G.D., M.C. Ball, S. Von Caemmerer and Z. Roksandic. 1982. Effect of salinity and humidity on the $\delta^{13}C$ value of halophytes - evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. *Oecologia*, **52**:121-124.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimniation and photosynthesis. *Annual Review of Plant Physiology*, **40**:503-537.

Farquhar, G.D., K.T. Hubick, A.G. Condon and R.A. Richards. 1989. Carbon isotope fractionation and plant water-use efficiency. **In:** P.W. Rundel, J.R. Ehleringer and K.A. Nagy (eds.). *Stable isotopes in ecological research*. Springer Verlag, Berlin.

Farquhar, G.D. and J. Lloyd. 1993. Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. **In:** J.R. Ehleringer, A.E. Hall and G.D. Farquhar (eds.). *Stable Isotopes and Plant Carbon-Water Relations*. Academic Press, San Diego.

Farquhar, G.D., J. Lloyd, J.A. Taylor, L.B. Flanagan, J.P. Syvertsen, K.T. Hubick, S.C. Wong and J.R. Ehleringer. 1993. Vegetation effects on the isotope composition of oxygen in atmospheric CO_2. *Nature*, **363**:439-443.

Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, **33**:317-345.

Feitzel, T.C., R.D. DeLaune and W.H. Patrick. 1988. Seasonal pore water dynamics in marshes of Barataria Basin,Louisiana. *Soil Science* , **52**:59-67.

Feng, X., R.V. Krishnamurthy and S. Epstein. 1993. Determination of D/H ratios of nonexchangeable hydrogen in cellulose: a method based on the cellulose-water exchange reaction. *Geochimica et Cosmochimica Acta*, **57**:4249-4256.

Field, E.M. 1993,a. Method for determining $\delta^{13}C$ in cellulose. Unpublished method notes, Subdepartment of Quaternary Research, Godwin Laboratory, University of Cambridge.

Field, E.M. 1993,b. Extraction of alpha-cellulose from oak. Unpublished method notes, Subdepartment of Quaternary Research, Godwin Laboratory, University of Cambridge. .

Field, E.M., V.R. Switsur and J.S. Waterhouse. 1993. An improved technique for the determination of oxygen stable isotope ratios in wood cellulose. *Applied Radiation and Isotopes*, **43**:322-326.

Fitzgerald, M.A. 1992. Evidence that abaxial leaf glands are the sites of salt secretion in leaves of the mangrove *Avicennia Marina* (Forsk.) Vierh. *New Phytologist*, **120**:1-7. .

Flanagan, L.B., J.R. Ehleringer and J.D. Marshall. 1992. Differential uptake of summer precipitation among co-occurring trees and shrubs in a pinyon-juniper woodland. *Plant, Cell and Environment*, **15**:831-836.

Flanagan, L.B. and R.L. Jeffries. 1989. Photosynthetic and stomatal responses of the halophyte, *Plantago maritima* L. to fluctuations in salinity. *Plant, Cell and Environment*, **12**:559-568.

Fletcher, R.L. 1987. Seaweeds of the British Isles, Vol. Vol.3. British Museum, Natural History, London.

Flowers, T.J., S.A. Flowers, M.A. Hajibagheri and A.R. Yeo. 1990. Salt tolerance in the halophytic wild rice, *Porteresia coarctata* Tateoka. *New Phytologist*, **114**:675-684.

Flowers, T.J., M.A. Hajibagheri and A.R. Yeo. 1991. Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for the Oertli hypothesis. *Plant, Cell and Environment*, **14**:319-325.

Francey, R.J. and G.D. Farquhar. 1982. An explanation of $^{13}C/^{12}C$ variations in tree rings. *Nature*, **297**:28-31.

Francey, R.J., R.M. Gifford, T.D. Sharkey and B. Weir. 1985. Physiological influences on carbon isotope discrimination in Houn Pine *Lagarostrobus franklinii* L. *Oecologia*, **66**:211-218.

Francey, R.J. and P.P. Tans. 1987. Latitudinal variation in O-18 of atmospheric CO_2. *Nature*, **327**:495-497.

Freyer, H.D. and N. Belacy. 1983. $^{13}C/^{12}C$ records in Northern hemispheric trees during the past 500 years - anthropogenic impact and climatic superposition. *Journal of Geophysical Research*, **88**:6844-6852.

Friedman, I., A.C. Redfield, B. Schoer and J. Harris. 1964. The variation of the deuterium content of natural waters in the hydrologic cycle. *Reviews of Geophysics*, **2**:177-224.

Galvez, A.F., P.J. Gulick and J. Dvorak. 1993. Characterization of the early stages of salt-stress responses in salt tolerant *Lophopyrum elongatum*, salt-sensitive wheat, and their amphiploid. *Plant Physiology*, **103**:257-265.

Van Geel, B. and A.A. Middledorp. 1988. Vegetational history of Carbury bog (Co. Kildare, Ireland) during the last 850 years and a test of the temperature indicator value of $^{2}H/^{1}H$ measurements of peat samples in relation to historical sources and meteorological data. *New Phytologist*, **109**:377-392.

Gehrels, W.R. 1994. Determining relative sea-level change from saltmarsh foraminifera and plant zones on the coast of Maine, USA. *Journal of Coastal Research*, **10**:990-1009.

Gehrels, W.R. and D.F. Belknap. 1993. Neotectonic history of eastern Maine evaluated from historic sea-level data and ^{14}C dates on saltmarsh peats. *Geology*, **21**:615-618.

Gehrels, W.R. and O. Van de Plassche. 1991. Origin of the paleovalley system underlying Hammock River Marsh, Clinton, Connecticut. *Journal of Coastal Research*, **Special Issue 11**:73-83.

Gibbs, R.D. 1958. Patterns in the seasonal water content of trees. **In:** K.V. Thimann, W.B. Critchfield and M.H. Zimmerman (eds.). *The physiology of forest trees.* Ronald Press, New York.

Glenn, E.P. 1987. Relationship between cation accumulation and water content of salt-tolerant grasses and a sedge. *Plant, Cell and Environment*, **10**:205-212.

Glenn, E.P., M.C. Watson, J.W. O'Leary and R.D. Alexson. 1992. Comparison of salt tolerance and osmotic adjustment of low-sodium and high-sodium subspecies of the C_4 halophyte *Atriplex canescens*. *Plant, Cell and Environment*, **15**:711-718.

Godwin, H. and L. Newton. 1938. The submerged forest at Borth and Ynyslas, Cardiganshire. *New Phytologist*, **37**:333-344.

Gorenflot, R. 1986. Degrees and levels of variation in chromosome number in *Phragmites australis*. *Geobotanisch Institiut im Technische Hochsch Stift Ruebel*, **0(87)**:53-65.

Gorham, J., L.L. Hughes and R.G. Wyn Jones. 1980. Chemical composition of saltmarsh plants from Ynys Mon (Anglesey): the concepts of physiotypes. *Plant, Cell and Environment*, **3**:309-318.

Gray, A.J. 1971. The ecology of Morecambe Bay. V. The saltmarshes of Morecambe Bay. *Journal of Applied Ecology*, **9**:207-220.

Gray, A.J. 1985. Adaptation in perennial coastal plants - with particular reference to heritable variation in *Puccinellia maritima* and *Ammophila arenaria*. *Vegetatio*, **61**:179-188.

Gray, A.J. 1987. Genetic change during succession in plants. **In:** A.J. Gray, M.J. Crawley and P.J. Edwards (eds.). *Colonization, Succession and Stability*. Blackwell, Oxford.

Gray, A.J. 1992. Saltmarsh plant ecology: zonation and succession revisited. **In:** J.R.L. Allen and K. Pye (eds.). *Saltmarshes*. Cambridge University Press, Cambridge.

Gray, A.J. and R.G.H. Bunce. 1971. The ecology of Morecambe Bay. VI. Soils and vegetation of the salt marshes: a multivariate approach. *Journal of Applied Ecology*, **9**:221-234.

Gray, A.J., R.J. Parsell and R. Scott. 1979. The genetic structure of plant populations in relation to the development of salt marshes. **In:** R.L. Jefferies and A.J. Davy (eds.). *Ecological processes in coastal environments*. Blackwell, Oxford.

Gray, J. and S.J. Song. 1984. Climatic implications of the natural variations of D/H in tree ring cellulose. *Earth and Planetary Science Letters*, **70**:129-138.

Gray, J. and P. Thompson. 1976. Climatic information from $^{18}O/^{16}O$ ratios in cellulose in tree rings. *Nature*, **262**:481-482.

Gray, J. and P. Thompson. 1977. Climatic information from $^{18}O/^{16}O$ analysis of cellulose, lignin and whole wood from tree rings. *Nature*, **270**:708-709.

Gray, J. and P. Thompson. 1978. Reply to Wigley T.M.L., B.M.Gray and P.M.Kelly, *Nature* **271**:92-93. *Nature*, **271**:93-94.

Grebmeier, J.M., L.W. Cooper and M.J. Deniro. 1990. Oxygen isotopic composition of bottom seawater and tunicate cellulose used as indicators of water masses in the northern Bering and Chukchi Seas. *Limnology and Oceanography*, **35**:1182-1195.

Gries, C., L. Kappen and R. Losch. 1990. Mechanism of flood tolerance in reed, *Phragmites australis* (Cav.) Trin ex Steudal. *New Phytologist*, **114**:589-593.

Griffiths, H. 1992. Carbon isotope discrimination and the integration of carbon assimilation pathways in terrestrial CAM plants. *Plant, Cell and Environment*, **15**:1051-1062.

Grime, J.P., J.G. Hodgson and R. Hunt. 1988. Comparative Plant Ecology. Unwin Hyman, London.

Groenhof, A.C., N. Smirnoff and J.A. Bryant. 1990. The appearance of a new molecular species of phosphoenolpyruvate carboxylase (PEPC) and the rapid induction of CAM in *Sedum telphium* L. *Plant, Cell and Environment*, **13**:437-445.

Gross, L.J., M.U.F. Kirschbaum and R.W. Pearcy. 1991. A dynamic model of photosynthesis in varying light taking account of stomatal conductance, C_3-cycle intermediates, photorespiration and Rubisco activation. *Plant, Cell and Environment*, **14**:881-893.

Guy, R.D. and D.M. Reid. 1986,a. Factors affecting $^{13}C/^{12}C$ ratios of inland halophytes.II. Ecophysiological interpretations of patterns in the field. *Canadian Journal of Botany*, **64**:2700-2707.

Guy, R.D. and D.M. Reid. 1986,b. Photosynthesis and the influence of CO_2-enrichment on $\delta^{13}C$ values in a C_3 halophyte. *Plant, Cell and Environment*, **9**:65-72.

Guy, R.D., D.M. Reid and H.R. Krouse. 1980. Shifts in carbon isotope ratios of two C_3 halophytes under natural and artificial conditions. *Oecologia*, **44**:241-247.

Guy, R.D., D.M. Reid and H.R. Krouse. 1986. Factors affecting $^{13}C/^{12}C$ ratios of inland halophytes.I. Controlled studies on growth and isotopic composition of *Puccinellia nuttaliana* L. *Canadian Journal of Botany*, **64**:2693-2699.

Guy, R.D. and R.L. Wample. 1983. Stable carbon isotope ratios of flooded and nonflooded sunflowers (*Helianthus annuus* L.). *Canadian Journal of Botany*, **62**:1770-1774.

Guy, R.D., P.G. Warne and D.M. Reid. 1989. Stable carbon isotope ratio as an index of water-use efficiency in C_3 halophytes - possible relationship to strategies for osmotic adjustment. **In:** P.W. Rundel, J.R. Ehleringer and K.A. Nagy (eds.). *Stable isotopes in ecological research*. Springer Verlag, Berlin.

Haines, E.B. 1976. Relation between the stable carbon isotope composition of fiddler crabs, plants, and soils in a saltmarsh. *Limnology and Oceanography*, **21**:880-883.

Handley, L.L. and J. Raven. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell and Environment*, **15**:965-985.

Hara, T., J. Van der Toorn and J.H. Mook. 1993. Growth dynamics and size structure of shoots of *Phragmites australis*, a clonal plant. *Journal of Ecology*, **81**:47-60.

Hardisty, J. 1990. Beaches; form and process. Unwin Hyman, London.

Haslam, S.M. 1970,a. The development of the annual population in *Phragmites communis* Trin. *Annals of Botany*, **34**:571-591.

Haslam, S.M. 1970,b. The performance of *Phragmites communis* Trin. in relation to water supply. *Annals of Botany*, **34**:867-877.

Haslam, S.M. 1970,c. Variation of population type in *Phragmites communis* Trin. *Annals of Botany*, **34**:147-158.

Haslam, S.M. 1972. Biological flora of the British Isles: *Phragmites communis* Trin. *Journal of Ecology*, **60**:585-610.

Haslett, S.K. 1994. Unpublished preliminary report on sites investigated for foramininferal assemblages. Environmental Research Centre, University of Durham. .

Healy, T. 1991. Coastal erosion and sea level rise. *Zeitschrift fur Geomorphologie N.F. Supplementas Bund*, **81**:15-29.

Heaton, T.H.E. 1987. The $^{15}N/^{14}N$ ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia*, **74**:236-246.

Heaton, T.H.E. 1990,a. Components of the stable isotope gas preparation lines. NERC Isotope Geosciences Laboratories Report Series, no.19, Unpublished NERC manual.

Heaton, T.H.E. 1990,b. Procedure: Vacuum Systems. Unpublished method notes, NERC Isotope Geoscience Laboratories, Keyworth. .

Heaton, T.H.E. 1991. Procedure and notes on the preparation of gases from organic matter. NERC Isotope Geosciences Laboratories Report Series, no.29, Unpublished NERC manual.

Hellings, S.E. and J.L. Gallagher. 1992. The effects of salinity and flooding on *Phragmites australis*. *Journal of Applied Ecology*, **29**:41-49.

Hemond, H.F. and D.G. Chen. 1990. Air entry in salt marsh sediments. *Soil Science* , **150**:459-468.

Hester, M.W. and I.A. Mendelsohn. 1989. Water relations and growth responses of *Uniola paniculata* (sea oats) to soil moisture and water-tale depth. *Oecologia*, **78**:289-296.

Heyworth, A. 1978. Submerged forests around the British Isles: their dating and relevance as indicators of post-glacial land and sea-level changes. **In:** J. Fletcher (ed.). *Dendrochronology in Europe*. British Archaeological Reports, Interim Series no.51, .

Heyworth, A. 1986. Submerged forests as sea-level indicators. **In:** O. Van de Plassche (ed.). *Sea level research: a manual for the collection and evaluation of data.* GeoBooks, Norwich.

Holmden, C. and K. Muehlenbachs. 1993. The $^{18}O/^{16}O$ ratio of 2-billion-year-old seawater inferred from ancient oceanic crust. *Science*, **259**:1733-1736.

Huiskes, A.H.L., J. Van Soelen and M.M. Markusse. 1985. Field studies on the variabity of populations of *Aster tripolium* L. in relation to saltmarsh vegetation. *Vegetatio*, **61**:163-169.

Hwang, Y.-H. and J.T. Morris. 1994. Whole-plant gas exchange responses of *Spartina alterniflora* (Poaceae) to a range of constant and transient salinities. *American Journal of Botany*, **81**:659-665.

Hydrographer of the Navy. 1995. Admiralty Tide Tables. Volume 1. Taunton.

Innes, J.B., I. Shennan and Y. Zong. 1993. The contemporary diatom and pollen flora of Kentra Bay, Argyll. Unpublished report to Scottish Natural Heritage, Environmental Research Centre, University of Durham. .

Ishibashi, M. and I. Terashima. 1995. Effects of continuous leaf wetness on photosynthesis: adverse aspects of rainfall. *Plant, Cell and Environment*, **18**:431-438.

Ish-Shalom, N., L.d.S.L. Sternberg, M. Ross, J. O'Brien and L. Flynn. 1992. Water utilization of tropical hardwood hammocks of the Lower Florida Keys. *Oecologia*, **92**:108-112.

Ish-Shalom-Gordon, N., G. Lin and L.d.S.L. Sternberg. 1992. Isotopic fractionation during cellulose synthesis in two mangrove species: salinity effects. *Phytochemistry*, **31**:2623-2626.

Jackson, M.B. and K.C. Hall. 1983. Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Plant, Cell and Environment*, **10**:121-130.

Jennings, A.E., A.R. Nelson, D.B. Scott and J.C. Aravena. 1995. Marsh foraminiferal assemblages in the Valdivia Estuary, South-central Chile, relative to vascular plants and sea-level. *Journal of Coastal Research*, **11**:107-123.

Jerling, L. 1985. Population dynamics of *Plantago maritima* along a distributional gradient on a Baltic seashore meadow. *Vegetatio*, **61**:155-161.

Johnsen, S.J., W. Dansgaard, H.B. Clausen and C.C. Longway. 1972. Oxygen isotope profiles through the Antarctic and Greenland ice sheets. *Nature*, **235**:429-434.

Johnson, S.R. and D.R. Young. 1992. Variation in tree ring width in relation to storm activity from mid-Atlantic barrier island populations of *Pinus taeda*. *Journal of Coastal Research*, **8**:99-104.

Jones, H.G. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant, Cell and Environment*, **8**:95-104.

Jones, H.G. and A. Sutherland. 1991. Stomatal control of xylem embolism. *Plant, Cell and Environment*, **14**:607-612.

Kamio, A. 1989. Characteristic of drying in the A6 field (natural plot) of Hachirogata Central Polder. *Journal of Yamagata Agriculture and Forestry Society*, **46**:27-32.

Kapos, V., G. Gonade, E. Matsui and R.L. Victoria. 1993. $\delta^{13}C$ as an indicator of edge effects in tropical rainforest reserves. *Journal of Ecology*, **81**:425-432.

Karim, M.A., E. Nawata and S. Shigenager. 1993. Influence of flooding with diluted seawater on morphological and physiological characteristics in hexaploid titricale. *Plant, Cell and Environment*, **16**:663-671.

Keeley, J.E. and D.R. Sandquist. 1992. Carbon: freshwater plants. *Plant, Cell and Environment*, **15**:1021-1035.

Keeling, C.D., W.G. Mook and P.P. Tans. 1979. Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. *Nature*, **277**:121-123.

Keeling, R.F. and S.R. Shertz. 1992. Seasonal and interannual variations in atmospheric oxygen and implications for the global carbon cycle. *Nature*, **358**:723-727.

Kelley, J.T., W.R. Gehrels and D.F. Belknap. 1995. Late Holocene relative sea-level rise and the geological development of tidal marshes at Wells, Maine, USA. *Journal of Coastal Research*, **11**:136-153.

Kelly, C.K. and F.I. Woodward. 1995. Ecological correlates of carbon isotope composition of leaves; a comparative analysis testing for the effects of temperature, CO_2 and O_2 partial pressures and taxonomic relatedness on $\delta^{13}C$. *Journal of Ecology*, **83**:509-515.

Kennicutt, M.C., R.R. Bidigrove, S.A. Macko and W.C. Keeney-Kennicutt. 1992. The stable isotopic composition of photosynthetic pigments and related biochemicals. *Chemical Geology*, **101**:235-245.

Khalil, A.A.M. and J. Grace. 1993. Does xylem sap ABA control the stomatal behaviour of water-stressed sycamore (*Acer pseudoplanatus* L.) seedlings? *Journal of Experimental Botany*, **44**:1127-1134.

Kicheva, M.I., T.D. Tsonev and L.P. Popova. 1994. Stomatal and nonstomatal limitations to photosynthesis in two wheat cultivars subjected to water stress. *Photosynthetica*, **30**:107-116.

Knapp, A.K. 1993. Gas exchange dynamics in C_3 and C_4 grasses: consequences of differences in stomatal conductance. *Ecology*, **74**:113-123.

Knight, J.D., N.J. Livingston and C. Van Kessel. 1994. Carbon isotope discrimination and water-use efficiency of six crops grown under wet and dryland conditions. *Plant, Cell and Environment*, **17**:173-179.

Kohorn, L.U., G. Goldstein and P.W. Rundel. 1994. Morphological and isotopic indicators of growth environment; variability in $\delta^{13}C$ in *Simmondsia chinesis* L., a dioecious desert shrub. *Journal of Experimental Botany*, **45**:1817-1822.

Korner, C., G.D. Farquhar and Z. Roksandic. 1988. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia*, **74**:623-632.

Korner, C., G.D. Farquhar and S.C. Wong. 1991. Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia*, **88**:30-40.

Kosters, E.C., G.L. Chmura and A. Bailey. 1987. Sedimentary and botanical factors influencing peat accumulation in the Mississippi delta. *Journal of the Geological Society*, **144**:423-434.

Kosugi, M. 1989. Salinity from the former Tokyo Bay in the Holocene based on diatom assemblages. *Quaternary Research*, **28**:19-26.

Van Kraalingen, D.W.G. 1990. Implications of non-uniform stomatal closure on gas exchange calculations. *Plant, Cell and Environment*, **13**:1001-1004.

Kramer, P.J. 1964. The role of water in wood formation. **In:** M.H. Zimmerman (ed.). *The formation of wood in forest trees.* Academic Press, New York.

Krishnamurthy, R.V. and S. Epstein. 1985. Tree ring D/H ratios from Kenya, East Africa and its palaeoclimatic significance. *Nature*, **317**:160-162.

Kubiske, M.E. and M.D. Abrams. 1993. Stomatal and nonstomatal limitations of photosynthesis in 19 temperate tree species on contrasting sites during wet and dry years. *Plant, Cell and Environment*, **16**:1123-1129.

Kuido, G. and K. Ito. 1988. Rhizome development of *Phragmites australis* in a reed community. *Ecological Research*, **3**:239-252.

Labeyrie, L. 1974. New approach to surface seawater palaeotemperatures using $^{18}O/^{16}O$ ratios in silica of diatom frustules. *Nature*, **248**:40-41.

Lawrence, J.R., S.D. Gedzelman, J.W.C. White, D. Smiley and P. Lazov. 1982. Storm trajectories in eastern US: D/H isotopic composition of precipitation. *Nature*, **296**:638-640.

Lawrence, J.R. and J.W.C. White. 1984. Growing season precipitation from D/H ratios of Eastern White Pine. *Nature*, **311**:558-560.

Leaney, F.W., C.B. Osmond, G.B. Allison and H. Ziegler. 1985. Hydrogen isotope composition of leaf water in C_3 and C_4 plants: its relationship to the hydrogen isotope comopsition of dry matter. *Planta*, **164**:215-220.

Leavitt, S.W. and S.R. Danzer. 1992. $\delta^{13}C$ variations in C_3 plants over the past 50,000 years. *Radiocarbon*, **34**:783-791.

Leavitt, S.W. and S.R. Danzer. 1993. Method for batch processing small wood samples to holocellulose for stable carbon isotope analysis. *Analytical Chemistry*, **65**:87-89.

Leavitt, S.W. and R.M. Kalin. 1992. A new tree-ring width, $\delta^{13}C$ and ^{14}C investigation of the Two Creeks site. *Radiocarbon*, **34**:792-797.

Leavitt, S.W. and A. Long. 1984. Sampling strategy for stable carbon isotope analysis of tree rings in pine. *Nature*, **311**:145-147.

Lecolle, P. 1985. The oxygen isotope composition of landsnail shells as a climatic indicator. Applications in hydrogeology and palaeoclimatology. *Chemical Geology*, **58**:157-181.

Lee, S.Y. 1990,a. Net aerial primary productivity, litter production and decomposition of the reed *Phragmites communis* in a nature reserve in Hong Kong: management implications. *Marine Ecology Progress Series*, **66**:161-174.

Lee, S.Y. 1990,b. Primary productivity and particulate organic matter flow in an estuarine wetland in Hong Kong. *Marine Biology*, **106**:453-464.

Lee, Y., H.J. Lee, R.C. Crain, A. Lee and S.J. Korn. 1994. Polyunsaturated fatty acids modulate stomatal aperture and two distinct K^+ channel currents in guard cells. *Cellular Signalling*, **6**:181-186.

de Leeuw, J. 1991. Factors influencing the soil salinity regime along an intertidal gradient. *Estuarine and Coastal Shelf Science*, **32**:87-97.

Leuning, R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. *Plant, Cell and Environment*, **18**:339-355.

Levinson, A.A and J.C. Ludwick. 1966. Speculation on the incorporation of boron into argillaceous sediments. *Geochimica et Cosmochimica Acta*, **30**:855-861.

Liao, C.T. and C.H. Lin. 1994. Effect of flooding stress on photosynthetic activities of *Momordica charantia*. *Plant Physiology and Biochemistry*, **32**:479-485.

Libby, L.B. and L.J. Pandolfi. 1977,a. Climate periods in trees, ice and tides. *Nature*, **266**:415-417.

Libby, L.M. and L.J. Pandolfi. 1977,b. Reply to Epstein, S. and C.Yapp, *Nature* **266**, 477-478. *Nature*, **266**:479.

Libby, L.M. and L.J. Pandolfi. 1978. Reply to Wigley, T.M.L., B.M.Gray and P.M.Kelly, *Nature* **271**, 92-93. *Nature*, **271**:94.

Libby, L.M., L.J. Pandolfi, P.H. Payton, J. Marshall, B. Becker and V. Giertz-Siebenlist. 1976. Isotope tree thermometers. *Nature*, **261**:284-288.

Lin, C.H. and C.H. Lin. 1992. Physiological adaptation of waxapple to waterlogging. *Plant, Cell and Environment*, **15**:321-328.

Lin, G. and L.d.S.L. Sternberg. 1993. Effects of salinity fluctuation on photosynthetic gas exchange and plant growth of the red mangrove (*Rhizophora mangle* L.). *Journal of Experimental Botany*, **44**:9-16.

Liss, P.S and M.J. Pointon. 1973. Removal of dissolved boron and silicon during estuarine mixing of sea and river waters. *Geochimica et Cosmochimica Acta*, **37**:1493-1498.

Livingston, N.J. and D.L. Spittlehouse. 1993. Carbon isotope fractionation in tree rings in relation to the growing season water balance. **In:** J.R. Ehleringer, A.E. Hall and G.D. Farquhar (eds.). *Stable Isotopes and Plant Carbon-Water Relations.* Academic Press, San Diego.

Lloyd, J., J.P. Syvertsen, P.E. Kriedemann and G.D. Farquhar. 1992. Low conductances for CO_2 diffusion from stomata to the sites of carboxylation in leaves of woody species. *Plant, Cell and Environment*, **15**:873-899.

Long, A.J. 1991. Holocene sea-level changes in the East Kent Fens. Unpublished Ph.D. Thesis, University of Durham.

Long, S.P., L.D. Incoll and H.W. Woolhouse. 1975. C4 photosynthesis in plants from cool temperate regions, with particular reference to *Spartina townsendii*. *Nature*, **257**:622-624.

Long, S.P. and C.F. Masson. 1983. Saltmarsh Ecology. Blackie and Son, Glasgow.

Long, S.P., W.F. Postl and H.R. Bolhar-Nordendorf. 1993. Quantum yield for uptake of carbon dioxide in C_3 vascular plants of contrasting habitats and taxonomic groupings. *Planta*, **189**:226-234.

Longstreth, D.J. and P.S. Nobel. 1979. Salinity effects on leaf anatomy: consequences for photosynthesis. *Plant Physiology*, **63**:700-703.

Lovelock, J. 1989. The Ages of Gaia. Oxford University Press, Oxford.

Lucas, P.W., B.W. Darvell, P.K.D. Lee, T.D.B. Yuen and M.F. Choong. 1995. The toughness of plant cell walls. *Philosophical Transactions of the Royal Society of London, Series B.*, **348**:363-372.

Luckman, B. and J. Gray. 1990. Oxygen isotope ratios from tree rings containing compression wood. *Quaternary Research*, **33**:117-121.

Luckman, B.H. and M.S. Kearney. 1986. Reconstruction of Holocene changes in Alpine vegetation and climate in the Malique Range, Jasper National Park, Alberta. *Quaternary Research*, **26**:244-261.

Maathius, F.J.M. and H.B.A. Prins. 1990. Patch clamp studies on root cell vacuoles of a salt-tolerant and a salt sensitive *Plantago* species. *Plant Physiology*, **92**:23-28.

Machler, F., J. Muller and M. Dubach. 1990. RuBPCO kinetics and the mechanism of CO_2 entry in C_3 plants. *Plant, Cell and Environment*, **13**:881-899.

Macko, S.A., M.H. Engel and Y. Qian. 1994. Early diagenesis and organic matter preservation - a molecular stable carbon isotope perpective. *Chemical Geology*, **114**:365-379.

MacRobbie, E.A.C. 1992. Calcium and ABA-induced stomatal closure. *Philosophical Transactions of the Royal Society of London, Series B.*, **338**:5-18.

Magaritz, M., J. Heeler and M. Volokita. 1981. Land-air boundary environment as recorded by the $^{18}O/^{16}O$ and $^{13}C/^{12}C$ isotope ratios in the shells of land snails. *Earth and Planetary Science Letters*, **52**:101-106.

Malone, S.R., H.S. Mayeux, H.B. Johnson and H.W. Polley. 1993. Stomatal density and aperture length in four plant species grown across a subambient CO_2 gradient. *American Journal of Botany*, **80**:1413-1418.

Mansfield, T.A. and D.C.R. DeSilva. 1994. Sensory systems in the roots of plants and their role in controlling stomatal function in the leaves. *Physiological Chemistry and Physics and Medical NMR*, **26**:89-99.

Mansfield, T.A., A.M. Hetherington and C.J. Atkinson. 1990. Some current aspects of stomatal physiology. *Annual Review of Plant Physiology*, **41**:55-75.

Marcum, K.B. and C.L. Murdoch. 1992. Salt tolerance of the coastal salt marsh grass, *Sporobolus virginicus* (L.) Kunth. *New Phytologist*, **120**:281-288.

Marino, B.D. and M.B. McElroy. 1991. Isotopic composition of atmospheric CO_2 inferred from carbon in C4 plant cellulose. *Nature*, **349**:127-131.

Martin, B., A. Bytnerowicz and Y.R. Thorstenson. 1988. Effects of air pollutants on the composition of stable carbon isotopes, $\delta^{13}C$ of leaves and wood, and on leaf injury. *Plant Physiology*, **88**:218-223.

Martin, B. and E.K. Sutherland. 1990. Air pollution in the past recorded in width and stable carbon isotope composition of annual growth rings of Douglas Fir. *Plant, Cell and Environment*, **13**:839-844.

Matheney, R.K. and L.P. Knauth. 1989. Oxygen-isotope fractionation between marine biogenic silica and seawater. *Geochimica et Cosmochimica Acta*, **53**:3207-3214.

Matsushita, N. and T. Matoh. 1991. Characterization of sodium exclusion mechanisms of salt-tolerant reed plants in comparison with salt-sensitive rice plants. *Physiologia Plantarum*, **83**:170-176.

Matsushita, N. and T. Matoh. 1992. Function of the shoot base of salt-tolerant reed (*Phragmites communis* Trinius) plants for sodium exclusion from the shoots. *Soil Science and Plant Nutrition*, **38**:565-571.

McManus, J. and S.A.K. Alizai. 1987. Variations in marsh surface levels in the Upper Tay Estuary. *Proceedings of the Royal Society of Edinburgh, Section B*, **92**:345-358.

McPherson, G.R., T.W. Boutton and A.J. Midwood. 1993. Stable carbon isotope analysis of soil organic matter illustrates vegetation change at the grassland /woodland boundary in southeastern Arizona, USA. *Oecologia*, **93**:95-101.

Megonigal, J.P. and F.P. Day. 1992. Effects of flooding on root and shoot production on Bald Cypress in large experimental enclosures. *Ecology*, **73**:1182-1193.

Meinzer, F.C., G. Goldstein, N.M. Holbrook, P. Jackson and J. Cavelier. 1993. Stomatal and environmental control of transpiration in a lowland tropical forest tree. *Plant, Cell and Environment*, **16**:429-436.

Meinzer, F.C., Z. Plaut and N.Z. Saliendra. 1994. Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. *Plant Physiology*, **104**:521-526.

Merwe, N.J. and E. Medina. 1989. Photosynthesis and $^{13}C/^{12}C$ ratios in Amazonian rain forests. *Geochimica et Cosmochimica Acta*, **53**:1091-1094.

Meyers, P. 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chemical Geology*, **114**:289-302.

Meyers, P. and S. Horie. 1993. An organic carbon isotopic glacial-postglacial change in atmospheric CO_2 in the sediments of Lake Biwa, Japan. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **105**:171-178.

Montieth, J.L. 1995. A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment*, **18**:357-364.

Mook, J.H. and J. Van der Toorn. 1982,a. The influence of environmental factors and management on stands of *Phragmites australis* I. Effects of burning, frost and insect damage on shoot density and shoot size. *Journal of Applied Ecology*, **19**:477-499.

Mook, J.H. and J. Van der Toorn. 1982,b. The influence of environmental factors and management on stands of *Phragmites australis* II. Effects on yield and its relationships with shoot density. *Journal of Applied Ecology*, **19**:501-517.

Morgan, V.I. 1982. Antarctic ice sheet surface oxygen isotope values. *Journal of Glaciology*, **28**:315-323.

Mortley, D.G., C.K. Bonsi, P.A. Lorefan, W.A. Hill and C.E. Morris. 1994. Relative humidity influences yield, edible biomass, and linear growth rate of sweetpotato. *Hortscience*, **29**:609-610.

Morton, R.A. (1981). Formation of storm deposits by wind-forced currents in the Gulf of Mexico and the North Sea. In S.D. Nio, R.T.E Shuttenhelm and T.J.C.E. van Weering (eds.), *Holocene marine sedimentation in the North Sea Basin.* Special Publication of the International Association of Sedimentologists number 5. Oxford, Blackwell.

Mullane, M.V., J.S. Waterhouse and V.R. Switsur. 1988. On the development of a novel method for the determination of stable isotope ratios in cellulose. *Applied Radiation and Isotopes*, **39**:1028-1035.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell and Environment*, **16**:15-24.

Murnick, D.E. and B.J. Peer. 1994. Laser-based analysis of carbon isotope ratios. *Science*, **263**:945-947.

Murray, J.W. 1979. British Nearshore Foraminiferids. Academic Press, London.

Murrray, J.W. 1980. The foraminifera of the Exe Estuary. *Essays on the Exe Estuary, Devonian Association Special Paper no.2.*

Nagy, Z. and G. Galiba. 1995. Drought and salt tolerance are not necessarily linked: A study on wheat varieties differing in drought tolerance under consecutive water and salinity stresses. *Journal of Plant Physiology*, **145**:168-174.

Nordt, L.C., T.W. Boutton, C.T. Hallmark and M.R. Waters. 1994. Late Quaternary vegetation and climate change in central Texas based on the isotopic composition of organic carbon. *Quaternary Research*, **41**:109-120.

Oenema, O. and R.D. DeLaune. 1988. Accretion rates in salt marshes in the Eastern Scheldt, SW Netherlands. *Estuarine and Coastal Shelf Science*, **26**:379-394.

O'Leary, M.H. 1981. Carbon isotope fractionation in plants. *Phytochemistry*, **20**:553-567.

O'Leary, M.H. 1993. Biochemical basis of carbon isotope fractionation. **In:** J.R. Ehleringer, A.E. Hall and G.D. Farquhar (eds.). *Stable Isotopes and Plant Carbon-Water Relations*. Academic Press, San Diego.

O'Leary, M.H., S. Madhavan and P. Pareth. 1992. Physical and chemical basis of carbon isotope fractionation in plants. *Plant, Cell and Environment*, **15**:1099-1104.

O'Leary, M.H., I. Treichel and M. Rooney. 1986. Short-term measurement of carbon isotope fractionation in plants. *Plant Physiology*, **80**:578-582.

de Oliveira, F.E.C. and A. Fletcher. 1980. Taxonomic and ecological relationships between rocky-shore and saltmarsh populations of *Pelvetia canaliculata* (Phaeophyta) at Four Mile Bridge, Anglesey, UK. *Botanica Marina*, **23**:409-417.

Ollendorf, A.L., S.C. Mulholand and G. Rapp. 1988. Phytolith analysis as a means of plant identification: *Arundo donax* and *Phragmites communis*. *Annals of Botany*, **61**:209-214.

Orson, R.A., R.S. Warren and W. Niering. 1987. Development of a tidal marsh in a New England river valley (Connecticut, USA). *Estuaries*, **10**:20-27.

Ostendorp, W. 1991. Damage by episodic flooding to *Phragmites* reeds in a prealpine lake: Proposal of a model. *Oecologia*, **86**:119-124.

Parker, P.L. 1964. The biogeochemistry of the stable isotopes of carbon in a marine bay. *Geochimica et Cosmochimica Acta*, **28**:1155-1164.

Pearcy, R.W. and S.L. Ustin. 1984. Effects of salinity on growth and photosynthesis of three California tidal marsh species. *Oecologia*, **62**:68-73.

Pearman, G.I., R.J. Francey and P.J.B. Fraser. 1976. Climatic implications of stable carbon isotopes in tree rings. *Nature*, **260**:771-773.

Peisker, M. and S.A. Henderson. 1992. Carbon: terrestrial C4 plants. *Plant, Cell and Environment*, **15**:987-1004.

Pennings, S.C. and R.M. Calloway. 1992. Saltmarsh plant zonation: the relative importance of competition and physical factors. *Ecology*, **73**:681-690.

Penuelas, J. and J. Azcon-Bieoto. 1992. Changes in leaf $\delta^{13}C$ of herbarium plant species during the last 3 centuries of CO_2 increase. *Plant, Cell and Environment*, **15**:485-489.

Penuelas, J. and R. Matamala. 1990. Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO2 increase. *Journal of Experimental Botany*, **41**:1119-1124.

Perera, L.K.R.R., T.A. Mansfield and A.J.C. Malloch. 1994. Stomatal responses to sodium ions in *Aster tripolium*: a new hypothesis to explain salinity regulation in above-ground tissues. *Plant, Cell and Environment*, **17**:335-340.

Perry, D.A. 1977. Oxygen isotope ratios in spruce cellulose. *Nature*, **266**:476-477.

Perry, E.A. 1972. Diagenesis and the validity of the boron palaeosalinity technique. *American Journal of Science*, **277**:150-160.

Pettersson, R. and A.J.S. McDonald. 1992. Effects of elevated carbon dioxide concentration on photosynthesis and growth of small birch plants (*Betula pendula* Roth.) at optimal nutrition. *Plant, Cell and Environment*, **15**:911-919.

Pezeshki, S.R. and R.D. DeLaune. 1988. Carbon assimilation in contrasting streamside and inland *Spartina alterniflora* salt marsh. *Vegetatio*, **76**:55-62.

Pezeshki, S.R., R.D. DeLaune and W.H. Patrick. 1987,a. Response of baldcypress (*Taxodium districhum* var.*distichum*) to increases in flooding salinty in Louisiana's Mississippi River deltaic plain. *Wetlands*, 7:1-10.

Pezeshki, S.R., R.D. DeLaune and W.H. Patrick. 1987,b. Gas exchange characteristics of Gulf of Mexico coastal marsh macrophytes. *Journal of Experimental Marine Biology and Ecology*, **111**:243-254.

Pezeshki, S.R., R.D. DeLaune and W.H. Patrick. 1988. Effects of salinity on leaf ionic content and photosynthesis of *Taxodium distichum* L. *American Midlands Naturalist*, **119**:185-192.

Pezeshki, S.R., R.D. DeLaune and W.H. Patrick. 1989. Effect of fluctuating rhizosphere redox potential on carbon assimilation of *Spartina alterniflora*. *Oecologia*, **80**:132-135.

Pezeshki, S.R., R.D. DeLaune and W.H. Partick. 1990. Differential response of selected mangroves to soil flooding and salinity: gas exchange and biomass partitioning. *Canadian Journal of Forest Research*, **20**:869-874.

Pezeshki, S.R., W.H. Patrick, R.D. DeLaune and E.D. Moser. 1989. Effects of waterlogging and salinity interaction on *Nyssa aquatica* seedlings. *Forest Ecology and Management*, **27**:41-52.

Phillips, J.D. 1987. Shoreline processes and establishment of *Phragmites australis* in a coastal plain estuary. *Vegetatio*, **71**:139-144.

Phleger, F.B. and J.S. Bradshaw. 1966. Sedimentary environments in a marine marsh. *Science*, **154**:1551-1553.

Pizzolata, T.D. 1993. Vascular system of male and perfect florets of *Phragmites australis* (Gramineae - Arundinoideae). *International Journal of Plant Sciences*, **154**:119-133.

Pizzolata, T.D. 1994. Procambial initiation for the vascular system of florets of *Phragmites* (Gramineae - Arundinoidae). *International Journal of Plant Sciences*, **155**:23-35.

Polderman, P.J.G. and R.A. Polderman-Hall. 1980. Algal communities in Scottish saltmarshes. *British Phycological Journal*, **15**:59-71.

Poole, I., J. Raven and J.D.B. Weyers. 1995. Variability in stomatal frequency and index of *Alnus glutinosa* (L.) Gaertn. leaves in relation to carbon dioxide partial pressures. Paper given at meeting of the Society of Experimental Biology, 3-7 April 1995, University of St.Andrews. .

Preston, T. 1992. The measurement of stable isotope natural abundance variations. *Plant, Cell and Environment*, **15**:1091-1097.

Price, J.S., M. Woo and B. Maxwell. 1990. Salinity of marshes along the James Bay coast, Ontario, Canada. *Physical Geography*, **10**:1-12.

Pulich, W., S. Richard, R. Anderson and P. Parker. 1985. Stable carbon isotope inventory of a brackish Texas estuary. *Eos*, **66**:1279.

Pye, K. 1992. Saltmarshes of the barrier coastline of North Norfolk, eastern England. **In:** J.R.L. Allen and K. Pye (eds.). *Saltmarshes*. Cambridge University Press, Cambridge.

Quick, W.P., M.M. Choves, R. Wendler, M. David, M.C. Rodrgues, J.A. Passaharinho, J.S. Pereira, M.P. Adcock, R.G. Leegood and M. Stiff. 1992. The effects of water stress on photosynthetic carbon metabolism in four species grown under field conditions. *Plant, Cell and Environment*, **15**:25-35.

Ramapogal, S. and J.B. Carr. 1991. Sugarcane proteins and messenger RNAs regulated by salt in suspension cells. *Plant, Cell and Environment*, **14**:47-56.

Ramesh, R., S.K. Bhattacharya and K. Gopalan. 1985. Dendrochronological implications of isotope coherence in trees from Kashmir Valley, India. *Nature*, **317**:802-804.

Ranwell, D.S. 1974. The saltmarsh to tidal woodland transition. *Hydrobiological Bulletin*, **8**:139-151.

Ranwell, D.S., E.C.F. Bird, J.C.E. Hubbard and R.E. Stebbings. 1964. *Spartina* salt marshes in southern England V. Tidal submergence and chlorinity in Poole Harbour. *Journal of Ecology*, **52**:627-642.

Rasulov, B.K. 1993. Regulation of photosynthetic carbon dioxide exchange in leaves during development of water deficit in the radical area of cotton plants. *Fiziologiya Rastenii*, **40**:668-675.

Raven, J. 1992. Present and potential uses of the natural abundance of stable isotopes in plant science, with illustrations from the marine environment. *Plant, Cell and Environment*, **15**:1083-1091.

Reed, D.J. 1991. Ponds and bays: natural processes of coastal marsh erosion in the Mississippi deltaic plain, Louisiana, USA. *Zeitschrift fur Geomorphologie N.F. Supplementas Bund*, **81**:41-51.

Ren, D.-T. and C.-L. Zhang. 1994. Seasonal changes of nucleic acid metabolism of different ecotypes of *Phragmites communis* in the corridor of Gansu Hexi. *Acta Botanica Sinica*, **36**:385-392.

Rengel, Z. 1992. The role of calcium in salt toxicity. *Plant, Cell and Environment*, **15**:625-632.

Rhebergen, L.J. and H.J.M. Nelissen. 1985. Ecotypic differentiation within *Festuca rubra* L. occurring in a heterogeneous coastal environment. *Vegetatio*, **61**:197-202.

Rico-Gray, V. and L.d.S.L. Sternberg. 1991. Carbon isotopic evidence for seasonal change in feeding habits of *Componotus planatus* Roger (Formicidae) in Yucatan, Mexico. *Biotropica*, **23**:93-95.

Rintamaki, E. and E.-M. Aro. 1985. Photosynthetic and photorespiratory enzymes in widely divergent plant species with special reference to the moss *Ceratodon purpureus*: Properties of ribulose biphosphate carboxyalse/oxyegenase, phosphoenolpyruvate carboxylase and glycolate oxidase. *Journal of Experimental Botany*, **36**:1677-1684.

Robe, W.E. and H. Griffiths. 1992. Seasonal variation in the ecophysiology of *Littorella uniflora* L. Ascherson in acidic and eutrophic habitats. *New Phytologist*, **120**:289-304.

Robertson, I. 1993. Extraction of alpha-cellulose from oak shavings. Unpublished method notes, Department of Archaeological Sciences, University of Bradford. .

Roozen, A.J.M. and V. Westhoff. 1985. A study on long-term saltmarsh succession using permanent plots. *Vegetatio*, **61**:23-32.

Rosenbaum, J.M. 1993. Room temperature oxygen isotope exchange between liquid CO_2 and H_2O. *Geochimica et Cosmochimica Acta*, **57**:3195-3198.

Ross, M.S., J.J. O'Brien and L.d.S.L. Sternberg. 1994. Sea level rise and the reduction in pine forests in the Florida Keys. *Ecological Applications*, **4**:144-156.

Roy, J. and H.A. Mooney. 1982. Physiological adaptation and plasticity to water stress of coastal and desert populations of *Heliotropium curassavicum* L. *Oecologia*, **52**:370-375.

Rozanski, R. 1985. Deuterium and O-18 in European groundwaters - links to atmospheric circulation in the past. *Chemical Geology*, **52**:349-363.

Rozema, J. and B. Blom. 1977. Effects of salinity and inundation on the growth of *Agrostis stolonifera* and *Juncus gerardii*. *Journal of Ecology*, **65**:213-222.

Ruiz, L.P. and T.A. Mansfield. 1994. A postulated role for calcium oxalate in the regulation of calcium ions in the vicinity of stomatal guard cells. *New Phytologist*, **127**:473-481.

Rundel, P.W., J.R. Ehleringer and K.A. Nagy, eds. 1989. Stable isotopes in ecological research. Springer Verlag, Berlin.

Russell, P.J., T.J. Flowers and M.J. Hutchings. 1985. Comparison of niche breadths and overlaps of halophytes on saltmarshes of differing diversity. *Vegetatio*, **61**:171-178.

Salim, M. 1989. Effects of salinity and relative humidity on growth and ionic relations of plants. *New Phytologist*, **113**:13-20.

Sauer, P.E. and L.d.S.L. Sternberg. 1994. Improved method for the determination of the oxygen isotopic composition of cellulose. *Analytical Chemistry*, **66**:2409-2411.

Schiegl, W.E. 1972. Deuterium content of peat as a palaeoclimatic recorder. *Science*, **175**:512-513.

Schiegl, W.E. 1974. Climatic significance of deuterium abundance in growth rings of *Picea*. *Nature*, **251**:582-584.

Schiegl, W.E. and J.C. Vogel. 1970. Deuterium content of organic matter. *Earth and Planetary Science Letters*, **7**:307-313.

Schmidt, H.-L. 1995. Controlling factors of isotopic patterns of natural compounds. Paper given at Scottish Crop Research Institute, Invergowrie on 24/4/1995.

Schonwitz, R., W. Stichler and H. Ziegler. 1986. $\delta^{13}C$ values of CO_2 from soil respiration on sites with crops of C_3 and C_4 type of photosynthesis. *Oecologia*, **69**:305-308.

Schultz, D.J. and J.A. Calder. 1976. Organic carbon $^{13}C/^{12}C$ variations in estuarine sediments. *Geochimica et Cosmochimica Acta*, **40**:381-385.

Schulze, E.-D., O.L. Lange, L. Kappen, M. Evenari and U. Buschbom. 1975. The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armenica* L. under desert conditions. II. The significance of leaf water status and internal carbon dioxide concentration. *Oecologia*, **18**:219-233.

Schuster, W.S. and R.K. Manson. 1990. An examination of the advantages of C_3-C_4 intermediate photosynthesis in warm environments. *Plant, Cell and Environment*, **13**:903-912.

Scott, D.B. and F.S. Medioli. 1978. Vertical zonations of marsh foraminifera as accurate indicators of former sea-levels. *Nature*, **272**:528-531.

Scott, D.B. and F.S. Medioloi. 1980. Living vs. total foraminifera populations: their relative usefulness in paleoecology. *Journal of Palaeontology*, **54**:814-831.

Scott, D.B. and F.S. Medioli. 1986. Foraminifera as sea-level indicators. **In:** O. Van de Plassche (ed.). *Sea level research: A manual for the collection and evaluation of data.* Geobooks, Norwich.

Seeman, J.R. and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaeseolus vulgaris*, L. *Planta*, **164**:151-162.

Shackleton, N.J. 1969. The last interglacial in the marine and terrestrial records. *Proceedings of the Royal Society of London, B*, **174**:135-154.

Shackleton, N.J., J.D.H. Wiseman and H.A. Buckley. 1973. Non-equilibrium isotopic fractionation between sea-water and planktonic foraminiferal tests. *Nature*, **242**:177-179.

Shennan, C., R. Hunt and E.A.C. MacRobbie. 1987,a. Salt toerance in *Aster tripolium* L. I. The effect of salinity on growth. *Plant, Cell and Environment*, **10**:59-65.

Shennan, C., R. Hunt and E.A.C. MacRobbie. 1987,b. Salt tolerance in *Aster tripolium* L. II. Ionic regulation. *Plant, Cell and Environment*, **10**:67-74.

Shennan, I. 1986 Flandrian sea-level changes in the Fenland, II: Tendencies of sea-level movement, altitudinal changes and local and regional factors. *Journal of Quaternary Science*, **1**: 155-179.

Shennan, I., J.B. Innes, A.J. Long and Y. Zong. 1995. Holocene relative sea-level changes and coastal vegetation history at Kentra Moss, Argyll, northwest Scotland. *Marine Geology*, **124**: 43-59.

Shennan, I. and M.J.Tooley. 1987. Conspectus of fundamental and strategic research on sea-level changes. Ch.13 in M.J.Tooley and I.Shennan (eds.), *Sea-level Changes.* Oxford, Blackwell.

Shumway, S.D. and M.D. Bertness. 1992. Salt stress limitation of seedling recruitment in a salt marsh plant community. *Oecologia*, **92**:490-497.

Siddiqui, A.A., P.K. Srivastav, Shova Beck, B.N. Brahmchari and K. Thangavelu. 1994. Genotypic variability and correlation studies of leaf characters in *Terminalia* species. *Indian Journal of Genetics and Plant Breeding*, **53**:85-90.

Siegenthaler, U. and U. Eicher. 1986. Stable oxygen and carbon isotope analyses. **In:** B.E. Berglund (ed.). *Handbook of Holocene Palaeoecology and Palaeohydrology.* J.Wiley and Sons, Chichester.

Singer, L.E. and D.C. Havill. 1993. Resistance to divalent manganese of saltmarsh plants. *Journal of Ecology*, **81**:797-806.

Singh, K. 1991. Tolerance of trees to soil salinity. *Journal of the Indian Society of Soil Science*, **39**:549-556.

Slocombe, S.P., G.C. Whitelam and W. Cockburn. 1993. Investigation of phosphenolpyruvate carboxylase (PEPlase) in *Mesembryanthemum crystallinum* L. in C_3 and CAM photosynthetic states. *Plant, Cell and Environment*, **16**:403-411.

Smedley, M.P., T.E. Dawson, J.P. Comstock, L.A. Donovan, D.E. Shernill, CS Cook and J.R. Ehleringer. 1991. Seasonal carbon isotope discrimination in a grassland community. *Oecologia*, **85**:314-320.

Van Smeerdijk, D.G. 1989. A palaeoecological and chemical study of a peat profile from the Assendelver Polder (The Netherlands). *Reviews of Palaeobotany and Palynology*, **58**:231-288.

Smith, A.G. and L.A. Morgan. 1989. A succession to ombrotrophic bog in the Gwent levels and its demise: A Welsh parallel to the peats of the Somerset Levels. *New Phytologist*, **112**:145-168.

Smith, B.N. and S. Epstein. 1970. Biogeochemistry of the stable isotopes of hydrogen and carbon in saltmarsh biota. *Plant Physiology*, **46**:738-742.

Smith, B.N. and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology*, **47**:380-384.

Sofer, Z. 1980. Preparation of carbon dioxide for stable carbon isotope analysis of petroleum fractions. *Analytical Chemistry*, **52**:1389-1391.

Sparling, J.H. 1967. The occurrence of *Schoenus nigricans* L. in blanket bogs. I: Environmental conditions affecting the growth of *S. nigricans* in blanket bog. *Journal of Ecology*, **55**:1-13.

Steers, J.A. 1953. The east coast floods. *Geographical Journal*, **119**:280-298.

Sternberg, L.d.S.L. 1989,a. Oxygen and hydrogen isotope ratios in plant cellulose:mechainisms and applications. **In:** P.W. Rundel, J.R. Ehleringer and K.A. Nagy (eds.). *Stable isotopes in ecological research*. Springer Verlag, Berlin.

Sternberg, L.d.S.L. 1989,b. Oxygen and hydrogen isotope measurements in plant cellulose analysis. **In:** H.F. Linskens and J.F. Jackson (eds.). *Modern methods of plant analysis*. New series, volume 10, Plant Fibers. Springer Verlag, Berlin.

Sternberg, L.d.S.L. and M.J. Deniro. 1983. Biogeochemical implications of the isotopic equilibrium factor between the oxygen atom of acetone and water. *Geochimica et Cosmochimica Acta*, **47**:2271-2274.

Sternberg, L.d.S.L., M.J. DeNiro and H.B. Johnson. 1986. Oxygen and hydrogen isotope ratios of water from photosynthetic tissues of CAM and C_3 plants. *Plant Physiology*, **82**:428-431.

Sternberg, L.d.S.L., M.J. DeNiro, D. McJunkin, R. Berger and J.E. Keeley. 1985. Carbon, oxygen and hydrogen isotope abundances in *Stylites* reflect its unique physiology. *Oecologia*, **67**:598-600.

Sternberg, L.d.S.L., N. Ish-Shalom-Gordon, M. Ross and J. O'Brien. 1991. Water relations of coastal plant communities near the ocean/freshwater boundary. *Oecologia*, **88**:305-310.

Sternberg, L.d.S.L., S.S. Mulkey and S.J. Wright. 1989. Oxygen isotope stratification in a tropical moist forest. *Oecologia*, **81**:51-56.

Sternberg, L.d.S.L. and P.K. Swart. 1987. Utilization of freshwater and ocean water by coastal plants of Southern Florida. *Ecology*, **68**:1898-1905.

Stiles, W. and E.C. Cocking. 1967. An introduction to the principles of plant physiology, Third edition . Methuen, London.

Stuiver, M. and T.F. Braziunas. 1987. Tree cellulose $^{13}C/^{12}C$ isotope ratios and climatic change. *Nature*, **328**:58-60.

Sukamar, R., R. Ramesh, R.K. Pont and G. Rajagopalan. 1993. A $\delta^{13}C$ record of late Quaternary climate change from tropical peats in southern India. *Nature*, **364**:703-706.

Surif, M.B. and J. Raven. 1989. Exogenous inorganic carbon sources for photosynthesis in seawater by members of the *Fucales* and the *Laminariales* (Phaeophyta): ecological and taxonomic implications. *Oecologia*, **78**:97-105.

Surif, M.B. and J.Raven. 1990. Photosynthetic gas exchange under emersed conditions in eulittoral and normally submersed members of the Fucales and Laminariales: Interpretation in relation to carbon isotope ratio and water use efficiency. *Oecologia,* **82**, 68-80.

Swart, P.K., L.d.S.L. Sternberg, R. Steinen and S.A. Harrison. 1989. Controls on the oxygen and hydrogen isotopic composition of the waters of Florida Bay, USA. *Chemical Geology*, **79**:113-123.

Switsur, V.R., J. Waterhouse, E.M. Field, T. Carter, M. Hall, A.M. Pollard, I. Robertson, J.R. Pilcher and T.H.E. Heaton. 1994. Stable isotope studies in tree rings of oak from the English Fenland and Northern Ireland. **In:** B.M. Funnell and R.L.F. Kay (eds.). *Palaeoclimate of the Last Glacial/Interglacial Cycle*. Natural Environment Research Centre, Swindon.

Tanaka, Y. 1991. Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. *Hydrobiologia*, **220**:119-130.

Tansley, A.G. 1939. *The British islands and their vegetation*. Cambridge University Press, Cambridge.

Tardieu, F. and W.J. Davies. 1992. Stomatal response to abscisic acid is a function of current plant water status. *Plant Physiology*, **98**:540-545.

Tardieu, F. and W.J. Davies. 1993. Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. *Plant, Cell and Environment*, **16**:341-349.

Tardieu, F., J. Zhang and D.J.G. Gowing. 1993. Stomatal control by both ABA in the xylem sap and leaf water status: a test of a model for droughted or ABA-fed field-grown maize. *Plant, Cell and Environment*, **16**:413-420.

Tardieu, F., J. Zhang, N. Kateji, O. Bethenod, S. Palmer and W.J. Davies. 1992. Xylem ABA controls the stomatal conductance of field-grown maize to soil compaction or soil drying. *Plant, Cell and Environment*, **15**:193-197.

Thomas, C., S.D. Davis and G. Tallman. 1991. Responses of stomata of senescing and nonsenescing leaves of *Nicotiana glauca* to changes in intercellular concentrations of leaf carbon dioxide. *Plant, Cell and Environment*, **14**:971-978.

Tiku, B.L. 1976. Effect of salinity on the photosynthesis of the halophytes *Salicornia rubra* and *Distichlis stricta*. *Physiologia Plantarum*, **37**:23-28.

Treichel, S. and P. Bauer. 1974. Different NaCl-dependences of the circadian CO_2-gas-exchange of some halophial growing coastal plants. *Oecologia*, **17**:87-95.

Trejo, C.L., W.J. Davies and L.d.M.P. Ruiz. 1993. Sensitivity of stomata to abscisic acid. *Plant Physiology*, **102**:497-502.

Troels-Smith, J. 1955. Characterization of Unconsolidated Sediments. Geological Survey of Denmark, Series IV, 3, Copenhagen.

Troughton, J.H. 1979. $\delta^{13}C$ as an indicator of carboxylation reactions. **In:** M. Gibbs and E. Latzko (eds.). *Photosynthesis II: Photosynthetic carbon metabolism and related processes*. Springer Verlag, Berlin.

Tukey, H.B. 1970. The leaching of substances from plants. *Annual Review of Plant Physiology*, **21**:305-324.

Tyree, M.T. and F.W. Ewers. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist*, **119**:345-360.

Urey, H.C. 1947. The thermodynamic properties of isotopic substances. *Journal of the Chemistry Society*, **152**:190-219.

Urey, H.C. 1948. Oxygen isopes in nature and in the laboratory. *Science*, **108**:489-496.

Vaquer, A. and P. Heurteaux. 1989. Recent modification of the aquatic vegetation in the Etang de Vaccares (Camargue, France) in relation with the human disturbances. *Annales Limnologia*, **25**:25-38.

Vernon, D.M., J.A. Ostrem and H.J. Bohnert. 1993. Stress perception and response in a facultative halophyte: regulation of salinity-induced genes in *Mesembryanthemum crystallinum*. *Plant, Cell and Environment*, **16**:437-444.

Vitousek, P.M., C.B. Field and P.A. Matson. 1990. Variation in foliar $\delta^{13}C$ in Hawaiian *Metrosideros polymorpha*: A case of internal resistance? *Oecologia*, **84**:362-370.

Vogel, J.C., A. Fuls and A. Danin. 1986. Geographical and envronmental distribution of C_3 and C_4 grasses in the Sinai, Negev and Judean deserts. *Oecologia*, **70**:258-265.

Walker, D. 1982. Vegetation's fourth dimension. *New Phytologist*, **90**:419-429.

Wang, H., C. Zhang and H. Liang. 1995. Seasonal changes of polyamines in habitat adaptation of different ecotypes of reed plants. *Oecologia*, **101**:119-123.

Warren, R.S. and W.A. Niering. 1993. Vegetation change on a northeast tidal marsh: interaction of sea-level rise and marsh accretion. *Ecology*, **74**:96-103.

Weisner, S.E.B. 1988. Factors affecting the internal oxygen supply of *Phragmites australis* (Cav.) Trin. ex Steudal. *Aquatic Botany*, **31**:329-336.

Welker, J.M. 1993. Carbon isotope discrimination and water-use efficiency. **In:** G.A.F. Hendry and J.P. Grime (eds.). *Methods in comparative plant ecology*. Chapman and Hall, London.

Welker, J.M., P.A. Wookey, A.N. Parsons, M.C. Press, T.V. Callaghan and J.A. Lee. 1993. Leaf carbon isotope discrimination and vegetative responses of *Dryas octopetala* to temperature and water manipulations in a High Arctic polar semi-desert, Svalbard. *Oecologia*, **95**:463-469.

Wetzel, R.G. 1988. Water as an environment for plant life. **In:** J.J. Symoens (ed.). *Vegetation of inland waters*. Kluwer Academic, Dordrecht.

White, J.W.C. 1993. Stable hydrogen isotope ratios in plants: A review of current theory and some potential applications. **In:** J.R. Ehleringer, A.E. Hall and G.D. Farquhar (eds.). *Stable Isotopes and Plant-Carbon Water Relations*. Academic Press, San Diego.

White, J.W.C., P. Ciais, R.A. Figge, R. Kenny and V. Markgraf. 1994. A high-resolution record of atmospheric CO_2 content from carbon isotopes in peat. *Nature*, **367**:153-156.

White, J.W.C., E.R. Cook, J.R. Lawrence and W.S. Broecker. 1985. The D/H ratios of sap in trees: Implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochimica Acta*, **49**:237-246.

Wigley, T.M.L., B.M. Gray and P.M. Kelly. 1978. Climatic interpretation of $\delta^{18}O$ and δD in tree rings. *Nature*, **271**:92-93.

Wilson, A.T. 1978. Reply to Wigley, T.M.L, B.M.Gray and P.M.Kelly, *Nature* **271**,92-93. *Nature*, **271**:93.

Wilson, A.T. and M.J. Grinsted. 1977. $^{13}C/^{12}C$ in cellulose and lignin as palaeothermometers. *Nature*, **265**:133-135.

Winter, K. 1974. The effect of NaCl salinity on growth and photosynthesis in the halophytes *Mesembryanthemum nodiflorum* L. and *Suaeda maritima* L. Dum. *Oecologia*, **17**:317-324.

Winter, K. 1979. Photosynthetic and water relationships of higher plants in a saline environment. **In:** R.L. Jeffries and A.J. Davy (eds.). *Ecological processes in coastal environments*. Blackwell, Oxford.

Wong, S.C., I.R. Cowan and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature*, **282**:424-426.

Woodell, S.R.J. and A. Dale. 1993. Biological flora of the British Isles: *Armeria maritima* (Mill.) Willd. (Statice armeria L.; S.maritima Mill.). *Journal of Ecology*, **81**:573-588.

Woodrow, I.E., J.T. Ball and J.A. Berry. 1990. Control of photosynthetic carbon dioxide fixation by the boundary layer, stomata and ribulose 1,5-biphosphate carboxylase/oxygenase. *Plant, Cell and Environment*, **13**:339-347.

Woodward, F.I. and F.A. Bazzaz. 1988. The response of stomatal density to CO_2 partial pressure. *Journal of Experimental Botany*, **39**:1771-1781.

Woodward, F.I. and T.M. Smith. 1994. Global photosynthesis and stomatal conductance: modelling the controls by soil and climate. *Advances in Botanical Research*, **20**:1-41.

Yakir, D. 1992. Varaitions in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. *Plant, Cell and Environment*, **15**:1005-1020.

Yakir, D., J. Berry and L. Giles. 1991. Autotrophy in maize husk leaves. Evaluation using natural abundance of stable isotopes. *Plant Physiology*, **97**:1196-1198.

Yakir, D., J.A. Berry, L. Giles and C.B. Osmond. 1994. Isotopic heterogeneity of water in transpiring leaves: identification of the component that controls the $\delta^{18}O$ of atmospheric O_2 and CO_2. *Plant, Cell and Environment*, **17**:73-80.

Yakir, D. and M.J. DeNiro. 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. *Plant Physiology*, **93**:325-332.

Yakir, D., M.J. DeNiro and J.E. Eprath. 1990. Effects of water stress on oxygen, hydrogen and carbon isotope ratios in 2 species of cotton plants. *Plant, Cell and Environment*, **13**:949-955.

Yakir, D., M.J. DeNiro and J.R. Gat. 1990. Natural deuterium and oxygen-18 enrichment in leaf water of cotton plants grown under wet and dry conditions: evidence for water compartmentation and its dynamics. *Plant, Cell and Environment*, **13**:49-56.

Yakir, D., A. Isaar, J. Gat, E. Adar, P. Trimborn and J. Wipp. 1994. ^{13}C and ^{18}O of wood from the Roman siege rampart in Masada, Israel (AD 70-73): Evidence for a less arid climate in the region. *Geochimica et Cosmochimica Acta*, **58**:3535-3539.

Yakir, D. and Y. Israeli. 1995. Reduced solar irradiance effects on net primary productivity (NPP) and the $\delta^{13}C$ and $\delta^{18}O$ values in plantations of *Musa* sp., *Musaceae*. *Geochimica et Cosmochimica Acta*, **59**:2149-2151.

Yakir, D. and Y. Yechieli. 1995. Plant invasion of newly hypersaline Dead Sea shores. *Nature*, **374**:803-805.

Yapp, C.J. and S. Epstein. 1977. Climatic implications of D/H ratios of meteoric water over North America (9500-22000 BP) as inferred from ancient wood cellulose. *Earth and Planetary Science Letters*, **34**:333-350.

Yapp, C.J. and S. Epstein. 1982,a. Climatic significance of the hydrogen isotope ratios in tree cellulose. *Nature*, **297**:636-639.

Yapp, C.J. and S. Epstein. 1982,b. A reexamination of cellulose carbon-bound hydrogen δD measurements and some factors affecting plant-water D/H relationships. *Geochimica et Cosmochimica Acta*, **46**:955-965.

Yeo, A.R. 1983. Salinity resistance: physiologies and prices. *Physiologia Plantarum*, **58**:214-222.

Youngman, A.L. and S.A. Heckthorn. 1992. Effect of salinity on water relations of two growth forms of *Suaeda calceoliformis*. *Functional Ecology*, **6**:686-692.

Zhang, J., J.D. Marshall and B.C. Jaquish. 1993. Genetic differentiation in carbon isotope discrimnation and gas exchange in *Pseudotsuga menziesii* L. *Oecologia*, **93**:80-87.

Ziegler, H., C.B. Osmond, W. Strichler and P. Trimborn. 1976. Hydrogen isotope discrimination in higher plants: Correlations with photosynthetic pathway and environment. *Planta*, **128**:85-92.

Ziska, L.H., R.B. Hutmacher, G.J. Hoffman and T.M. DeJong. 1989. Changes in leaf water status associated with salinity in mature, field-grown *Prunus salinica*. *Physiologia Plantarum*, 77:141-149.

Ziska, L.H., J.R. Seeman and T.M. DeJong. 1990. Salinity induced limitations in *Prunus salicina*, a deciduous tree species. *Plant Physiology*, **93**:864-870.

Zong, W., R. Chen, K. Taniguili and K. Kondo. 1991. A chromosome study in intraspecific polyploidy of *Phragmites australis* and its related species. *Kromosomo*, **2**:2168-2172.

Zong, Y. 1993. Flandrian sea-level changes and impacts of projected sea-level rise on the coastal lowlands of Morecambe Bay and the Thames Estuary, U.K. Unpublished Ph.D. Thesis, University of Durham.

Zrenner, R. and M. Stiff. 1991. Comparison of the effect of rapidly and gradually developing water-stress on carbohydrate metabolism in spinach leaves. *Plant, Cell and Environment*, **14**:939-946.

Appendix 1

Surface sediment analysis methods.

i. Loss on drying (%).

In the field samples are collected in sealable bags and on return to the laboratory are immediately weighed before being dried in an oven at 100°C for 24 hours. The samples are re-weighed and the proportion of the original wet weight which has been lost is calculated.

ii. Loss on ignition (%).

Weighed samples of oven-dried material (see i., above) are put into weighed ceramic crucibles and heated to 800°C for 4 hours in a muffle furnace. Once cooled they are either immediately re weighed or placed in a dessicator for weighing at a later date. The proportion of the original dry weight which has been lost is calculated.

iii. Salinity (parts per thousand).

A 5 - 15g. weighed sample of oven dried material is mixed with distilled water in a 5:1 or 10:1 ratio (water (ml.): sample (g)), the more water being used for samples with a high organic matter content. This is mixed thoroughly before being placed in a sonic bath for 20 minutes. The supernatant is filtered off and either analysed immediately or kept refrigerated.

The analysis for salinity is by titration of chloride against a mercuric nitrate standard where 1ml. HgNO3 standard = 1 mg Cl^-. The volume of supernatant used in the titration depends on the ratio of sample: water and whether the sample is from a saltmarsh

or freshwater sediment. Typical values are 2ml. for saltmarsh samples and 5ml. for others. To this aliquot enough distilled water is added to make a total of 50ml. On a magnetic stirrer add 10 drops of mixed indicator and then drop-by-drop add 0.05M nitric acid until the colour changes to yellow, when 1ml. excess of the acid is added. Titrate with mercuric nitrate standard until a blue-violet colour persists, noting the volume of standard added. Three blank repeats are needed to normalise the values.

Salinity in parts per thousand is calculated using the following information:

A = (1000/ ml.s aliquot used) × (ml.s titrate used in sample – ml.s titrate used in blank)

B = 100 – loss on drying (%) of sample used

C = (weight of dry sediment used/ B) × 100

D = weight of dry sediment used × ratio of distilled water : sample

E = C – weight of dry sediment used

F = (D/E) × A = concentration of Cl^- in supernatant in parts per million

G = F/1000

H = G × 1.80655 = salinity of supernatant in parts per thousand.

Appendix 2.

Collection and preparation of samples for counting leaf stomatal densities.

Plant material which is collected should be kept as fresh as possible and returned to the laboratory as quickly as possible. If preparation cannot proceed immediately place the leaves in a refrigerator, but ideally they should be used within a few days of collection.

Using a clear nail varnish paint the part of the leaf to be analysed and allow the varnish to set, but not to harden. With a sharp pair of tweasers peel off the layer of varnish and attach to a microscope slide ready for examination at 400× magnification.

Appendix 3

Isolation of α-cellulose from bulk plant material.

This method follows Sternberg (1989b; see also Field 1993b, Robertson 1993). Dried plant material is ground and boiled in distilled water for 2 hours. Under a fume hood the temperature is reduced to 70°C and to each beaker 0.5ml. of concentrated acetic acid and 1g. of mercuric chloride are added. This is repeated 4 times hourly until the plant material is bleached white when it is thoroughly washed with distilled water. The samples are immersed in 17% (weight) sodium hydroxide for 45 minutes and then thoroughly washed before a 10 minute immersion in 10% (volume) acetic acid. A further wash is followed by drying in at c.50°C, after which the cellulose is ready for stable isotope analysis (appendices 4 and 5).

Appendix 4

Production and isolation of CO_2 from α-cellulose for $\delta^{13}C$ analysis.

4 - 6 mg. of α-cellulose (see appendix 3) and an excess (c.1g.) of pre-heated copper filings are put into 9mm outside diameter quartz glass necked cuvettes which have been marked with the sample number and previously heated for 2 hours at 600°C. Each cuvette is pumped to vacuum ($<10^{-3}$ mbar) and sealed. In a muffle furnace the sealed vessels are heated to 850°C for 2 hours and then cooled slowly back to room temperature.

Once cooled the vessels are scored with a glass cutter and placed in a cracker on a vacuum line. The line is pumped out and then isolated from the pump. A dewar flask containing a dry ice/acetone slush is placed over the first trap on the line and one containing liquid nitrogen is put on the second. The vessel is broken and water vapour condenses in trap 1. CO_2 condenses in the second trap and any trace impurities (such as nitrogen) which are registered on the vacuum gauge are pumped away.

Liquid nitrogen is placed around the collection vessel and is taken away from the second trap. Once all the CO_2 has re-condensed, the collection vessel is isolated and, depending upon design, is either physically removed or sealed using an oxyacetylene torch. The CO_2 within the collection vessel is then ready for analysis in a gas isotope ratio mass-spectrometer (see also Sofer 1980, Field 1993a, Heaton 1990a, b, 1991; cf. Murnick & Peer 1994).

Appendix 5

Production and isolation of CO_2 from α-cellulose for $\delta^{18}O$ analysis.

This method follows Sauer and Sternberg (1994, cf. Siegenthaler & Eicher 1986, Mullane *et al.* 1988, Edwards *et al.* 1994, Field *et al.* 1993). Into pre-heated break-seal quartz glass ampules put 6-10mg. of α-cellulose (see appendix 3) and 0.375g. of mercuric chloride. Attach break-seals to vacuum line and pump to vacuum. Place a beaker around each break-seal add an immersion heater and cover with water. Turn on heater and boil for 20-30 minutes to drive off all water from sample. Allow to cool and remove beaker before sealing under vacuum.

Heat ampules at 500°C for 6 hours in a muffle furnace and once cooled place each break-seal ampule into a magnetic breaker unit on a vacuum line. Into a necked collection vessel put 2g. of zinc and attach this to the line before pumping out. Isolate the breaker and the trap and gently heat the zinc until it sublimates and covers the walls of the collecting vessel. (The zinc will scavenge chlorine from the HCl during later heating.) Pump out line fully and then isolate pump, vacuum gauge and collection vessel. Put liquid nitrogen around trap containing small glass balls to increase surface area. Crack break-seal with magnet to release the cocktail of CO, CO_2 and HCl, allowing CO_2 and HCl to condense in trap.

Immerse spark chamber in liquid nitrogen and allow gas to enter. Increase potential difference across electrodes to 5000 volts and maintain until blue spark begins to flicker. Take away liquid nitrogen from spark chamber and warm with heat gun. The CO which has been converted to CO_2 will condense in the trap. Replace the liquid nitrogen around the spark chamber and repeat process until the blue spark has disappeared and all the CO has been converted to CO_2 (cf. Rosenbaum 1993). Remove liquid nitrogen, heat

spark chamber and allow CO_2 to condense in trap. Remove any trace impurities by briefly opening line to pump. Isolate line and remove liquid nitrogen from trap, replacing it around the collection vessel which is now opened to the line. Warm trap to speed movement of CO_2 and HCl and after 5 minutes seal and remove collection vessel.

Heat collection vessel to 200°C for 2 hours, allow to cool and score with glass cutter. On second line pump out new set of collection vessels and cracker with scored vessel containing gaseous mix of CO_2 and H. Put liquid nitrogen on trap and crack vessel. Allow CO_2 to condense in trap, pump off H and re-isolate line. Remove liquid nitrogen from trap, place around collection vessel and allow CO_2 to re-condense. Isolate, seal and remove collection vessel from line. The CO_2 within the collection vessel is then ready for analysis in a gas isotope ratio mass-spectrometer to establish the $\delta^{18}O$ value.

Appendix 6

Tidal data recorded at Heysham 1975-1994;

incidence of inundation at or above Mean High Water Spring Tide (4.5 m O.D.)

and upto Highest Astronomical Tide (5.6 m O.D.).

m. O.D.	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6
1994	132	107	87	72	53	37	22	20	13	8	4	1
1993	126	101	87	76	62	51	42	32	22	13	7	3
1992	114	92	71	61	51	42	35	26	17	9	3	2
1991	125	102	76	60	40	26	16	11	7	4	3	1
1990	136	113	85	61	49	38	31	26	19	11	9	4
1989	116	98	81	64	50	40	32	27	20	14	11	9
1988	103	83	72	59	49	43	33	27	16	15	10	6
1987	113	86	60	45	37	28	22	15	12	8	5	3
1986	121	101	83	66	51	30	22	13	7	5	2	1
1985	100	82	66	52	39	30	18	12	8	6	5	4
1984	124	107	94	83	71	55	49	35	25	16	10	6
1983	140	108	99	78	57	46	34	21	18	14	9	5
1982	153	133	104	73	50	32	19	7	3	2	1	0
1981	156	126	104	86	77	65	52	42	33	23	17	7
1980	140	120	105	91	79	61	52	43	34	29	23	5
1979	146	129	107	86	69	56	41	34	26	21	15	7
1978	149	126	109	81	63	43	28	19	10	7	5	1
1977	153	132	117	94	69	58	38	22	11	10	8	4
1976	139	127	102	85	68	53	39	24	19	13	7	4
1975	108	94	89	67	57	43	33	22	15	10	6	5

Appendix 7.
Roudsea Marsh Surface Sediment Analysis, 06/04/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	---	---	---	---
sample 2	---	---	---	---
sample 3	---	---	---	---
sample 4	---	---	---	---
sample 5	---	---	---	---
mean±s.d.	---	---	---	---

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	50.94	11.84	3881.3	7.01
sample 2	47.00	10.50	4352.8	7.86
sample 3	42.47	9.78	3779.3	6.83
sample 4	44.21	8.05	4429.4	8.00
sample 5	48.68	10.32	4417.2	7.98
mean±s.d.	46.66±3.39	10.10±1.37	4172.0±315.3	7.54±0.57

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	77.90	31.74	1032.7	1.87
sample 2	78.48	40.52	1039.3	1.88
sample 3	78.60	33.28	1021.0	1.84
sample 4	70.43	22.85	1515.7	2.74
sample 5	77.61	35.16	761.6	1.38
mean±s.d.	76.60±3.48	32.71±6.43	1074.0±273.1	1.94±0.49

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	83.72	79.04	128.3	0.23
sample 2	81.83	73.72	97.7	0.18
sample 3	85.66	84.95	97.1	0.18
sample 4	86.48	67.01	59.4	0.11
sample 5	77.91	61.40	90.7	0.16
mean±s.d.	83.12±3.43	73.22±9.36	94.7±24.5	0.17±0.04

Roudsea Marsh Surface Sediment Analysis, 22/04/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	55.33	11.26	1356.3	2.45
sample 2	50.53	10.06	988.8	1.79
sample 3	49.03	10.86	1684.1	3.04
sample 4	50.20	7.83	1617.0	2.92
sample 5	50.87	8.51	1361.8	2.46
mean±s.d.	51.19±2.41	9.71±1.47	1401.6±274.0	2.53±0.49

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	47.12	9.48	2132.3	3.85
sample 2	50.90	10.49	2054.7	3.71
sample 3	47.72	10.21	2202.1	3.98
sample 4	52.58	10.64	3318.9	6.00
sample 5	43.38	9.32	2036.1	3.68
mean±s.d.	48.34±3.57	10.03±0.60	2348.8±546.3	4.24±1.02

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	80.22	33.07	586.8	1.06
sample 2	70.01	26.67	475.5	0.86
sample 3	73.04	25.84	764.1	1.38
sample 4	77.16	34.71	201.3	0.36
sample 5	80.27	38.40	673.5	1.22
mean±s.d.	76.14±4.53	31.74±5.37	546.2±225.4	0.99±0.41

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	82.98	77.15	73.8	0.13
sample 2	77.98	58.14	90.4	0.16
sample 3	85.13	78.38	111.8	0.20
sample 4	81.35	72.59	96.3	0.17
sample 5	84.56	78.68	65.7	0.12
mean±s.d.	82.4±2.88	72.99±8.65	87.6±18.3	0.16±0.03

Roudsea Marsh Surface Sediment Analysis, 05/05/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	48.40	10.24	3065.1	5.54
sample 2	52.47	10.86	3646.1	6.59
sample 3	56.42	10.51	3533.8	6.38
sample 4	51.29	11.22	3775.1	6.82
sample 5	49.66	10.23	3700.0	6.68
mean±s.d.	51.65±3.09	10.61±0.43	3544.0±281.8	6.40±0.51

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	52.69	10.63	4624.2	8.35
sample 2	55.74	8.84	3990.1	7.21
sample 3	57.08	12.52	4812.3	8.69
sample 4	45.44	10.55	4562.7	8.24
sample 5	54.58	10.97	2995.8	5.41
mean±s.d.	53.11±4.58	10.70±1.31	4197.0±738.3	7.58±1.37

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	82.15	36.49	1656.8	2.99
sample 2	69.39	28.97	1213.1	2.19
sample 3	74.75	30/62	1511.6	2.73
sample 4	78.52	37.89	1401.9	2.53
sample 5	72.99	26.32	1711.5	3.09
mean±s.d.	75.56±4.94	32.06±4.95	1499.0±200.8	2.71±0.36

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	81.44	81.82	113.9	0.21
sample 2	78.01	52.34	95.8	0.17
sample 3	81.96	74.76	114.5	0.21
sample 4	76.28	70.16	93.3	0.17
sample 5	76.00	58.53	56.8	0.10
mean±s.d.	78.74±2.82	67.52±11.99	94.9±23.4	0.17±0.04

Roudsea Marsh Surface Sediment Analysis, 19/05/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	42.87	11.61	8262.3	14.93
sample 2	45.08	11.71	9198.0	16.61
sample 3	31.64	10.15	8480.2	15.32
sample 4	36.97	13.01	7416.3	13.40
sample 5	42.45	9.61	5323.4	9.61
mean±s.d.	39.80±5.45	11.22±1.35	7736.0±1490.9	13.98±2.69

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	38.00	13.38	15540.8	28.08
sample 2	41.37	13.85	7227.8	13.06
sample 3	33.52	12.05	16610.1	30.01
sample 4	43.82	12.22	7468.0	13.49
sample 5	42.42	12.45	10825.1	19.56
mean±s.d.	39.83±4.13	12.79±0.78	11534.4±4399	20.84±7.95

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	81.87	41.36	1029.7	1.86
sample 2	74.28	29.59	1359.1	2.46
sample 3	78.18	37.23	1032.7	1.87
sample 4	78.00	36.05	959.0	1.73
sample 5	78.84	37.16	677.7	1.22
mean±s.d.	78.23±2.71	36.28±4.25	1011.6±242.8	1.83±0.44

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	79.98	84.15	170.2	0.31
sample 2	79.10	73.32	90.0	0.16
sample 3	82.52	82.17	118.6	0.21
sample 4	71.33	57.59	88.4	0.16
sample 5	80.09	85.06	29.8	0.05
mean±s.d.	78.60±4.26	76.46±11.53	99.4±51.1	0.18±0.09

Roudsea Marsh Surface Sediment Analysis, 08/06/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	46.17	11.47	5217.4	9.43
sample 2	39.88	12.50	8102.9	14.64
sample 3	42.88	12.10	7493.0	13.54
sample 4	47.76	12.35	5086.2	9.19
sample 5	49.20	15.25	11435.2	20.66
mean±s.d.	45.18±3.78	12.73±1.46	7466.9±2592.3	13.49±4.68

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	54.23	15.33	6583.2	11.89
sample 2	35.06	10.94	10696.8	19.32
sample 3	49.76	13.10	9743.1	17.60
sample 4	40.51	9.97	7893.3	14.26
sample 5	43.72	10.30	12003.9	21.69
mean±s.d.	44.66±7.55	11.93±2.26	9384.1±2167.0	16.95±3.91

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	74.26	28.79	528.6	0.95
sample 2	72.10	27.09	977.1	1.77
sample 3	78.62	34.58	278.7	0.50
sample 4	73.26	30.17	410.6	0.74
sample 5	72.84	29.72	894.9	1.62
mean±s.d.	74.22±2.58	30.07±2.78	618.0±304.8	1.12±0.55

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	77.12	76.89	142.4	0.26
sample 2	78.14	80.50	67.1	0.12
sample 3	72.27	55.22	184.2	0.33
sample 4	74.52	71.31	102.6	0.19
sample 5	77.83	79.89	119.6	0.21
mean±s.d.	75.97±2.52	72.76±10.46	123.2±43.8	0.22±0.08

Roudsea Marsh Surface Sediment Analysis, 22/06/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	53.64	12.95	1123.6	2.03
sample 2	57.24	12.38	1157.9	2.09
sample 3	51.16	12.71	1599.0	2.89
sample 4	61.21	16.75	839.7	1.52
sample 5	57.64	11.50	1616.8	2.92
mean±s.d.	56.18±3.88	13.26±2.03	1267.4±334.5	2.29±0.60

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	48.65	10.91	2295.7	4.15
sample 2	56.46	9.20	1291.7	2.33
sample 3	47.88	10.47	1986.6	3.59
sample 4	55.10	10.08	1039.0	1.88
sample 5	54.07	11.31	2144.9	3.87
mean±s.d.	52.43±3.91	10.39±0.81	1751.6±553.5	3.16±1.00

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	73.08	34.73	736.7	1.33
sample 2	72.65	29.94	452.0	0.82
sample 3	74.82	33.85	622.6	1.12
sample 4	70.80	28.90	247.5	0.45
sample 5	76.58	35.57	267.6	0.48
mean±s.d.	73.59±2.20	32.60±2.99	465.3±215.1	0.84±0.39

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	77.19	64.93	76.8	0.14
sample 2	70.31	76.10	135.1	0.24
sample 3	76.93	75.72	126.0	0.23
sample 4	77.74	76.00	63.0	0.11
sample 5	79.19	80.67	78.8	0.14
mean±s.d.	76.27±3.45	74.68±5.83	96.0±32.3	0.17±0.06

Roudsea Marsh Surface Sediment Analysis, 11/07/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	46.36	8.93	2024.8	3.66
sample 2	47.63	9.85	1237.0	2.23
sample 3	51.04	9.18	1342.9	2.43
sample 4	56.91	8.96	1268.2	2.29
sample 5	49.38	8.26	1435.2	2.59
mean±s.d.	50.26±4.11	9.04±0.57	1461.6±324.0	2.64±0.59

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	55.21	11.31	3245.1	5.86
sample 2	47.42	11.81	6846.9	12.37
sample 3	59.49	14.06	1259.8	2.28
sample 4	53.93	10.42	2605.5	4.71
sample 5	50.43	9.21	2359.1	4.26
mean±s.d.	53.30±4.61	11.36±1.80	3263.3±2127.6	5.90±3.84

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	71.84	29.64	252.5	0.46
sample 2	71.83	29.08	421.6	0.76
sample 3	72.01	26.79	485.9	0.88
sample 4	69.70	24.04	534.3	0.97
sample 5	74.92	31.16	460.3	0.83
mean±s.d.	72.06±1.86	28.14±2.78	434.9±112.8	0.79±0.20

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	81.61	70.97	85.6	0.15
sample 2	83.73	89.61	124.4	0.22
sample 3	82.96	76.99	410.2	0.74
sample 4	83.82	90.58	88.8	0.16
sample 5	82.34	83.07	72.9	0.13
mean±s.d.	82.89±0.94	82.24±8.35	156.4±143.2	0.28±0.26

Roudsea Marsh Surface Sediment Analysis, 27/07/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	49.95	8.71	8737.5	15.78
sample 2	55.36	10.36	8628.0	15.59
sample 3	47.01	10.56	7467.7	13.49
sample 4	56.20	4.80	6001.1	10.84
sample 5	49.41	8.91	6220.1	11.24
mean±s.d.	51.59±4.00	8.67±2.32	7410.9±1289.4	13.39±2.33

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	54.08	11.74	5752.7	10.39
sample 2	46.36	8.97	7491.8	13.53
sample 3	64.01	13.75	7885.6	14.25
sample 4	55.53	17.20	5725.9	10.34
sample 5	47.19	11.26	5903.2	10.66
mean±s.d.	53.43±7.17	12.58±3.09	6551.9±1049.3	11.84±1.90

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	69.29	27.03	542.9	0.98
sample 2	65.89	26.33	1126.0	2.03
sample 3	73.03	35.00	215.1	0.39
sample 4	70.72	31.06	641.7	1.16
sample 5	71.50	31.30	528.1	0.95
mean±s.d.	70.09±2.70	30.14±3.54	610.8±329.5	1.10±0.60

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	81.87	86.00	75.3	0.14
sample 2	80.85	85.12	199.0	0.36
sample 3	81.13	90.20	120.9	0.22
sample 4	80.27	90.40	127.8	0.23
sample 5	81.16	91.27	153.2	0.28
mean±s.d.	81.06±0.58	88.60±2.82	135.2±45.4	0.24±0.08

Roudsea Marsh Surface Sediment Analysis, 10/08/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	49.40	10.31	6529.9	11.80
sample 2	55.39	12.05	5033.6	9.09
sample 3	54.72	11.65	11088.3	20.03
sample 4	52.84	11.93	9951.4	17.98
sample 5	46.97	9.58	10556.3	19.07
mean±s.d.	51.86±3.59	11.10±1.10	8631.9±2685.4	15.59±4.85

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	49.78	12.69	9104.8	16.45
sample 2	50.33	11.11	5970.7	10.79
sample 3	44.51	8.02	8415.1	15.20
sample 4	47.45	10.51	6894.1	12.45
sample 5	51.96	10.46	5547.3	10.02
mean±s.d.	48.81±2.89	10.55±1.68	7186.4±1536.2	12.98±2.78

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	66.70	23.25	1023.5	1.85
sample 2	69.95	26.67	741.0	1.34
sample 3	70.68	26.68	580.8	1.05
sample 4	65.27	25.68	824.8	1.49
sample 5	68.23	25.88	710.1	1.28
mean±s.d.	68.17±2.24	25.63±1.41	776.0±163.8	1.40±0.30

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	76.26	92.12	244.8	0.44
sample 2	79.80	84.46	126.6	0.23
sample 3	73.66	58.22	128.7	0.23
sample 4	74.24	66.88	194.3	0.35
sample 5	79.57	89.79	30.8	0.06
mean±s.d.	76.71±2.89	78.29±14.95	145.0±80.7	0.26±0.15

Roudsea Marsh Surface Sediment Analysis, 24/08/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	48.39	9.07	6212.6	11.22
sample 2	49.73	7.99	7025.5	12.69
sample 3	48.04	9.35	8030.9	14.51
sample 4	50.32	8.62	5430.0	9.81
sample 5	55.40	9.90	4447.9	8.03
mean±s.d.	50.38±2.96	8.99±0.72	6229.4±1386.7	11.25±2.51

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	51.04	11.18	7985.7	14.43
sample 2	51.85	9.33	7568.4	13.67
sample 3	46.35	10.00	7726.3	13.95
sample 4	49.66	9.79	5651.3	10.21
sample 5	49.21	9.69	6115.2	11.05
mean±s.d.	49.62±2.11	10.00±0.70	7009.4±1051.6	12.66±1.90

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	64.34	27.53	692.8	1.25
sample 2	72.01	27.31	524.7	0.95
sample 3	68.85	25.75	429.8	0.78
sample 4	66.12	21.63	576.5	1.04
sample 5	72.99	31.10	416.3	0.75
mean±s.d.	68.86±3.71	26.66±3.43	528.0±113.6	0.95±0.21

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	81.35	87.49	188.0	0.34
sample 2	79.15	91.04	205.5	0.37
sample 3	79.78	88.44	263.6	0.48
sample 4	79.57	82.25	118.1	0.21
sample 5	81.76	84.61	303.4	0.55
mean±s.d.	80.32±1.16	86.77±3.41	215.7±71.4	0.39±0.13

Roudsea Marsh Surface Sediment Analysis, 12/09/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	50.46	10.03	3117.1	5.63
sample 2	52.27	9.00	1438.2	2.60
sample 3	58.29	8.69	2862.2	5.17
sample 4	58.50	8.36	3547.0	6.41
sample 5	51.17	8.74	3411.5	6.16
mean±s.d.	54.14±3.94	8.96±0.64	2875.2±846.0	5.19±1.52

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	53.62	9.96	2940.9	5.31
sample 2	55.64	19.20	3906.6	7.06
sample 3	57.17	8.83	3015.4	5.45
sample 4	50.12	10.99	4528.2	8.18
sample 5	46.06	9.31	6704.4	12.11
mean±s.d.	52.52±4.47	11.66±4.29	4219.1±1537.3	7.62±2.78

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	74.55	28.84	1596.0	2.88
sample 2	75.20	27.98	1698.4	3.07
sample 3	74.72	26.82	1243.4	2.25
sample 4	77.04	35.64	1408.2	2.54
sample 5	70.77	22.37	1662.4	3.00
mean±s.d.	74.46±2.29	28.33±4.79	1521.7±191.6	2.75±0.35

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	85.37	17.27	106.3	0.19
sample 2	70.52	67.43	150.5	0.27
sample 3	80.44	58.20	68.1	0.12
sample 4	86.04	91.43	68.1	0.12
sample 5	85.54	86.66	121.7	0.22
mean±s.d.	81.58±6.59	64.20±29.56	102.9±35.5	0.19±0.06

Roudsea Marsh Surface Sediment Analysis, 05/10/94.

Zone 0	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	49.57	9.18	5341.1	9.65
sample 2	62.09	12.14	8364.7	15.11
sample 3	55.36	11.02	6632.3	11.98
sample 4	61.85	11.26	5551.3	10.03
sample 5	53.91	10.78	6326.6	11.43
mean±s.d.	56.56±5.38	10.88±1.08	6443.2±1199.1	11.64±2.17

Zone 1	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	37.07	8.81	8912.4	11.43
sample 2	41.98	10.10	6253.9	11.30
sample 3	45.81	10.07	7777.8	14.05
sample 4	43.13	9.60	5241.3	9.47
sample 5	42.15	8.67	7205.5	13.02
mean±s.d.	42.03±3.17	9.45±0.68	7078.2±1407.4	12.79±2.54

Zone 2	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	77.60	31.63	707.2	1.28
sample 2	68.33	21.00	428.7	0.77
sample 3	68.73	25.71	432.2	0.78
sample 4	74.11	35.41	401.7	0.73
sample 5	87.16	34.42	195.2	0.35
mean±s.d.	75.19±7.73	29.63±6.13	433.0±182.2	0.78±0.33

Zone 3	LOD(%)	LOI(%)	Cl^- (ppm)	Salinity(‰)
sample 1	79.88	85.15	171.3	0.31
sample 2	77.40	71.62	116.8	0.21
sample 3	80.29	85.26	230.8	0.42
sample 4	79.13	75.97	400.9	0.72
sample 5	78.41	84.08	143.2	0.26
mean±s.d.	79.02±1.16	80.42±6.25	212.6±113.5	0.38±0.20

Appendix 8.
Roudsea Marsh *Phragmites australis* density and minimum/maximum height of aerial shoots.
Zone 1, sampled 05/10/94. One quadrat = 4 divisions of 50x50cm.

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
1.1	12	28	130
1.2	4	67	94
1.3	8	41	127
1.4	15	33	122
Σ1.1 - 1.4 (=1m^2)	39	28	130

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
2.1	19	49	146
2.2	19	40	145
2.3	25	29	133
2.4	15	26	135
Σ2.1 - 2.4 (=1m^2)	78	26	146

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
3.1	13	34	124
3.2	8	58	81
3.3	16	48	116
3.4	8	49	123
Σ3.1 - 3.4 (=1m^2)	45	34	124

Roudsea Marsh *Phragmites australis* density and minimum/maximum height of aerial shoots. Zone 2, sampled 05/10/94. One quadrat = 4 divisions of 25x25cm.

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
1.1	19	175	226
1.2	22	185	230
1.3	17	184	271
1.4	17	197	248
Σ1.1 - 1.4 (=1m^2)	75	175	271

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
2.1	21	212	289
2.2	22	188	265
2.3	23	182	256
2.4	14	193	261
Σ2.1 - 2.4 (=1m^2)	80	188	256

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
3.1	22	208	267
3.2	11	213	249
3.3	13	214	279
3.4	20	209	271
Σ3.1 - 3.4 (=1m^2)	66	208	249

Roudsea Marsh *Phragmites australis* density and minimum/maximum height of aerial shoots. Zone 3, sampled 05/10/94. One quadrat = 4 divisions of 25x25cm.

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
1.1	16	81	211
1.2	21	89	155
1.3	18	118	206
1.4	14	124	262
Σ1.1 - 1.4 (=1m^2)	69	81	262

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
2.1	16	110	251
2.2	29	151	247
2.3	20	102	275
2.4	17	95	222
Σ2.1 - 2.4 (=1m^2)	82	95	275

Quadrat number	No. of living stems	Minimum height (cm.)	Maximum height (cm.)
3.1	14	136	209
3.2	30	111	249
3.3	24	134	255
3.4	20	103	272
Σ3.1 - 3.4 (=1m^2)	88	103	272

Appendix 9
Roudsea Marsh *Phragmites australis* plant habits.
Zone 1,sampled 05/10/94.

Plant no.	Height (cm.)	No. of leaves	Length of leaf no. 1 (cm.)
1	71	7	16
2	117	10	16
3	71	10	11
4	77	10	12
5	109	11	14
6	58	9	9
7	123	10	16
8	94	11	16
9	124	11	19
10	94	9	17
11	68	9	12
12	63	8	16
13	114	10	13
14	39	7	11
15	55	8	10
mean±s.d.	85.1±27.44	9.3±1.35	13.9±2.95

Roudsea Marsh *Phragmites australis* plant habits.
Zone 2,sampled 05/10/94.

Plant no.	Height (cm.)	No. of leaves	Length of leaf no. 1 (cm.)
1	243	11	36
2	272	10	39
3	238	12	36
4	234	11	35
5	242	12	36
6	241	10	36
7	175	11	26
8	220	11	29
9	187	11	30
10	181	10	29
11	215	12	38
12	253	11	36
13	240	11	32
14	243	11	31
15	195	11	27
mean±s.d.	225.3±28.74	11.0±0.65	33.1±4.15

Roudsea Marsh *Phragmites australis* plant habits.
Zone 3,sampled 05/10/94.

Plant no.	Height (cm.)	No. of leaves	Length of leaf no. 1 (cm.)
1	242	15	43
2	212	11	35
3	177	10	38
4	216	13	33
5	216	15	36
6	209	13	30
7	203	14	31
8	183	15	27
9	240	15	41
10	239	13	35
11	208	15	29
12	189	14	29
13	220	15	34
14	251	13	36
15	201	12	40
mean±s.d.	213.7±22.05	13.5±1.60	34.5±4.73

Appendix 10

Roudsea Marsh *Phragmites australis* stomatal density raw counts.
Zone 1, leaf 1,sampled on 05/10/94. View size = 84496μm^2.

Plant #	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
1	79	79	87	89	73	82	89	78	86	78	82.0±5.48
2	75	93	89	86	87	87	94	83	86	88	86.5±5.42
3	78	79	73	77	79	76	81	76	75	82	77.6±2.76
4	70	72	67	58	67	57	63	62	68	58	64.2±5.37
5	68	80	73	72	84	73	69	71	76	79	74.5±5.15
6	64	63	62	67	60	70	63	74	69	59	65.1±4.77
7	83	84	87	87	91	85	89	91	82	91	87.0±3.43
8	74	80	68	76	77	77	70	71	77	70	74.0±4.00
9	79	76	76	78	69	60	77	69	75	66	72.5±6.20
10	71	79	82	81	80	75	72	69	83	73	76.5±5.08
11	71	79	77	73	67	72	69	67	64	72	71.1±4.61
12	70	70	69	66	63	62	74	68	66	73	68.4±4.43
13	78	76	78	67	69	77	78	70	76	76	74.5±4.17
14	62	57	51	59	57	62	63	61	67	54	59.3±4.69
15	64	59	57	56	51	54	60	67	69	67	60.4±6.11

Roudsea Marsh *Phragmites australis* stomatal density raw counts.
Zone 2, leaf 1, sampled on 05/10/94. View size = 84496μm^2.

Plant #	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
1	67	87	83	69	89	93	83	75	93	82	82.1±9.19
2	85	74	89	84	92	85	82	81	90	78	84.0±5.54
3	83	83	90	87	89	74	78	82	85	77	82.8±5.25
4	76	89	86	82	80	79	77	74	85	87	81.5±5.10
5	77	80	84	81	84	90	85	92	84	90	84.7±4.79
6	83	80	77	89	82	75	83	87	76	84	81.6±4.62
7	71	62	77	68	79	71	67	71	64	59	68.9±6.28
8	67	81	75	75	76	73	85	70	70	66	73.8±5.98
9	87	84	72	73	71	71	69	74	79	78	75.8±6.01
10	70	64	68	68	77	65	66	63	63	58	66.2±5.07
11	79	78	75	75	77	69	79	84	85	82	78.3±4.74
12	69	76	75	69	69	69	68	61	63	71	69.0±4.59
13	60	61	59	65	62	63	72	57	72	65	63.6±5.08
14	63	67	66	59	73	56	61	67	63	75	65.0±5.91
15	72	68	62	68	61	68	66	61	64	65	65.5±3.60

Roudsea Marsh *Phragmites australis* stomatal density raw counts. Zone 3, leaf 1, sampled on 05/10/94. View size = 84496μm^2.

Plant #	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
1	97	101	100	105	95	100	94	98	101	106	99.7±3.89
2	98	91	94	92	85	85	92	96	87	96	91.6±4.65
3	92	88	84	89	86	81	82	82	84	88	85.6±3.60
4	96	90	94	89	88	83	87	97	93	84	90.1±4.82
5	81	84	82	80	84	80	83	80	86	80	82.0±2.16
6	86	79	72	78	74	76	71	75	73	78	76.2±4.37
7	77	74	77	75	71	76	77	71	75	69	74.2±2.90
8	74	78	75	70	72	74	70	68	76	69	72.6±3.31
9	80	86	87	86	84	88	79	86	79	84	83.9±3.38
10	80	76	84	83	86	77	76	88	82	84	81.6±4.22
11	94	93	86	89	87	86	84	84	89	87	87.9±3.41
12	80	78	81	79	82	81	79	85	85	74	80.4±3.27
13	90	95	94	97	98	97	99	93	92	102	95.7±3.59
14	85	88	92	85	90	87	92	90	84	82	87.5±3.47
15	85	77	78	75	71	81	84	88	87	83	80.9±5.53

Appendix 11a.
Roudsea Marsh *Phragmites australis* intra-plant comparisons.
Zones 1, 2 and 3, sampled on 05/10/94. Plant numbers, heights and length of leaves analysed.

Zone 1	Height (cm.)	Leaf 1 (cm.)	Leaf 3 (cm.)	Leaf 5 (cm.)	Leaf 7 (cm.)
Plant no. 2	117	16	19	21	22
Plant no. 7	123	16	22	27	24
Plant no. 9	124	19	22	22	22
Plant no. 10	94	17	19	19	17
Plant no. 13	114	13	17	21	20

Zone 2	Height (cm.)	Leaf 1 (cm.)	Leaf 3 (cm.)	Leaf 5 (cm.)	Leaf 7 (cm.)
Plant no. 1	243	36	30	40	35
Plant no. 2	272	39	40	41	35
Plant no. 8	220	29	35	38	31
Plant no. 13	240	32	24	29	25
Plant no. 14	243	31	25	30	36

Zone 3	Height (cm.)	Leaf 1 (cm.)	Leaf 3 (cm.)	Leaf 5 (cm.)	Leaf 7 (cm.)
Plant no. 2	212	43	36	38	35
Plant no. 4	216	33	33	36	35
Plant no. 6	209	30	34	37	40
Plant no. 10	239	35	39	36	36
Plant no. 13	220	34	39	38	37

Appendix 11b.

Roudsea Marsh *Phragmites australis* intra-plant comparisons of stomatal density. Zone 1. View size = 84496μm^2. Sampled 05/10/94.

Plant no. 2	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	75	93	89	86	87	87	94	83	86	88	86.5±5.42
Leaf no. 3	82	87	79	79	85	69	78	74	77	80	79.0±5.16
Leaf no. 5	83	80	79	85	79	78	78	79	75	73	78.9±3.45
Leaf no. 7	49	47	50	46	45	49	45	41	47	49	46.8±2.70

Plant no. 7	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	83	84	87	87	91	85	89	91	82	91	87.0±3.43
Leaf no. 3	71	64	70	70	74	73	72	69	72	67	70.2±2.97
Leaf no. 5	77	71	71	75	60	68	73	72	75	77	71.9±5.07
Leaf no. 7	61	69	65	63	62	61	60	63	56	58	61.8±3.61

Plant no. 9	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	79	76	76	78	69	60	77	69	75	66	72.5±6.20
Leaf no. 3	77	73	70	80	69	74	67	70	74	68	72.2±4.16
Leaf no. 5	74	72	70	69	74	77	68	72	63	62	70.1±4.79
Leaf no. 7	67	64	59	54	51	54	55	47	52	48	55.1±6.51

Plant no. 10	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	71	79	82	81	80	75	72	69	83	73	76.5±5.08
Leaf no. 3	72	69	71	69	71	71	64	74	69	74	70.4±2.91
Leaf no. 5	73	68	63	68	68	66	68	64	63	62	66.3±3.37
Leaf no. 7	67	64	59	63	57	54	64	63	60	67	61.8±4.24

Plant no. 13	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	78	76	78	67	69	77	78	70	76	76	74.5±4.17
Leaf no. 3	73	79	79	73	77	75	78	77	77	76	76.4±2.17
Leaf no. 5	75	80	76	72	84	70	74	75	75	76	75.7±3.92
Leaf no. 7	64	59	59	54	63	64	59	62	58	54	59.6±3.69

Roudsea Marsh *Phragmites australis* intra-plant comparisons of stomatal density. Zone 2. View size = 84496μm^2. Sampled 05/10/94.

Plant no. 1	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	67	87	83	69	89	93	83	75	93	82	82.1±9.19
Leaf no. 3	79	80	77	79	74	70	68	80	82	77	76.6±4.58
Leaf no. 5	74	77	69	65	66	83	76	72	75	71	72.8±5.41
Leaf no. 7	75	77	74	69	66	64	70	81	68	74	71.8±5.29

Plant no. 2	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	85	74	89	84	92	85	82	81	90	78	84.0±5.54
Leaf no. 3	84	84	78	82	87	79	84	85	78	82	82.3±3.09
Leaf no. 5	88	80	77	86	76	86	85	85	75	85	81.3±5.21
Leaf no. 7	56	49	59	59	49	51	57	62	54	50	54.6±4.70

Plant no. 8	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	67	81	75	75	76	73	85	70	70	66	73.8±5.98
Leaf no. 3	94	93	81	88	90	87	85	87	81	82	86.8±4.66
Leaf no. 5	74	79	72	78	77	75	83	77	73	71	75.9±3.63
Leaf no. 7	66	60	58	60	60	48	58	57	53	64	58.4±5.13

Plant no. 13	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	60	61	59	65	62	63	72	57	72	65	63.6±5.08
Leaf no. 3	63	64	68	66	69	71	67	69	68	72	67.7±2.83
Leaf no. 5	74	77	77	74	79	79	81	66	73	74	75.4±4.25
Leaf no. 7	54	48	60	51	51	41	50	46	45	48	49.4±5.21

Plant no. 14	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	63	67	66	59	73	56	61	67	63	75	65.0±5.91
Leaf no. 3	78	77	76	69	76	63	77	70	72	66	72.4±5.23
Leaf no. 5	73	70	67	77	75	74	80	81	72	83	75.2±5.07
Leaf no. 7	70	66	62	62	51	59	53	61	53	64	60.1±6.15

Roudsea Marsh *Phragmites australis* intra-plant comparisons of stomatal density. Zone 3. View size = 84496μm^2. Sampled 05/10/94.

Plant no. 2	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	98	91	94	92	85	85	92	96	87	96	91.6±4.65
Leaf no. 3	68	80	83	79	77	68	70	72	67	74	73.8±5.69
Leaf no. 5	66	69	76	69	70	59	69	67	60	62	66.7±5.17
Leaf no. 7	52	53	54	49	45	46	39	44	45	47	47.4±4.65

Plant no. 4	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	96	90	94	89	88	83	87	97	93	84	90.1±4.82
Leaf no. 3	68	69	63	71	64	66	69	70	68	64	67.2±2.78
Leaf no. 5	75	82	75	76	74	75	75	76	76	73	75.7±2.41
Leaf no. 7	72	79	79	71	75	72	71	71	79	72	74.1±3.57

Plant no. 6	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	86	79	72	78	74	76	71	75	73	78	76.2±4.37
Leaf no. 3	67	67	77	73	66	66	72	62	59	67	67.6±5.25
Leaf no. 5	68	68	70	67	67	74	70	73	70	59	68.6±4.12
Leaf no. 7	64	66	67	61	69	59	56	66	66	62	63.6±4.03

Plant no. 10	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	80	76	84	83	86	77	76	88	82	84	81.6±4.22
Leaf no. 3	74	68	79	73	72	69	76	72	68	75	72.6±3.60
Leaf no. 5	75	79	74	84	75	77	71	72	86	79	77.2±4.89
Leaf no. 7	64	70	72	64	72	74	69	78	61	79	70.3±5.98

Plant no. 13	view1	view2	view3	view4	view5	view6	view7	view8	view9	view10	mean±s.d.
Leaf no. 1	90	95	94	97	98	97	99	93	92	102	95.7±3.59
Leaf no. 3	84	92	91	93	91	82	88	85	78	84	86.8±4.96
Leaf no. 5	74	84	87	83	76	82	74	78	84	85	80.7±4.79
Leaf no. 7	75	79	71	77	78	68	79	77	77	84	76.5±4.43

Appendix 12.

Collection and preparation of samples for establishing contemporary foraminiferan populations.

In the field an area 10cm. × 10cm. is marked out and surface sediment to a depth of 1cm. is collected from this area. It is placed in to a glass jar and an equal volume of a preservative (for example absolute alcohol) is added. On return to the laboratory the jars are kept chilled prior to preparation.

Using a pressurized mist spray wash all silt and clay sized fractions through a sieve of 63-80μm. diameter mesh. All material of >2mm. in diameter can also be discarded, leaving the sand-sized fraction including the foraminifera tests.

Examination is by a low-power microscope using an independent light source.

Appendix 13.

**Kentra Bay levelling data for zones KB1 - KB7 in metres O.D.
Two repeats, October 1993 and July 1994.**

Zone	KB1	KB2	KB3	KB4	KB5	KB6	KB7
31/10/93	1.01	1.23	1.29	1.46	1.57	1.88	2.30
08/07/94	0.98	1.18	1.32	1.48	1.63	1.85	2.25

Appendix 14.

**Kentra Bay loss on ignition (%) data from three repeat samples,
October 1993 - July 1994.**

Zone	KB1	KB2	KB3	KB4	KB5	KB6	KB7
31/10/93	1.25	2.44	1.05	1.06	13.87	13.57	35.28
07/03/94	0.47	0.64	0.52	1.82	8.84	23.99	35.03
08/07/94	1.41	1.34	1.20	3.10	13.32	17.61	41.75

Appendix 15.

Kentra Bay foraminifera counts by zone from three repeats, 10/93 - 07/94.
Count base = 300 tests or 10g. of sediment.

30/10/93	KB1	KB2	KB3	KB4	KB5	KB6	KB7
Elphidium williamsonii	70	310	148	40	4	0	0
Trochammina inflata	0	0	0	8	12	4	15
Trochammina macrescens	0	0	0	9	38	299	87
Milliamina fusca	0	0	0	18	10	1	14
Haynesina germanica	0	0	0	0	0	0	0
sum	70	310	148	75	64	304	116
g. sediment analysed	10.36	8.52	10.13	10.32	10.43	5.47	10.84
foram.'s per 10g. sediment	68	364	146	73	61	556	107

07/03/94	KB1	KB2	KB3	KB4	KB5	KB6	KB7
Elphidium williamsonii	1	302	92	18	6*	0	0
Trochammina inflata	0	4	1	1	17	13	0
Trochammina macrescens	1	1	0	7	82	282	75
Milliamina fusca	0	5	0	15	29	1	4
Haynesina germanica	0	0	0	1	0	0	0
sum	2	312	93	42	134	296	79
g. sediment analysed	10.44	9.13	11.14	11.04	10.08	8.63	10.45
foram.'s per 10g. sediment	2	342	84	38	133	343	76

08/07/94	KB1	KB2	KB3	KB4	KB5	KB6	KB7
Elphidium williamsonii	3	12	11	8	0	0	0
Trochammina inflata	0	0	0	4	12	65	2
Trochammina macrescens	0	0	0	4	39	48	14
Milliamina fusca	0	0	0	1	1	2	0
Haynesina germanica	0	0	0	1	0	0	0
sum	3	12	11	18	52	115	16
g. sediment analysed	14.28	12.08	10.64	10.89	10.65	10.83	10.40
foram.'s per 10g. sediment	2	10	10	17	49	106	15

* All very corroded and of ≈ size.

Appendix 16.

Kentra Bay stomatal density raw data.
Sampled March 1994, view size = 84496μm.

Armeria maritima, KB4.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	10	13	12	14	16	13.0±2.24
leaf 2	8	7	6	10	9	8.0±1.58
leaf 3	7	12	10	9	9	9.4±1.82
leaf 4	8	9	10	12	10	9.8±1.48
leaf 5	9	7	10	11	11	9.6±1.67
leaf 6	8	7	8	6	6	7.0±1.00
leaf 7	15	10	14	9	16	12.8±3.11
leaf 8	12	13	13	15	11	12.8±1.48
leaf 9	7	9	11	8	10	9.0±1.58
leaf 10	13	12	10	13	11	11.8±1.30

Armeria maritima, KB5.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	8	11	13	14	8	10.8±2.77
leaf 2	5	6	6	6	8	6.2±1.10
leaf 3	15	17	15	16	17	16.0±1.00
leaf 4	6	10	10	10	6	8.4±2.19
leaf 5	6	7	5	7	9	6.8±1.48
leaf 6	8	13	12	8	10	10.2±2.28
leaf 7	6	8	8	10	8	8.0±1.41
leaf 8	5	7	5	8	10	7.0±2.12
leaf 9	9	7	9	9	9	8.6±0.89
leaf 10	10	10	8	11	9	9.6±1.14

Armeria maritima, KB6.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	10	12	13	12	13	12.0±1.22
leaf 2	7	6	9	5	8	7.0±1.58
leaf 3	6	5	8	9	11	7.8±2.39
leaf 4	10	9	10	10	7	9.2±1.30
leaf 5	7	7	8	7	8	7.4±0.55
leaf 6	8	7	7	9	6	7.4±1.14
leaf 7	9	7	9	9	9	8.6±0.89
leaf 8	7	7	8	6	5	6.6±1.14
leaf 9	5	7	7	7	6	6.4±0.89
leaf 10	7	6	6	8	7	6.8±0.84

Armeria maritima, KB7.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	6	7	7	8	9	7.4±1.14
leaf 2	7	6	7	7	8	7.0±0.71
leaf 3	5	6	6	6	5	5.6±0.55
leaf 4	5	6	6	5	5	5.4±0.55
leaf 5	8	7	8	8	6	7.4±0.89
leaf 6	8	10	11	12	9	10.0±1.58
leaf 7	9	10	12	15	10	11.2±2.39
leaf 8	5	7	6	6	8	6.4±1.14
leaf 9	8	8	8	9	7	8.0±0.71
leaf 10	7	8	6	6	7	6.8±0.84

Glaux maritima, KB5.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	7	11	9	10	10	9.4±1.52
leaf 2	11	9	10	10	11	10.2±0.84
leaf 3	7	6	8	8	5	6.8±1.30
leaf 4	7	7	6	8	9	7.4±1.14
leaf 5	7	9	6	7	5	6.8±1.48
leaf 6	5	7	7	8	6	6.6±1.14
leaf 7	8	9	7	10	12	9.2±1.92
leaf 8	7	6	6	7	8	6.8±0.84
leaf 9	11	7	7	9	11	9.0±2.00
leaf 10	11	10	8	9	9	9.4±1.14

Glaux matitima, KB6.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	9	7	6	7	6	7.0±1.22
leaf 2	6	8	6	7	6	6.6±0.89
leaf 3	6	8	9	7	8	7.6±1.14
leaf 4	6	6	7	7	5	6.2±0.84
leaf 5	6	6	7	8	8	7.0±1.00
leaf 6	6	8	8	4	9	7.0±2.00
leaf 7	9	11	10	13	9	10.4±1.67
leaf 8	7	7	5	5	6	6.0±1.00
leaf 9	4	6	6	7	8	6.2±1.48
leaf 10	7	6	5	6	6	6.0±0.71

Glaux maritima, KB7.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	9	10	7	8	9	8.6±1.14
leaf 2	9	8	8	10	12	9.4±1.67
leaf 3	12	9	9	9	9	9.6±1.34
leaf 4	8	9	10	7	12	9.2±1.92
leaf 5	9	8	9	10	14	10.0±2.35
leaf 6	8	12	12	11	9	10.4±1.82
leaf 7	10	10	9	7	8	8.8±1.30
leaf 8	7	6	6	8	9	7.2±1.30
leaf 9	12	13	11	9	10	11.0±1.58
leaf 10	7	7	8	7	9	7.6±0.89

Plantago maritima, KB6.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	30	24	26	17	26	24.6±4.77
leaf 2	24	20	22	19	20	21.0±2.00
leaf 3	20	22	16	20	17	19.0±2.45
leaf 4	18	17	18	19	19	18.2±0.84
leaf 5	22	25	30	29	25	26.2±3.27
leaf 6	18	21	25	27	27	23.6±3.97
leaf 7	20	18	21	21	17	19.4±1.82
leaf 8	19	20	18	16	18	18.2±1.48
leaf 9	18	21	15	19	21	18.8±2.49
leaf 10	17	21	21	19	18	19.2±1.79

Plantago maritima, KB7.

	view 1	view 2	view 3	view 4	view 5	mean±s.d.
leaf 1	17	19	19	20	21	19.2±1.48
leaf 2	33	30	29	32	28	30.4±2.07
leaf 3	24	19	25	26	28	24.4±3.36
leaf 4	21	22	17	20	20	20.0±1.87
leaf 5	30	39	29	30	37	33.0±4.64
leaf 6	20	23	20	21	24	21.6±1.82
leaf 7	21	16	18	21	7	16.6±5.77
leaf 8	16	16	15	19	17	16.6±1.52
leaf 9	17	17	21	22	17	18.8±2.49
leaf 10	28	27	26	26	21	25.6±2.70

Appendix 17.

Kentra Bay contemporary stomatal density data, by species and zone. Average values from five views per leaf, see appendix 16 for raw data. Sampled March 1994, view size = 84496μm.

Armeria maritima

Zone	leaf 1	leaf 2	leaf 3	leaf 4	leaf 5	leaf 6	leaf 7	leaf 8	leaf 9	leaf 10	mean±s.d.
KB 4	13.0	8.0	9.4	9.8	9.6	7.0	12.8	12.8	9.0	11.8	10.3±2.15
KB 5	10.8	6.2	16.0	8.4	6.8	10.2	8.0	7.0	8.6	9.6	9.2±2.83
KB 6	12.0	7.0	7.8	9.2	7.4	7.4	8.6	6.6	6.4	6.8	7.9±1.68
KB 7	7.4	7.0	5.6	5.4	7.4	10.0	11.2	6.4	8.0	6.8	7.5±1.83

Glaux maritima

Zone	leaf 1	leaf 2	leaf 3	leaf 4	leaf 5	leaf 6	leaf 7	leaf 8	leaf 9	leaf 10	mean±s.d.
KB 4	----	----	----	----	----	----	----	----	----	----	------
KB 5	9.4	10.2	6.8	7.4	6.8	6.6	9.2	6.8	9.0	9.4	8.2±1.40
KB 6	7.0	6.6	7.6	6.2	7.0	7.0	10.4	6.0	6.2	6.0	7.0±1.31
KB 7	8.6	9.4	9.6	9.2	10.0	10.4	8.8	7.2	11.0	7.6	9.2±1.18

Plantago maritima

Zone	leaf 1	leaf 2	leaf 3	leaf 4	leaf 5	leaf 6	leaf 7	leaf 8	leaf 9	leaf 10	mean±s.d.
KB 4	----	----	----	----	----	----	----	----	----	----	------
KB 5	----	----	----	----	----	----	----	----	----	----	------
KB 6	24.6	21.0	19.0	18.2	26.2	23.6	19.4	18.2	18.8	19.2	20.8±2.92
KB 7	19.2	30.4	24.4	20.0	33.0	21.6	16.6	16.6	18.8	25.6	22.6±5.65

Appendix 18.

Kentra Bay *Armeria maritima* salt gland densities by zone.
Sampled July 1994, view size = 84496μm.

Zone KB4	view 1	view 2	view 3	view 4	view 5	mean ± s.d.
leaf 1	1	2	2	1	1	1.4±0.55
leaf 2	2	1	3	1	1	1.6±0.89
leaf 3	2	3	0	2	2	2.0±1.22
leaf 4	4	5	3	3	3	3.6±1.14
leaf 5	1	2	0	1	3	1.4±1.14
leaf 6	0	0	0	1	1	0.4±0.55
leaf 7	1	2	2	2	2	1.8±0.45
leaf 8	1	1	0	0	0	0.4±0.55
leaf 9	1	4	3	2	1	2.2±1.30
leaf 10	1	1	2	4	2	2.0±1.22

Zone KB5	view 1	view 2	view 3	view 4	view 5	mean ± s.d.
leaf 1	3	4	2	3	3	3.0±0.71
leaf 2	1	1	1	3	2	1.6±0.89
leaf 3	3	4	3	4	4	3.6±0.55
leaf 4	2	2	2	1	3	2.0±0.71
leaf 5	2	4	3	1	3	2.6±1.14
leaf 6	3	3	2	3	4	3.0±0.71
leaf 7	2	0	0	1	0	0.6±0.89
leaf 8	2	2	2	2	2	2.0±0.00
leaf 9	3	3	3	4	2	3.0±0.71
leaf 10	2	3	4	2	3	2.8±0.84

Zone KB6	view 1	view 2	view 3	view 4	view 5	mean ± s.d.
leaf 1	3	1	1	4	2	2.2±1.30
leaf 2	2	0	1	1	2	1.2±0.84
leaf 3	2	3	3	1	3	2.4±0.89
leaf 4	2	2	3	3	1	2.2±0.84
leaf 5	2	3	3	1	2	2.2±0.84
leaf 6	1	2	2	2	4	2.2±1.10
leaf 7	1	2	3	1	3	2.0±1.00
leaf 8	1	1	0	2	3	1.4±1.14
leaf 9	3	2	3	1	2	2.2±0.84
leaf 10	1	0	1	0	2	0.8±0.84

Zone KB7	view 1	view 2	view 3	view 4	view 5	mean ± s.d.
leaf 1	3	2	2	1	0	1.6±1.14
leaf 2	2	1	3	2	3	2.2±0.84
leaf 3	2	2	3	3	2	2.4±0.55
leaf 4	1	1	2	2	2	1.6±0.55
leaf 5	2	3	2	2	1	2.0±0.71
leaf 6	1	3	1	2	1	1.6±0.89
leaf 7	2	3	1	1	3	2.0±1.00
leaf 8	2	2	1	2	1	1.6±0.55
leaf 9	2	2	1	1	3	1.6±0.55
leaf 10	2	1	2	2	1	1.6±0.55

Appendix 19.

Kentra Bay contemporary $\delta^{13}C$ data, by species and vegetation zone.

Puccinellia maritima

Zone	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	mean±s.d.
KB4	-26.09	-25.99	-26.12	-26.42	-26.41	----	-26.21±0.20
KB5	-27.54	-27.63	-27.57	-27.50	-27.67	----	-27.58±0.07
KB6	-26.06	-26.28	-26.62	-26.15	-26.26	-26.39	-26.29±0.20
KB7	----	----	----	----	----	----	------

Pelvetia canaliculata (?)

Zone	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	mean±s.d.
KB4	-17.87	-17.93	-17.49	-17.63	-17.66	----	-17.72±0.18
KB5	-18.76	-19.17	-19.47	-19.20	-19.19	----	-19.16±0.25
KB6	-20.20	-20.14	-20.63	-20.94	-21.00	-20.60	-20.59±0.36
KB7	----	----	----	----	----	----	------

Armeria maritima

Zone	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	mean±s.d.
KB4	-23.65	-24.58	-23.80	-24.07	-23.98	-23.35	-23.91±0.42
KB5	-25.28	-25.67	-25.85	-25.06	-26.14	-25.52	-25.59±0.39
KB6	-25.14	-25.36	-24.70	-24.29	-23.37	-24.14	-24.50±0.73
KB7	-25.59	-24.04	-24.19	-24.06	-23.92	-24.83	-24.44±0.65

Glaux maritima

Zone	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	mean±s.d.
KB4	----	----	----	----	----	----	------
KB5	-24.51	-24.82	-24.14	-23.93	-24.20	-24.77	-24.40±0.36
KB6	-23.75	-24.54	-23.77	-23.76	-23.90	-23.88	-23.93±0.30
KB7	-25.30	-24.61	-25.11	-24.49	-25.07	-24.05	-24.77±0.47

Plantago maritima

Zone	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	mean±s.d.
KB4	----	----	----	----	----	----	------
KB5	----	----	----	----	----	----	------
KB6	-27.88	-27.90	-28.06	-27.38	-27.86	-27.48	-27.76±0.27
KB7	-27.34	-26.67	-26.81	-26.15	-26.62	-26.49	-26.68±0.39

Juncus gerardii

Zone	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	mean±s.d.
KB4	----	----	----	----	----	----	------
KB5	----	----	----	----	----	----	------
KB6	----	----	----	----	----	----	------
KB7	-23.83	-24.95	-25.23	-25.09	-24.37	-25.09	-24.76±0.55