Chapter 2

Materials & Experimental Techniques

This chapter describes the various materials used and the experimental techniques employed for the present study. The topics covered are:

1. Collection and locations of the sediment cores.
2. A brief introduction to foraminifera and their separation procedure from the sea sediments, for use in AMS Radiocarbon dating as well as stable oxygen and carbon isotopic analyses.
3. Radiocarbon chronologies of the sediment cores.
4. Application and suitability of various isotopic and chemical proxies used in this study.
5. Analytical schemes used to measure various proxies, their precisions and accuracies.

2.1. Collection and locations of the sediment cores:

For the present study three sediment cores were collected viz. SS3827 G, SS4018 G and SK145-9 representing three different regions of the Arabian Sea. The cores SS3827 G and SS4018 G were obtained from the equatorial Indian Ocean and the western Arabian Sea respectively during the Ocean Research Vessel Sagar Sampada cruise no. SS152 and SS164 during 1997 and 1998 respectively. Another sediment core SK145-9 was collected from the eastern continental margin of the Arabian Sea during the ORV Sagar Kanya cruise no. SK145 during the year 1999. The locations of the various cores are shown in Fig.2.1 and other particulars are summarized in Table 2.1.

Table 2.1. Particulars of the sediment cores analyzed in this study.

S. No.	Core Name	Type	Location (Lat., Long.)	Length (cm)	Water depth	Resolution	Age limit (a BP)
1.	SK 145-9	Piston core	12.6^{0}N, 74.3^{0}E	400 (252)*	400 m	~50 years per cm	13,180
2.	SS 3827 G	Gravity Core	3^0 42'N, 75^0 54.5'E	196 (100)*	3118 m	~350 years per cm	34,730
3.	SS 4018 G	Gravity Core	13^{0}21.8'N, 53^{0}15.4'E	130 (130)*	2830 m	~150 years per cm	19,020

- Values in bracket denote the dated length

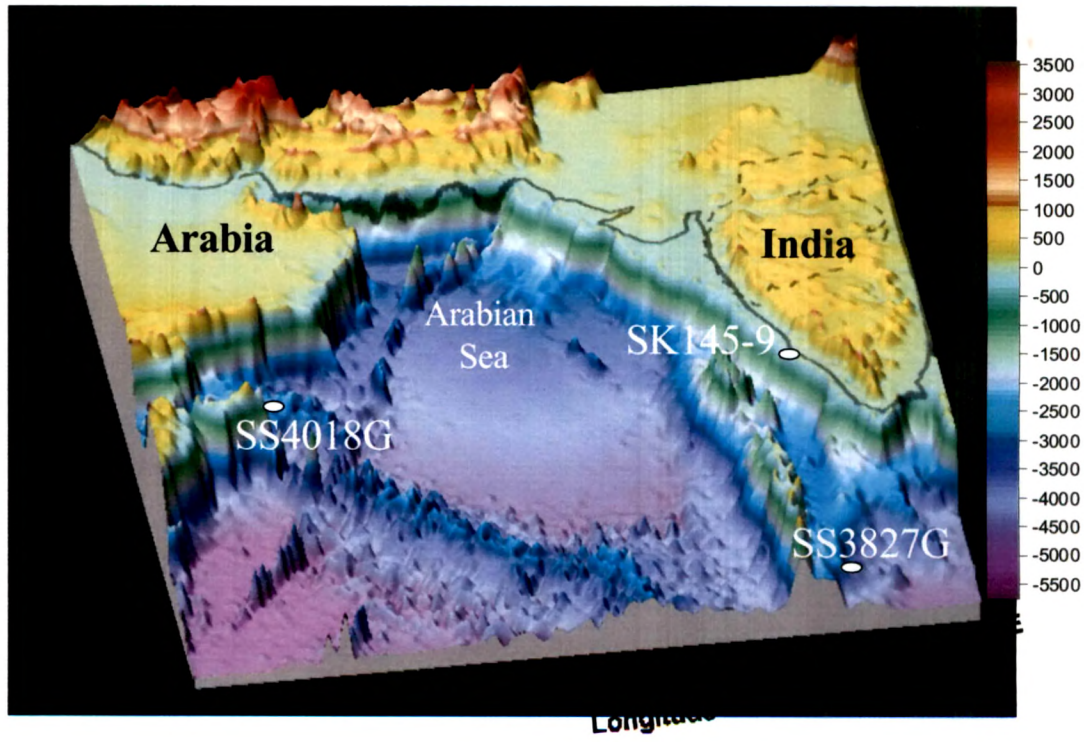


Fig. 2.1. Locations of the sediment cores employed in this study along with the Arabian Sea bottom topography; colour code for altitude and depth in meters, shown on the right.

The cores were sampled onboard with thin perspex sheets and were sealed in the plastic bags, which were then brought back to laboratory for further analysis. The core SK 145-9 was sampled at every cm in the top 50 cm and thereafter the sampling was done at every two cm. In SS 3827 G, sampling was performed at every cm in the top 130 cm and thereafter at every two cm for the remaining length of the core. In SS 4018 G sampling was carried out at every two cm for the entire length of the core.

2.2. Foraminifera and their separation procedure:

Foraminifera are eukaryotic (possessing a distinct nucleus), unicellular (single celled, characterized by the absence of tissue or organs) organisms belonging to the Phylum **Protozoa**. They secrete calcium carbonate shells of incredible beauty and structural complexity, which get preserved in sea sediments as fossils. Their size ranges from 0.01 mm (10 μ) to 10 mm but the average representative size range is 0.1 mm (100 μ) to 1.0 mm. The living organism consists of protoplasm encapsulated in the shell, which is further divided into different chambers. The wall separating one chamber from another is called as **septum**. There is a hole in the septum through which the protoplasm extends throughout the shell. This hole is called **foramen**, from which the name foraminifera (Latin, *foramen* = hole; *ferre* = to bear) is derived. The protoplasm extends outside the test through an opening known as **aperture** and surrounds the shell as a network of branching pseudopodia. The typical life spans of foraminifera are 2-4 weeks. Foraminifera are further divided into "Spinose" and "Non-Spinose" forms. "Spinose" forms are those that bear spines and "Non-Spinose" form lack them. Usually spines get broken and do not get fossilized with the shell.

Nearly all of the foraminifera are marine organisms and are found at all the latitudes. Highest diversity occurs in tropical regions, which decreases with increasing latitude to a single species in the true Antarctic and Arctic waters. They are both **planktonic** and **benthic**. Benthics are the bottom dwelling form and have existed since the Cambrian time (~570 Ma). They may be sessile i.e. constantly attached to the bottom or vagile i.e. free bottom-dwelling organisms. They inhabit all depths ranging from continental shelf to abyssal plain. Planktonic foraminifera float in the water column. They are more recent in origin than benthics and have existed since Jurassic (~200 Ma). Most of the planktonic species inhabit the top 150 m of the water column but their habitat can be upto 1000 m. On the basis of depth habitats,

planktonic foraminifera can be grouped into three categories viz. "Shallow-water", "Intermediate-water" and "Deep-water" dwelling species. "Shallow-water" species live predominantly in the upper 50 m of the water column. They are mainly spinose forms and include all the species of genus *Globigerinoides* and some species of *Globigerina*. "Intermediate-water" species include both spinose forms and non-spinose forms that inhabit the upper 100 m but predominantly dwell in the 50-100 meters of the water column. "Deep-water" group consist of those species, which live in the euphotic zone as the juveniles, and predominantly below 100 m as adults e.g. all the species of genus *Globorotalia*. Several planktonic foraminifera called as "symbiont bearing" are known to host algae as symbionts within their shells. The species that host dinoflagellates (unicellular, 5-10 μ size, brownish-yellow colour) are *G.ruber*, *G.sacculifer*, *G.conglobatus* and *O.universa*. The species hosting chrysophycophytes (yellow-green eukaryotic algae, 2-3 μ size) are *G.siphonophera* and *G.humilis*. There are some species that are known to sometime possess or lack chrysophycophytes as symbionts. They are called as "symbionts facultative" and include the species *G.inflata*, *G.menardii*, *N.dutertrei*, *P.obliquiloculata* and *G.glutinata*. On the other hand, species like *H.pelagica*, *N.pachyderma*, *G.bulloides*, *G.truncatulinoides* and *G.hirusta* are not known to host symbionts and are called as "symbiont barren" (Hemleben et al, 1988).

2.2.1. Separation of foraminifera:

About 5-10 g of sediment samples were taken in a 400 ml beaker filled with ~200 ml of distilled water. Around ½ cc of 30% H_2O_2 was added to oxidize any organic matter that might be present in the sediments. Thereafter ½ spoonful of Calgon powder (sodium hexametaphosphate) was added. Calgon acts as a dispersing agent and helps in removing the agglutinated inorganic particles. They were then warmed at 60-80°C for an hour. Care was taken that the mixtures don't get boiled while heating. Thereafter the beakers were kept for 4-5 hours so that total disintegration of the sediments took place. Subsequently wet sieving was carried out using sieves of the sizes 250 μ and 500 μ. The size fraction (500 μ to 250 μ) was transferred to 50ml beakers containing ~25 ml distilled water and were ultrasonically cleaned to remove the remaining inorganic particles sticking to the shells. The excess water was decanted and the shells were dried at 90°C. Thereafter they were kept in a

furnace at 400°C for ~8 hours to remove any volatile organic matter still adhering to the shells. The dry bulk fraction thus obtained is stored in pre-cleaned plastic vials. The planktonic species required are then handpicked under a stereoscopic microscope from the dry bulk fraction. While picking foraminifera care was taken to pick the shells falling approximately in the size range 450 μ to 350 μ and all the chosen shells were more or less in the same size range.

2.3.Radiocarbon chronologies:

The chronologies of the sediments cores studied in this work have been obtained using Radiocarbon dating method (Libby, 1955). The radiocarbon (^{14}C) produced in the atmosphere is oxidized to $^{14}CO_2$. The chemical cycle of the CO_2 in the ocean is governed by a series of equilibria as shown below:

$$^{14}CO_2\,(g) \leftrightarrow {}^{14}CO_2\,(aq.) \leftrightarrow H_2{}^{14}CO_3 \leftrightarrow H^+ + H^{14}CO_3^- \leftrightarrow 2H^+ + {}^{14}CO_3^{2-}$$

(dissolved) (carbonic acid) (bicarbonate ion) (carbonate ion)

The bicarbonate ions, which are the dominant species in the oceans at the prevailing pH, are removed by the microorganisms to secrete calcium carbonate shells. These calcitic shells of microorganisms contain the radioactive carbon, and are used for radiocarbon dating. We chose planktonic foraminifera namely *Globigerinoides ruber, Globigerinoides sacculifer, Orbulina universa* and *Neogloboquadrina dutertrei.* We have selected planktonic foraminifera for our study because they inhabit the surface and near surface oceans (up to ~100 m) and therefore readily incorporate changes occurring in the surface ocean into their calcitic shells. For AMS radiocarbon dating about 10mg of foraminifera are required. This means approximately 200 individuals have to be handpicked for every date. C-14 dating was carried out at 12 depths for SS 3827 G, at 15 depths for SS 4018 G & 11 depths for SK145-9. It was done using the Accelerator Mass Spectrometer at NSF Arizona AMS Facility, University of Arizona, USA in collaboration with A.J.T. Jull and G.S. Burr (Linick et al, 1986; Jull et al, 1989; Somayajulu et al, 1999). The fundamental difference between conventional radiocarbon dating such as Liquid scintillation method and AMS technique is that in the former, decay of the atom is counted whereas in the latter the atoms themselves are counted. This allows us to measure even small samples, which helps in improving the accuracy relative to the bulk

method by the absence of various contaminants that are usually present in the bulk samples. Accelerator mass spectrometry differs from the conventional mass spectrometry in the energies to which the ions are accelerated. In mass spectrometers the energies are in thousands of eV (keV) whereas in the AMS they are in millions of eV (MeV). The practical consequence of having higher energies is that ambiguities in the identification of the atomic and molecular ions with the same mass (isobaric effects) are removed. Thus we can measure isotopic ratio for specific elements to a level of 1 in 10^{15}. The chronologies of the three cores are given in table 2.2 on the next page.

ΔR is the value that has to be added to the global mean reservoir age (400 yrs.) to obtain the age correction needed for the local reservoir effect. The radiocarbon ages have been calibrated to calendar ages using the calibration program "Calib 4.1 (INTCAL 98)" (Stuiver et al, 1998). Radiocarbon ages beyond 24 ka BP were also calculated using the "Glacial polynomial" (Bard, E. 1998) but the age difference came out to be only 2 years so the Calib 4.1 ages have been retained. The errors given are 1 standard deviation.

Table 2.2. Radiocarbon ages

Sample Details	Radiocarbon Age (a BP)	Calibrated Age (a BP)
Core SK 145-9, ΔR= 100 ± 30		
Depth (0-2) cm	844 ± 36	410 ± 80
Depth (23-25) cm	1899 ± 56	1330 ± 80
Depth (40-41) cm	2506 ± 39	2030 ± 70
Depth (50-52) cm	3210 ± 41	2860 ± 70
Depth (78-80) cm	3952 ± 53	3820 ± 80
Depth (124-126) cm	6308 ± 48	6650 ± 60
Depth (160-162) cm	8124 ± 66	8450 ± 90
Depth (174-176) cm	8891 ± 64	9210 ± 270
Depth (210-212) cm	9359 ± 57	9840 ± 230
Depth (228-230) cm	9922 ± 58	10430 ± 380
Depth (250-252) cm	11913 ± 73	13180 ± 350
Core SS 3827 G, ΔR= 100 ± 30		
Depth (4-5) cm	3692 ± 48	3460 ± 70
Depth (11-12) cm	5338 ± 52	5590 ± 40
Depth (19-20) cm	5788 ± 52	6100 ± 90
Depth (39-40) cm	13523 ± 87	15550 ± 220
Depth (59-60) cm	17780 ± 100	20450 ± 340
Depth (79-80) cm	24040 ± 180	27680 ± 210
Depth (99-100) cm	30280 ± 440	34730 ± 510
Core SS 4018 G, ΔR= 163 ± 30		
Depth (2-4) cm	1116 ± 38	540 ± 30
Depth (10-12) cm	3097 ± 58	2720 ± 30
Depth (20-22) cm	5227 ± 45	5440 ± 80
Depth (32-34) cm	7375 ± 74	7660 ± 70
Depth (42-44) cm	8734 ± 49	9000 ± 160
Depth (50-52) cm	9558 ± 55	10120 ± 220
Depth (60-62) cm	10543 ± 60	10930 ± 380
Depth (60-62) cm	10543 ± 60	10930 ± 380
Depth (72-74) cm	11383 ± 58	12860 ± 130
Depth (80-82) cm	12036 ± 67	13220 ± 300
Depth (92-94) cm	13170 ± 91	14720 ± 570
Depth (102-104) cm	13613 ± 75	15580 ± 220
Depth (112-114) cm	14719 ± 77	16850 ± 250

2.4. Isotopic and chemical proxies employed in this study:

Isotopic proxies that have been studied are stable oxygen and carbon isotopes in selected species of planktonic foraminifera and nitrogen isotopes in the sedimentary organic matter. Chemical proxies studied are calcium carbonate (weight %), organic carbon (wt %) and the C/N ratio.

The variations in the physical and chemical properties due to the presence of different isotopes (the so called "isotope effect") arise due to very small, albeit finite mass differences among them. These differences are most significant for lighter elements such as hydrogen, carbon, nitrogen, oxygen etc. Urey (1947) was the first to explain the variations in physico-chemical properties in isotopic systems on the basis of thermodynamic considerations and proposed that a paleotemperature scale can be constructed based on the fractionation of oxygen isotopes in the calcites.

2.4.1. Oxygen isotopes:

Oxygen has three stable isotopes viz. ^{16}O, ^{17}O and ^{18}O with 99.763%, 0.0375% and 0.1995% abundances respectively. Usually the ratio $^{18}O/^{16}O$ is determined as ^{18}O has higher abundance than ^{17}O and greater mass difference with ^{16}O. The fractionation (defined as the relative partitioning of the heavier and lighter isotopes between two co-existing phases) of these isotopes in nature is caused by an equilibrium or kinetic process. Kinetic fractionation is associated with incomplete and unidirectional processes such as evaporation, diffusion, biologically mediated reactions etc. No isotopic equilibrium is attained in this case. Equilibrium fractionation is a special case of chemical equilibrium reaction in which there is no net reaction but an exchange of isotopes takes place. In this case isotopes can move to and fro and equilibrium is attained when there is no more change in the isotopic ratios with time. In the case of foraminifera, calcite is precipitated from water and the following isotopic exchange process takes place:

$$1/3\ CaC^{16}O_3 + H_2{}^{18}O \leftrightarrow 1/3\ CaC^{18}O_3 + H_2{}^{16}O$$

The equilibrium constant "K" for this reaction can be written as:

$$K = [CaC^{18}O_3]^{1/3}[\ H_2{}^{16}O] / [CaC^{16}O_3]^{1/3}\ [H_2{}^{18}O]$$

$$\text{or, } K = ([CaC^{18}O_3] / [CaC^{16}O_3])^{1/3} / ([H_2{}^{18}O] / [\ H_2{}^{16}O])$$

Thus equilibrium constant can be expressed as the ratio of $^{18}O/^{16}O$ in the carbonate phase to that in the water. This leads us to the concept of fractionation factor "α" that will better represent the partitioning of isotopes between two phases. Fractionation factor is defined as the ratio of isotopes in one phase to the other co-existing phase. In the $CaCO_3$-H_2O system, the fractionation factor is defined as:

$$\alpha_{CaCO_3\text{-}H_2O} = R_{CaCO_3} / R_{H_2O} = 1.031 \text{ at } 25^{\circ}C$$

R_{CaCO_3} is $^{18}O/^{16}O$ in calcite and R_{H_2O} is $^{18}O/^{16}O$ in water. The fractionation factor is related to equilibrium constant by:

$$\alpha = K^{1/n}$$

where "n" is the number of atoms exchanged. For the sake of simplicity, isotope exchange reactions can be written in the way that only one atom is exchanged and then fractionation factor "α" becomes identical to equilibrium constant "K". The fractionation factor is temperature dependent in such a way that calcites precipitated from water of constant isotopic composition but at different temperatures will possess different $^{18}O/^{16}O$ ratios. This is the basis for the paleotemperature scale, by which various empirical paleotemperature equations are deduced. The overall effect is that approximately 0.25‰ depletion in carbonate $\delta^{18}O$ occurs for every $1^{0}C$ temperature increase (Erez and Luz, 1983). The absolute abundances of minor isotopes as well as absolute values of isotope ratios cannot be determined precisely enough for geochemical purposes. Moreover to aid the measurements in mass spectrometers, the isotope abundances are reported in "δ" values, which are relative differences of isotopic ratios from an international standard expressed in per mil (‰) units:

$$\delta_A = [(R_A/R_{st})-1] \times 10^3 \text{ ‰}$$

Where,

R_A is the ratio of the abundances of the less abundant (heavier e.g. ^{18}O) to more abundant (lighter e.g. ^{16}O) isotope in the sample, and

R_{st} is the ratio of the abundances of less abundant (heavier) to more abundant (lighter) isotope in the standard (e.g. $^{18}O/^{16}O$ in the standard)

For oxygen isotopic studies two international standards viz. SMOW and PDB are in the use. SMOW (Standard Mean Ocean Water) is a hypothetical standard with $\delta^{18}O$ value close to the modern mean seawater value. The value of SMOW was

defined by Craig (1961) with respect to an existing water standard NBS-1 (distilled Potomac river water) with $\delta^{18}O$ value of –7.94 ‰ vs. SMOW. As SMOW does not actually exist as a real water sample so it can't be used for calibrating laboratory measurements. Therefore a water sample with values identical to SMOW is distributed by IAEA (International Atomic Energy Agency), Vienna that is called V-SMOW. H.Craig of the University of California prepared it by mixing distilled ocean water (collected from the Pacific Ocean, 0^0 lat.,180^0 long, in July,1967) with small amounts of other waters to adjust the isotopic ratios to the required values (Gonfiantini R, 1981). It has $\delta^{18}O$ value of 0 ‰ with respect to SMOW (by definition). This scale has been used for oxygen isotope analyses involving waters, silicates, phosphates, high temperature carbonate etc. The PDB standard is used in the low temperature carbonate studies. It is a carbonate obtained from fossilized rostrum (cigar shaped internal shell) of *Belemnitella americana*, a belemnite (an extinct cephalopod) from the Peedee Formation of Cretaceous Period in South Carolina, USA. It was the laboratory working standard used by Urey's group in the University of Chicago in the 1950s for developing the initial paleotemperature scale (McCrea, 1950). Over the decades, the original PDB standard has been exhausted and is no longer available. To overcome this problem IAEA introduced a hypothetical standard Vienna-PDB (V-PDB) with values nearly identical to PDB and it is defined by its relationship to carbonate reference materials NBS-19 and NBS-20. NBS-19 is calcium carbonate from a marble of geologically unidentified origin with $\delta^{18}O$ and $\delta^{13}C$ values of –2.20 ‰ and +1.95 ‰ respectively with respect to V-PDB. NBS-20 is a homogenized sample of Solenhofen limestone of Jurassic Period from Southern Germany with $\delta^{18}O$ and $\delta^{13}C$ values of –4.14 ‰ and -1.06 ‰ with respect to V-PDB (Hoefs, 1997). The conversion equations of $\delta^{18}O_{(PDB)}$ to $\delta^{18}O_{(SMOW)}$ and *vice-versa* are (Coplen et al,1983):

$$\delta^{18}O_{(SMOW)} = 1.03091\ \delta^{18}O_{(PDB)} + 30.91$$

$$\delta^{18}O_{(PDB)} = 0.97002\ \delta^{18}O_{(SMOW)} - 29.98$$

Erez and Luz (1983) determined the following empirical temperature equation by comparing isotopic composition of the planktonic foraminifera *Globigerinoides sacculifer* with the actual growth temperature:

$$T^{o}c = 17.0 - 4.52\,(\delta^{18}O_c - \delta^{18}O_w) + 0.03\,(\delta^{18}O_c + \delta^{18}O_w)^2$$

T, $\delta^{18}O_c$, $\delta^{18}O_w$, are the estimated temperature (^{0}C), the isotopic composition of the shell carbonate and the seawater respectively. But there are various complications that inhibit the widespread application of paleotemperature scale. To apply the paleotemperature equation we must know the $\delta^{18}O$ value of the seawater with which a given specimen of $CaCO_3$ has equilibrated. This is not easy to establish with certainty because the isotopic composition of seawater as a whole depends on the amount of ice stored on the continents, which gives rise to the so called "ice volume effect". When water evaporates from the ocean surface, the water vapour gets enriched in the lighter isotopes, as vapour pressure of $H_2{}^{16}O$ is more than $H_2{}^{18}O$. Kinetic effects also take place that further depletes the vapour phase in the heavier isotope. As more and more evaporation takes place and as this isotopically lighter water gets locked in the form of continental ice sheets, the remaining ocean water gets more and more enriched in the heavier isotope. It is believed that during the Last Glacial Maximum the average seawater $\delta^{18}O$ was 1.2 ‰ heavier than present (Labeyrie et al, 1987, Fairbanks et al, 1989).

The depletion of heavier isotope in freshwater gives rise to the correlation of salinity with $\delta^{18}O$. As evaporation takes place, the salinity as well as $\delta^{18}O$ of the seawater increases. In the Arabian Sea it has been found that 1‰ increase in salinity causes a 0.33 ‰ increase in $\delta^{18}O$ of water (Duplessy et al, 1981, Sarkar et al, 2000). Separately analyzing planktonic and benthic foraminifera from the same site can help to resolve the ice volume effect. The $\delta^{18}O$ values of the planktonics reflect the changes in both the temperature as well as $\delta^{18}O$ of water whereas the bottom water is mostly believed to be unaffected by the temperature changes, hence the benthics could record only the change in the isotopic composition of seawater.

There are several effects associated with the biology of foraminifera that cause deviation from the equilibrium. Isotopic disequilibrium effects can be classified as either metabolic or kinetic (McConnaughey, 1989 a, b). Metabolic effects result from the incorporation of ambient dissolved carbon and oxygen (produced due to respiration or photosynthesis) into the shell material. Kinetic effects are due to the preferential uptake of lighter isotopes during the hydration and hydroxylation of CO_2

and are generally associated with rapid calcification. These effects, collectively called "vital effects", include effects due to respiration, ontogeny, secretion of gametogenic calcite etc. The respiration products are depleted in the heavier isotopes (Lane and Doyle, 1956) and their utilization for shell secretion results in depleted $\delta^{18}O$ values. Progressive $\delta^{18}O$ enrichment from juvenile to mature chambers has been observed and is assigned to the incorporation of the respired CO_2 during early calcification. The higher metabolic rates in the juvenile specimen would cause the strongest depletions, which then decreases in the adult specimen that has reduced metabolism (Berger et al, 1978; Wefer and Berger, 1991). Furthermore, planktonic foraminifera inhabit different depths at various stages of their ontogeny. In the later part of their lifecycles they tend to secrete shells at deeper and cooler waters resulting in enhanced $\delta^{18}O$ values (Bouvier-Soumagnac and Duplessy, 1985; Emiliani, 1971). Towards the end of their life cycle foraminifera secrete gametogenic calcite. Before gamete release, foraminifera move to deeper, cooler waters and secrete a layer of calcite that is enriched in $\delta^{18}O$ over its shell. This calcite layer can comprise 18 to 28% of the shell mass of foraminifera (Be, 1980; Duplessy et al, 1981). Furthermore $\delta^{18}O$ has been found to vary with varying light intensities (photosynthetic activities in the symbiont carrying foraminifera) and carbonate ion concentrations. The $\delta^{18}O$ was found to decrease with increasing irradiance that caused enhanced photosynthesis. Enhanced rate of skeletogenesis ensues increased photosynthesis that favours stronger kinetic fractionation resulting in depleted $\delta^{18}O$ (Spero and Lea, 1993; Wefer and Berger, 1991). $\delta^{18}O$ in foraminiferal carbonate decreases with increasing carbonate ion concentration that was ascribed to a-biological, kinetic fractionation effect (Spero et al, 1997) as it was also observed in rapidly precipitating inorganic $CaCO_3$ (McCrea, 1950). The disequilibria due to vital effects can be avoided by choosing the particular species, which are known to precipitate their shells in equilibrium with seawater and by picking mature shells from a particular size range. Also diagenetic alteration after the death of the organism can modify its isotopic composition significantly. The $\delta^{18}O$ values of planktonic foraminifera from warm subtropical seas are particularly sensitive to alteration as the secondary calcite precipitates in equilibrium with the cold pore fluids at the sediment-water interface (Schrag, 1999). Also shells may get

recrystallized at the micron scale (preserving the shell features and ornamentation) unlike the previous case in which euhedral calcite crystals precipitate on the outer and inner surfaces of the shell (Pearson et al, 2001). This obstacle can be overcome by selected pristine shells, which appear glassy under the microscope and are without any overgrowths.

All foraminifera used in this study have been carefully handpicked to as far as possible avoid most of the above complications.

2.4.2. Carbon isotopes:

Carbon has two stable isotopes viz. ^{12}C and ^{13}C with an abundance of 98.89% and 1.11% respectively. The two main carbon reservoirs in the earth are organic matter (2000 Giga Ton) and the sedimentary carbonates (72,000,000 GT) and are characterized by different carbon isotopic signatures because of the two distinct processes controlling them (Craig, 1953). Kinetic isotope effects during photosynthesis cause preferential uptake of ^{12}C in the organic matter. On the other hand, isotope equilibrium exchange reactions within the inorganic carbon system " atmospheric CO_2 – dissolved bicarbonate – solid carbonate" cause enrichment in the heavier isotope (Hoefs, 1997) in the carbonates. The carbonate equilibria in the marine system can be represented by the following equations (Faure, 1986):

$$CO_2\ (g) \leftrightarrow CO_2\ (aq.)$$

$$CO_2\ (aq.) + H_2O \leftrightarrow H^+ + HCO_3^-\ (aq.)$$

$$CaCO_3\ (s) + H^+ \leftrightarrow Ca^{2+}\ (aq.) + HCO_3^-\ (aq.)$$

These equilibrium reactions govern the carbon and oxygen isotopic variations among various chemical species. Total dissolved inorganic carbon (DIC) comprises of HCO_3^- (aq.), CO_3^{2-} and CO_2 (aq.). The net equation for carbonate precipitation comes out to be:

$$Ca^{2+}\ (aq.) + 2HCO_3^-\ (aq.) \leftrightarrow CaCO_3\ (s) + H_2O + CO_2\ (g)$$

The equilibrium fractionation factors for carbon isotope in this system at 20°C are (Emrich et al, 1970):

$$\alpha_{CaCO3\ (s)-\ HCO3^-\ (aq.)} = 1.00185$$

$$\alpha_{HCO3^-\ (aq.)\ -\ CO2\ (g)} = 1.00838$$

$$\alpha_{CaCO3\ (s)\ -\ CO2\ (g)} = 1.01017$$

At low temperatures the largest fractionation occurs between the dissolved CO_2 and the bicarbonate ion. The bicarbonate ion is the dominant species at the prevailing pH of seawater (7.5 – 8.3) and DIC can be conveniently approximated by HCO_3^- (aq.). The isotopic enrichment factor for calcite vs. bicarbonate is (Grossman, 1984):

$$\varepsilon_{CaCO3\text{-}HCO3^-}(‰) = 10.51 - 2980/T, \text{ where T is temperature in Kelvin}$$

This relationship gives a temperature dependence of 0.035 ‰ increase in $\delta^{13}C$ per degree Celsius increase in temperature, which is same as that given by Emrich et al (1970). Thus temperature has a negligible effect on $\delta^{13}C$ of calcite in comparison to the effect due to the productivity variations. The profile of $\delta^{13}C_{DIC}$ in the water column is governed by oceanic productivity (Kroopnick, 1974). Phytoplanktons prefer lighter isotope (^{12}C) during photosynthesis enriching the ambient surface seawater in the heavier isotope (^{13}C). As the organism dies it settles to the deeper water where it gets oxidized releasing CO_2 depleted in ^{13}C.Thus the DIC at the surface waters has the highest $\delta^{13}C$ with a minimum at the base of the thermocline after which it increases a little and thereafter stays more or less uniform. The organisms secreting calcareous shells in equilibrium with the ambient water will record these isotopic signatures. Thus a higher $\delta^{13}C$ value probably corresponds to an enhanced rate of photosynthesis in the euphotic layer that indicates an increase in productivity.

As $\delta^{13}C$ has a steep gradient in the water column so the species secreting their shells at different depths in equilibrium with the ambient water during their ontogeny will exhibit deviation from the surface seawater equilibrium values (Kroopnick, 1972). Disequilibria in the $\delta^{13}C$ values are caused by vital effect as discussed in the case of oxygen isotopes (see Sec. 2.4.1). Respiration products are depleted in heavier isotopes, the incorporation of which causes depletion relative to equilibrium (Weber and Woodhead, 1970; Vinot-Bertouille and Duplessy, 1973; Grossman, 1987). Higher metabolic rates have been found to cause increased depletion in $\delta^{13}C$ values (Wefer and Berger, 1991, Oritz et al, 1996). Symbionts present in the foraminifera carry out photosynthesis in which they preferentially utilize the lighter isotope. With increased irradiance photosynthesis also increases resulting in enhanced $H^{13}CO_3^-$ in the ambient microenvironment, resulting in chambers enriched in $\delta^{13}C$ (Spero and Lea, 1993).

$\delta^{13}C$ in foraminiferal carbonate decreases with increasing carbonate ion concentration similar to the effect experienced by $\delta^{18}O$ that was ascribed to be a-biological, kinetic fractionation effect (Spero et al, 1997). Diagenetic alteration at the sediment-water interface or beneath the sediments will shift $\delta^{13}C$ values toward the lighter side as degradation of organic matter produces ^{12}C depleted CO_2 making the ambient water depleted in the heavier isotope.

To avoid the above-mentioned problems as far as possible, pristine foraminifera from a certain size range have been chosen.

2.4.3. Nitrogen isotopes:

Nitrogen has two isotopes viz. ^{14}N and ^{15}N with abundances of 99.64% and 0.36% respectively. The standard for measuring the nitrogen isotopes is the atmospheric N_2. Nitrogen gets into the biological system by the process of photosynthesis. Nitrogen fixation involves the reduction of N_2 to ammonia (NH_3), NH_4^+ or any other nitrogen compound by the microorganisms (e.g. *Rhizobium*) under anaerobic conditions. Nitrogen fixing bacteria are called as diazotrophs. "Nitrification" is the conversion of the NH_3, NH_4^+ to nitrate (NO_3^-) or nitrite (NO_2^-) by the bacteria of *Nitrosamonas* and *Nitrobacter*. If ammonia is directly assimilated then it is called as "ammonia assimilation" but free ammonium ions can't exist for long in the aerobic soils as they are oxidized to nitrate or nitrite and hence nitrification is the dominant step. Denitrification is the process by which nitrate is reduced to any gaseous nitrogen species, generally N_2 or nitrous oxide (N_2O) under the anaerobic conditions (Deuser et al, 1978; Naqvi and Noronha, 1991).

In the world oceans there are three regions that support perennial anaerobic conditions in the water depths of ~200 m to ~1000 m called as oxygen minima zone (OMZ) with oxygen concentration falling below 0.5 ml/l. This is due to the occurrence of high overhead productivity that consumes the available oxygen during their descent/degradation. These regions are the eastern tropical North Pacific (ETNP), eastern tropical South Pacific and the Arabian Sea (Christensen et al, 1987; Naqvi, 1987, 1994). Due to lack of oxygen in OMZ, the anaerobic bacteria utilize NO_3^- for the decomposition of organic matter. During this process they preferentially consume NO_3^- with lighter isotope (^{14}N), thus enriching the residual nitrate in the heavier isotope, which gets upwelled to the sea surface and is taken by the organisms

as a nutrient. The $\delta^{15}N$ of the organic matter is governed by the isotopic composition of the source NO_3^- that is upwelled from below and fractionation experienced by NO_3^- during its uptake by the phytoplanktons (Altabet, 1994). In the oxic waters of the euphotic zone the $\delta^{15}N$ of the nitrate is 5 to 6 ‰ (Liu and Kaplan, 1989) whereas in the regions receiving waters upwelled from the OMZ the $\delta^{15}N$ is in the excess of 18 ‰ (Cline and Kaplan, 1975) that explains the highly enriched $\delta^{15}N$ values observed in the particulate organic matter (Kumar et al, 2004). This enriched $\delta^{15}N$ signature is preserved even when the organic matter settles down and get preserved in sea sediments (Saino and Hattori, 1987). Thus a high $\delta^{15}N$ can be related to increased denitrification, which in turn is controlled by the climate induced productivity increase (Ganeshram et al, 1995). There are some species of algae that can directly fix atmospheric nitrogen. *Trichodesmium* is a good example. The $\delta^{15}N$ of organic matter that have contribution from organisms that directly fix N_2 is close to 0 –2 ‰ (Capone et al, 1997).

2.4.4. Biogenic Proxies:

These include $CaCO_3$ (wt. %), C_{org} (wt. %) and the C/N ratio in the sea sediments. The overhead rain of calcitic shells is a major constituent of the sea sediments. Sediments are composed of minute foraminiferal shells, called as *Globigerina* ooze, and cover most of the open ocean floor. It has been observed that during the monsoon season in the Arabian Sea, 50 – 60 % of the total flux to the bottom is composed of calcitic material (Nair et al, 1989). Thus calcium carbonate percentage in the sea sediments can indicate the overhead productivity provided the core has been raised from depths above the lysocline (~3800 m in the Arabian Sea, Kolla et al, 1976; Peterson and Prell, 1985) and there is no contamination from the terrigenous inputs (Sirocko et al, 1993).

Organic Carbon (C_{org}) preserved in the sea sediments is derived from the particulate organic carbon (POC, the carbon content of particulate organic matter) and is a manifestation of the overhead primary productivity if there are no alterations after the deposition (Muller and Suess, 1979, Calvert and Pederson, 1992; Schulz et al, 1998). Around ~5% of the POC fixed by photosynthesis in the euphotic zone is exported to the deep waters where it undergoes further degradation and only 10%

reaches the ocean bed. Further degradation takes place at the sediment-water interface and just below the sediments (~ 50 cm, the diagenetically active sediment layer) and on an average less than 10% of the POC flux reaching the sediment bed (0.05% - 0.1% of the total surface productivity) gets ultimately preserved (Chester, 2003). The preservation of organic carbon improves drastically in the regions underlying OMZ and in the regions where reducing conditions prevail at the sediment-water interface. It is believed that a high sedimentation rate supports enhanced preservation as it rapidly removes the organic matter from the diagenetically active zone and shields it from oxidizing agents (Heinrichs, 1992).

Organic matter in the sea sediments is derived from the marine as well as terrestrial sources. It is necessary to ascertain the source of the organic carbon before interpreting its downcore variations as due to paleoproductivity. The elemental composition of phytoplanktons and zooplanktons were given by Redfield (1934, 1958) that are called as "Redfield ratios". The Redfield ratios for marine organic matter are C:N:P = 106:16:1 that yields a C/N value of 6.6. Recently this estimate was revised by Takahasi et al (1985) who gave the ratio as C:N:P = 122 (±18):16:1 that provide a range of 6.5 to 8.7 as the C/N ratio. The C/N ratio of recent sediments comprising marine organic matter has a value of 8 to 10 whereas the ancient sediments have a value of 12-15 (Mackenzie, 1980). Terrestrial sources provide a variety of particulate and dissolved organic matter to the oceans that have some compounds exclusive to terrestrial biota such as lignin, chitin etc. Organic matter derived from land plants has C/N values between 20 and 100 (Premuzic et al, 1982; Meyers, 1994). Diagenetic alteration of organic matter tends to lower the C/N ratio as nitrogenous compounds breakdown to produce ammonia, which is retained by clay minerals (Muller, 1977) whereas CO_2 diffuses out.

2.5. Analytical scheme followed to measure various proxies, their precisions and accuracies:

The following schemes were employed for measuring the various proxies:

2.5.1. $\delta^{18}O$ and $\delta^{13}C$ measurements on foraminifera:

For measuring $\delta^{18}O$ and $\delta^{13}C$, CO_2 is fed into the mass spectrometer. There are two basic approaches to extract CO_2 from the purified carbonate. The first approach

involves thermal decomposition of carbonate but it is not preferred as the resulting CO_2 exhibits wide isotopic variations.

The other technique involves the acid decomposition of the carbonate with 100% phosphoric acid at different temperatures between 25°C and 150°C (McCrea, 1950; Wachter and Hays, 1985; Rosenbaum and Sheppard 1986; Swart et al, 1991) as shown:

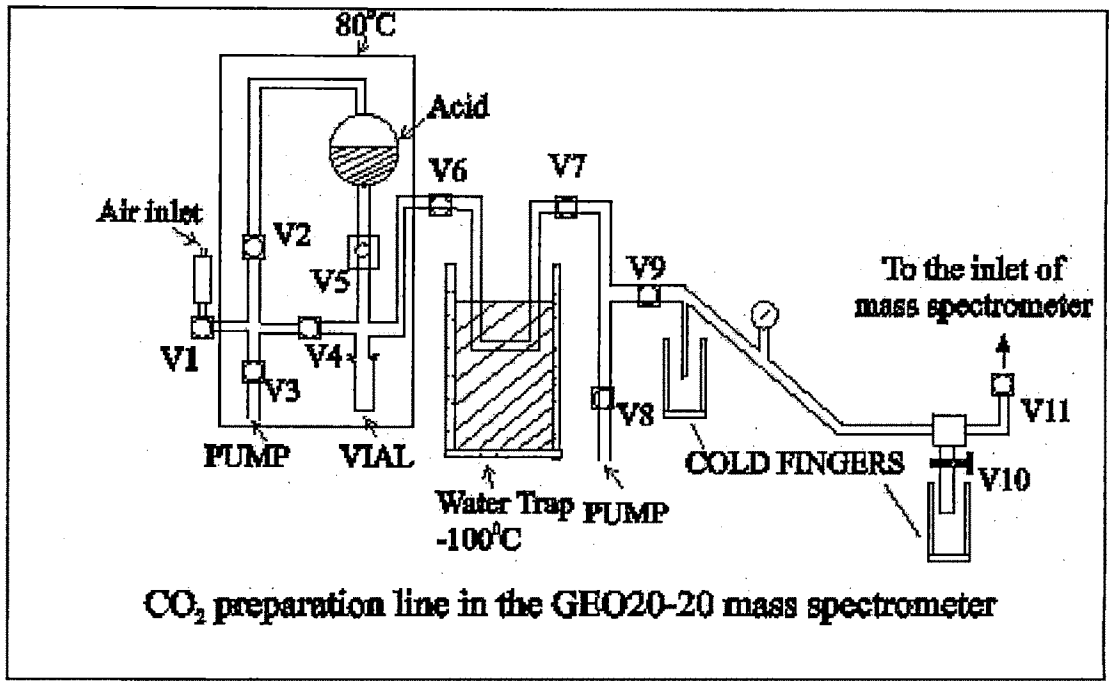
$$3CaCO_3 + 2H_3PO_4 \rightarrow 3\ CO_2 + 3H_2O + Ca_3(PO_4)_2$$

This reaction shows that only two-thirds of the carbonate oxygen is liberated in the product CO_2. The oxygen isotopic fraction factor for CO_2-calcite system during acid decomposition at 25°C is 1.01025 (Rosenbaum and Sheppard 1986). Many precautions have to be taken to follow the acid decomposition technique. The salt produced ($Ca_3(PO_4)_2$) should be soluble in the solution produced by the interaction of the acid with the carbonate, or else a protective covering will form on the latter that will inhibit further reaction. Also, the acid should not produce radicals in the mass range 44-46, which excludes nitric acid (HNO_3) that decomposes to form nitrous oxide (NO_2). Most importantly, isotopic exchange between water and CO_2 is known to take place that necessitates an acid that has no free water. For these reasons, orthophosphoric acid is chosen as it has an extremely low vapour pressure and also the concentration of water is very low in comparison to other acids. The acid was prepared following Coplen et al (1983).

The mass spectrometer used in this study is the Europa–Scientific GEO 20 –20 stable isotope ratio mass spectrometer with an attached Carbonate Preparation System (CAPS). The schematic diagram of CO_2 preparation using the CAPS (from the CAPS manual) and that of mass spectrometer inlet system (from the GEO 20-20 manual) are shown in Fig. 2.2. CAPS system had a factory fitted aluminum carousel that can hold upto 24 sample vials and is supposed to measure them automatically. But it never worked as intended due to some inherent mechanical defect. Therefore all the measurements were made manually. The total CO_2 production assembly was maintained at 80°C for rapid acid digestion of the carbonates. At first, air was put into the acid reservoir by opening the valves V1 and V2. Thereafter sample vial is connected to the acid dosing assembly. Then the valves V4 and V3 were opened to evacuate the vial to medium vacuum of 10^{-3} torr using a rotary pump. In the meantime

rest of the preparation line was pumped to high vacuum (of the order of ~10^{-8} torr) by keeping V6 closed and opening other valves that connect the lines to turbo pumps. When the total line was under vacuum and the temperature is 80°C, then acid was dropped for 0.175 second (~0.5 ml) on the sample. V6 was closed as soon as the reaction was over, which took around 1-2 minutes. The evolved gases were immediately removed from the reaction vial and are passed through the water trap between V6 and V7 that is kept at –100°C. Here H_2O molecules were frozen out and the remaining, moisture free CO_2 was frozen at the cold fingers (CF) using the liquid nitrogen (-196°C) trap. If the gas amount was very low, then the CF near V9 was used or else CF near V10 was used. After that any residual gases that may be present above the frozen CO_2 (at V9 or V10) were pumped out. This cryogenically cleaned CO_2 was then fed into the mass spectrometer inlet system.

The mass spectrometer sample inlet line was first brought to medium vacuum and then to high vacuum by opening V21 (connects to rotary pump) and V20 (connects to turbo pump) respectively. The V22 was closed and the sample gas was put into the variable sample reservoir by opening V11, V12 and V13. There the gas was left for 2 minutes for equilibration. Thereafter the V12 was closed and the gas was introduced into the mass spectrometer via a changeover valve by opening V14. The reference reservoir stores the reference gas, which is called CD Standard. It is a CO_2 gas prepared by reacting around 50g of mixed foraminiferal assemblage from a sediment core (raised from the Arabian Sea during a cruise of RV Charles Darwin in January, 1997) with orthophosphoric acid at 25°C (Ghosh, 2000). Its $\delta^{18}O$ and $\delta^{13}C$ values with respect to PDB are 1.07‰ and 1.7‰ respectively. The changeover valve acts as dual inlet in the way that it allows the sample gas and reference gas to alternately enter the mass spectrometer at preset intervals and when one is connected to the mass spectrometer the other is connected to waste line so that both the reference and the sample gas face the same amount of depletion. The variable volume reservoirs were adjusted to give a current of around 10 nanoampere for the major beam (mass 44) and the current was kept the same for both the sample and the reference gas.



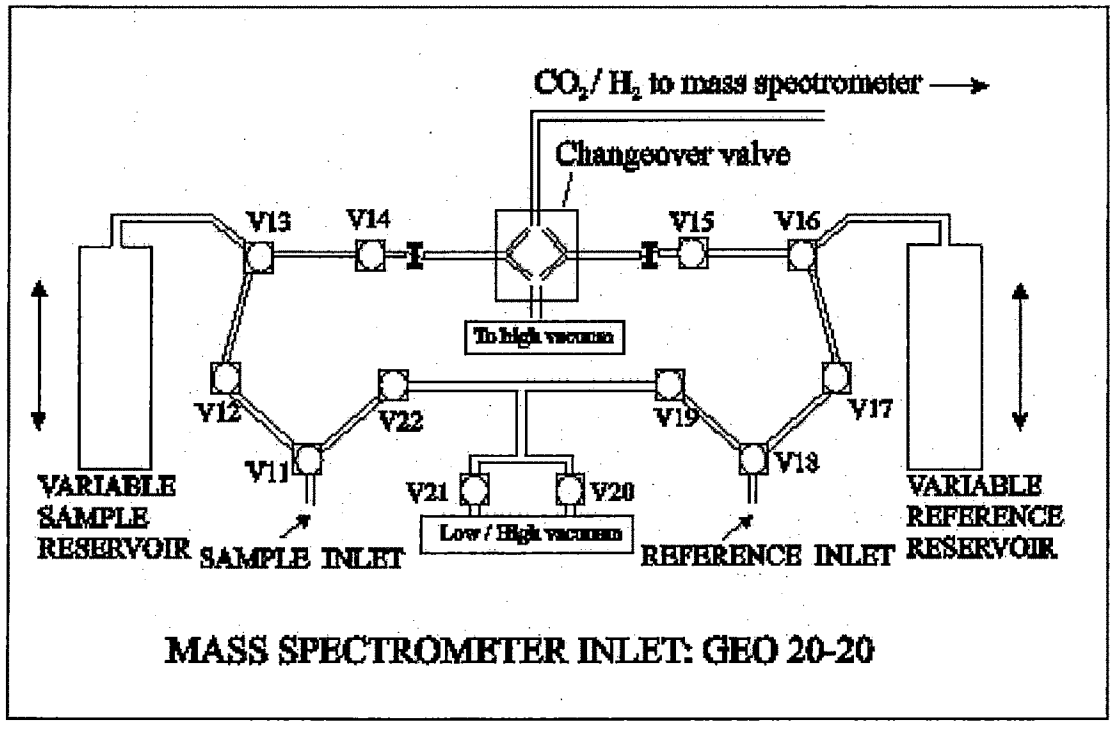


Fig 2.2. Schematic diagram of CO_2 preparation using CAPS and the Mass Spectrometer inlet system.

The predominant species of CO_2 belongs to the mass 44 viz. $^{12}C^{16}O_2$(100%) followed by mass 45 viz. $^{13}C^{16}O_2$ (93.67%), $^{12}C^{16}O^{17}O$ (6.33%) and mass 46 viz. $^{12}C^{16}O^{18}O$ (99.79), $^{13}C^{16}O^{17}O$ (0.205), $^{12}C^{17}O_2$ (0.003%) (Craig, 1957). Given in the parentheses is the relative abundance of that particular isotopic species with respect to other isotopic species with the same mass. The masses 47, 48 and 49 constitute only 0.0052% of natural CO_2 and are usually neglected. It is evident that ^{13}C dominates in the mass 45 and ^{18}O in the mass 46. GEO 20-20 mass spectrometer is a triple collector system having three faraday cups for collecting ions corresponding to three beams of masses 44, 45, and 46. Thus both $\delta^{18}O$ and $\delta^{13}C$ can be measured simultaneously under the same focusing conditions. The ion beams falling at the three collectors are converted to current using the appropriate electronic circuits and the ratio of currents are related to δ values as:

$\delta_{45} = [((I_{45}/I_{44})_{sam}/ (I_{45}/I_{44})_{std}) - 1]\ 10^3$ and $\delta_{46} = [((I_{46}/I_{44})_{sam}/ (I_{46}/I_{44})_{std}) - 1]\ 10^3$ where I_{44}, I_{45} and I_{46} are the currents generated due the beams of masses 44, 45 and 46 respectively. These current ratios are converted to $\delta^{13}C$ and $\delta^{18}O$ using the Craig correction (Craig, 1957):

$$\delta^{13}C = 1.06754400\ \delta_{45} - 0.03600782\ \delta^{18}O$$

$$\delta^{18}O = 1.00096600\ \delta_{46} - 0.00206322\ \delta^{13}C$$

In order to check the precision, a lab standard called as Z-Carrara (ZC-2002) was run at least three times daily viz. at the start of the measurement, in the middle and at the end of the day's measurement. It is a homogenized, fine carbonate powder (<63μ) from an Italian marble kindly provided by Prof. N.J. Shackleton. The $\delta^{13}C$ and $\delta^{18}O$ values of the Z-Carrara have been determined by repeated measurements and are 2.11‰ and -2.11‰ respectively. All the sample values have been corrected with respect to Z-Carrara on a daily basis to account for the variation in the machine and carbonate preparation line conditions. We measure sample (sam) as well as Z- Carrara (ZC) with respect to reference gas (rg) i.e. ${}_{rg}\delta^{sam}$ and ${}_{rg}\delta^{ZC}$. δ value for reference gas with respect to Z-Carrara can be calculated as follows:

$$_{ZC}\delta^{rg} = \{(1/\ 1 + {}_{rg}\delta^{ZC}\ 10^{-3}) - 1\}\ 10^{3}$$

Now, sample δ values with respect to Z-Carrara can be calculated using the formula:

$$_{ZC}\delta^{sam} = {}_{rg}\delta^{sam} + {}_{ZC}\delta^{rg} + {}_{rg}\delta^{sam}\ {}_{ZC}\delta^{rg}\ 10^{-3}$$

Z-Carrara values with respect to PDB ($_{PDB}\delta^{ZC}$) are known hence sample δ values with respect to PDB can be calculated as:

$$_{PDB}\delta^{sam} = {}_{ZC}\delta^{sam} + {}_{PDB}\delta^{ZC} + {}_{ZC}\delta^{sam}\ {}_{PDB}\delta^{ZC}\ 10^{-3}$$

Table 2.3 gives the Z-Carrara $\delta^{13}C$ and $\delta^{18}O$ values measured from 19th June, 2003 to 4th April, 2004.

The precision on $\delta^{13}C$ measurement is ± 0.1‰ while for $\delta^{18}O$ it is ±0.2 ‰. Uncertainties given are one standard deviation. Another standard that was run occasionally to check the stability of the machine is the Check Standard with $\delta^{13}C$ and $\delta^{18}O$ values of 0.33‰ and 2.33‰ with respect to PDB.

Table 2.3. Z-Carrara $\delta^{13}C$ and $\delta^{18}O$ values with respect to PDB in per mil, measured from 19.7.03 to 4.4.04:

$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$
1.98	-2.26	2.05	-2.07	1.94	-2.12	1.99	-2.11
2.04	-2.21	2.06	-2.03	1.92	-2.30	1.98	-2.14
1.99	-2.35	2.05	-2.02	2.10	-1.78	1.96	-2.17
1.90	-2.45	2.01	-2.21	1.98	-1.89	1.87	-2.17
1.92	-2.63	1.93	-2.44	1.97	-2.03	1.87	-2.25
1.84	-2.32	2.01	-2.14	2.02	-2.08	2.09	-1.79
1.95	-2.35	2.04	-2.00	1.96	-2.05	1.90	-2.15
1.96	-2.47	1.92	-2.32	1.96	-2.06	1.92	-1.87
1.92	-2.49	1.97	-2.29	2.03	-1.92	1.97	-2.25
1.99	-2.36	2.08	-1.98	2.22	-2.17	2.03	-1.97
1.92	-2.30	2.03	-1.96	2.18	-2.07	1.92	-2.16
2.03	-2.08	1.90	-2.24	2.25	-1.96	1.94	-2.11
2.10	-1.77	1.81	-2.51	2.07	-2.13	1.97	-2.00
2.03	-2.12	1.89	-2.26	1.94	-2.09	1.85	-2.19
2.06	-1.89	1.89	-2.32	2.04	-2.22	1.95	-2.17
1.99	-2.00	1.82	-2.34	1.99	-2.31	2.02	-2.00
2.11	-1.85	1.98	-2.22	2.12	-2.14	1.88	-2.22
1.98	-2.12	2.09	-1.73	2.06	-2.35	1.94	-2.09
1.99	-2.09	2.04	-1.90	2.02	-2.43		
1.99	-1.93	2.04	-2.11	2.02	-2.21		
2.00	-1.86	2.00	-2.03	2.00	-2.25		
2.05	-2.05	1.81	-1.94	2.08	-1.96		
2.08	-1.92	2.06	-1.89	2.03	-2.08		
1.90	-2.32	2.03	-2.01	1.98	-2.15		
1.90	-2.35	2.02	-1.95	1.84	-2.36		
2.07	-1.76	1.99	-2.06	1.98	-2.12		
2.08	-1.95	1.96	-2.08	2.02	-2.22		
2.10	-1.82	2.03	-2.00	1.95	-2.24		
2.10	-1.88	2.00	-1.97	1.97	-2.19		
2.06	-2.09	2.09	-1.94	1.95	-2.31		
2.06	-1.88	1.93	-2.13	2.04	-1.87		
2.03	-1.94	1.99	-1.91	2.03	-2.01		
2.10	-1.70	2.01	-1.91	2.17	-1.96		
2.07	-1.86	2.01	-2.03	1.98	-2.12		
2.06	-1.81	1.99	-2.20	1.97	-2.15		
2.06	-1.75	2.06	-1.90	2.08	-1.76		
2.07	-1.87	2.00	-1.88	1.92	-2.08		
2.06	-1.82	1.93	-2.25	1.98	-1.94		
2.07	-1.83	1.79	-1.89	1.89	-2.28		

Mean $\delta^{13}C = 2.00 \pm 0.08$; Mean $\delta^{18}O = -2.09 \pm 0.19$, total no. of measurements=135

2.5.2. Calcium Carbonate and Organic Carbon measurements:

Calcium carbonate in the sea sediments from the cores SS 3827 G and SS 4018 G were measured using the *UIC* Coulometer, Model 5012 (UIC Inc., IL, USA). CO_2 is evolved from the sediments by reacting the carbonate present in them with 40% orthophosphoric acid at 70°C. It is purified and analyzed following the standard procedure (Bhushan et al, 2001). The standard used is the Na_2CO_3. Calcium carbonate in the core SK 145-9 was estimated using the EDTA titration method (Vogel, 2002). Around 0.1g sediment sample was taken to which was added 10 ml of 2% acetic acid. The leachate obtained was titrated with 0.01M EDTA solution using the 0.4% Eriochrome Black-T indicator. The $CaCO_3$ concentration obtained is divided by the weight of the sediment to obtain the value in weight %.

Total carbon and total nitrogen were measured using a *Fisons* NA 1500 NC Elemental Analyzer (Fisons Inc., Italy). Around 10-15 mg of sediment sample is packed in a tin foil, which is released into a combustion chamber at 1020°C. The evolved CO_2 and nitrogen oxides are then passed through a reduction chamber maintained at 650 °C that contains metallic copper. After further purification the gases are passed though a gas chromatograph that release them sequentially. The gases enter a thermal conductivity detector, which generate electric signals proportional to the concentrations of the gases present. A calibration curve is prepared using Deer River Black Shale as a standard, having 2.53% carbon and 0.12% nitrogen (Bhushan et al, 2001). Again the values are expressed in weight % by dividing by the weight of the sediment taken. Organic Carbon was calculated by subtracting inorganic carbon values (obtained from coulometer and EDTA titration) from the total carbon values i.e.,

$$C_{org} = C_{total} - C_{inorg}$$

The precision in measuring $CaCO_3$, Total Carbon and Nitrogen are 3%, 4% and 6%. The precision calculated for C_{org} is 5%. All the uncertainties quoted are 1σ.

2.5.3. Nitrogen isotope measurements:

For this study, an Elemental Analyzer (Flash EA 1112 Series, CE Instruments, Italy) interfaced with Finnigan Delta Plus continuous flow mass spectrometer (Thermo Quest Finnigan, Germany) via Conflo III was used. For isotopic analysis,

adequate amount of the sediment sample (5 to 50 mg, depending on the nitrogen concentration) was wrapped in a silver foil and dropped into the combustion furnace of the elemental analyzer. There flash combustion takes place in the presence of 5-grade oxygen (99.999%). The evolved gases pass through reduction chamber containing metallic copper that reduces the oxides of nitrogen to N_2. Thereafter the gases pass through a water adsorbant and a gas chromatograph that introduces pure N_2 in the mass spectrometer where ^{15}N abundance was determined. Dry helium (99.999%) acted as a carrier gas. The $\delta^{15}N$ values have a systematic offset of approximately -3.5‰ as evident by comparison with other studies in the same region (e.g. Altabet et al, 2002; Altabet et al, 1995; Ganeshram et al, 2000), probably due to the low nitrogen content of the sediment samples. But this offset is not going to affect the interpretations as we are looking at trends exhibited by relative variations in these values and not the absolute values. The precision of isotopic measurement is 0.38 ‰ with a standard error of 0.08 ‰ obtained by making multiple measurements of ammonium sulphate standard, IAEA–N–2 [$(NH_4)_2SO_4$, No. 342] as shown in the Table 2.4. The error reported is 1σ.

Table 2.4. Ammonium sulphate standard $\delta^{15}N$ values in per mil with respect to air.

δ15N	δ15N
19.96	19.74
19.75	20.82
19.80	19.80
20.43	19.95
20.72	19.74
20.03	20.07
19.78	20.44
20.67	19.85
20.60	19.71
20.05	

Mean $\delta^{15}N = 20.10 \pm 0.38$, total no. of measurements = 19

Standard error = 0.08 ‰, 1 standard deviation