CHAPTER II

EXPERIMENTAL TECHNIQUES

The primary goal of this thesis, as outlined in the earlier chapter, is to analyze recent corals from the Indian coast for their proxy climatic records. Such a study involves the collection of corals, determination of their chronology and their analysis. The proxies chosen for measurements in this work are: stable oxygen and carbon isotopes, radiocarbon and cadmium. This chapter briefly outlines the procedures used in this work for sample collection and their analyses. Wherever possible the procedures available in literature were used, in some cases they were modified to suit our requirements.

II.1 SAMPLING AND CHRONOLOGY

Live coral heads were collected from two locations in the Indian coast viz., Covered and Amini islands belonging to Lakshadweep archipelago (10°N,73°E) and from the Pirotan island (22.6°N,70°E) in the Gulf of Kutch (Fig 2.1a). These corals were collected from depths of ~1 m during low tide. In addition to samples from these two sites, another coral from the Stanley Reef (19°15' S, 148°07'E) was also analyzed, the location of which is shown in Fig 2.1b. This coral was provided to us by Dr J M Lough for stable isotopic analyses. The corals (from the Indian coast) were dislodged from their site of growth with a hammer and chisel and brought ashore. The polyps were removed by washing with tap water. The coral head was air dried and cut into two halves (Fig 2.2).

To obtain time series records of proxy climate parameters preserved in the coral it is essential to determine its chronology precisely. We have done this through X-radiography and oxygen isotope systematics.

X-radiography of coral skeleton, originally developed by Knutson *et al.* (1972) is widely used to determine their growth rates and pattern. For X-radiography, a section of about 1 cm thick (Fig 2.2) was sliced along the principal growth axis from the cut

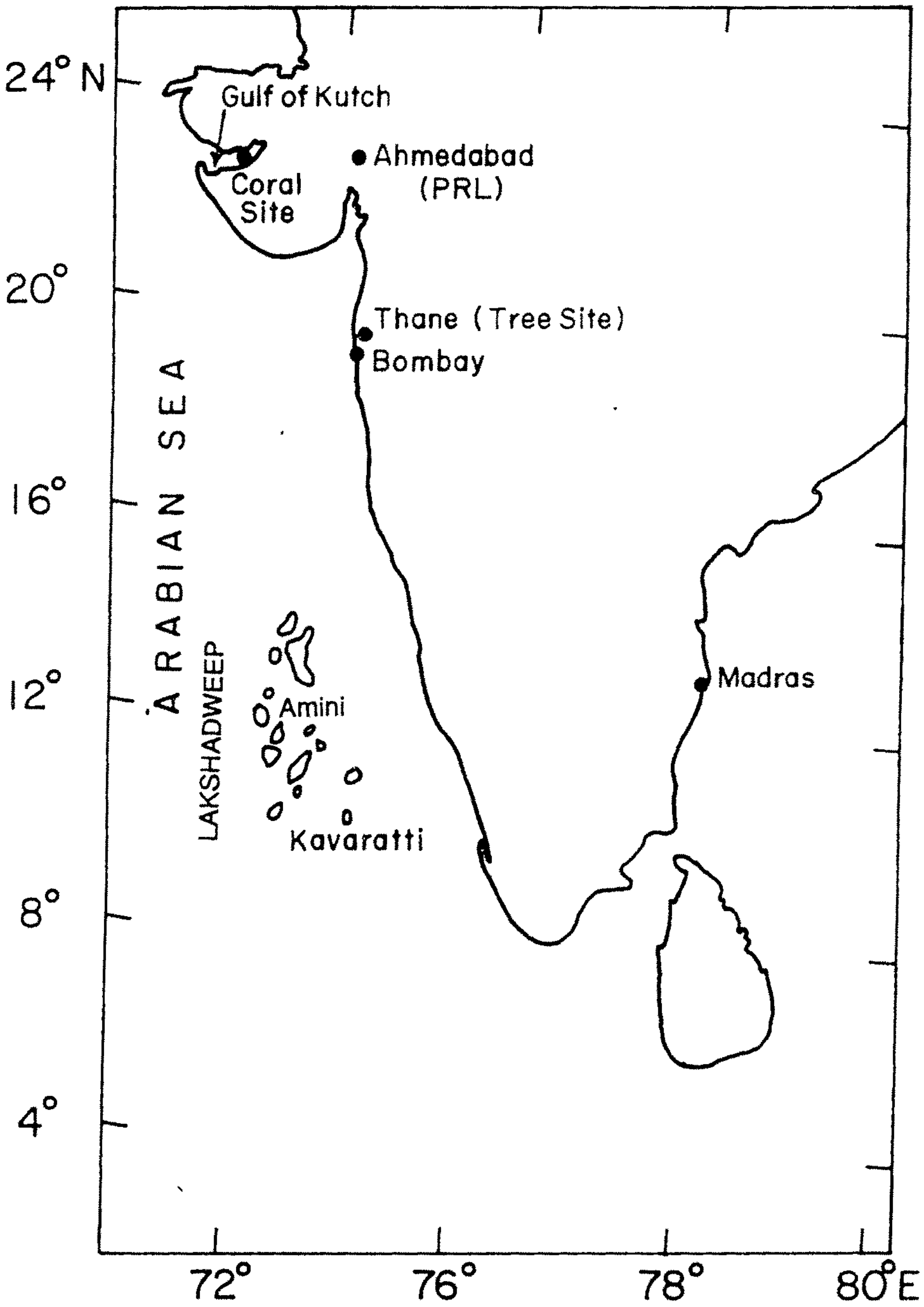

Fig 2.1a Location of corals from the Indian coast and tree sampled for proxy climate records. The tree stem slice was provided by Dr G B Pant of the Indian Institute of Tropical Meteorology.

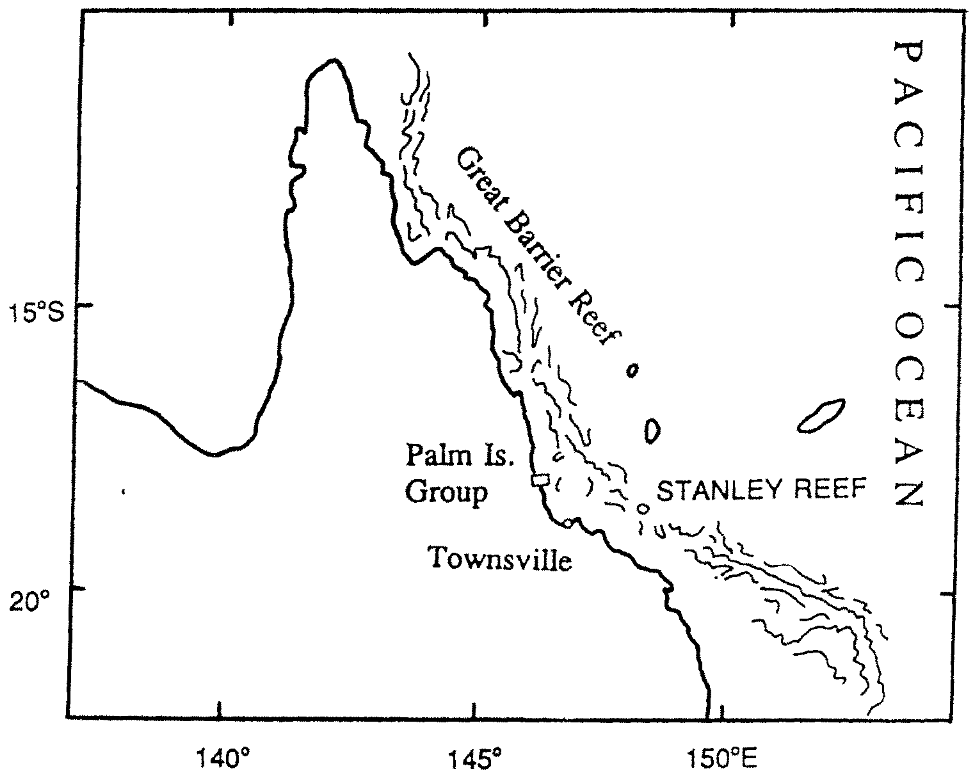

Fig 2.1b Location of coral sampled from the Stanley Reef, western Pacific. The sample was provided for isotopic studies by Dr J M Lough of the Australian Institute of Marine Science.

face of one of the halves. The slice was washed thoroughly with tap water and then in distilled water, followed by ultrasonic agitation to remove adhering particles. The cleaned slice is dried at 90°C for ~12 hours. This slice is X-rayed on Kodak medical film at a source to film distance of one metre and an applied voltage of about 46-48 KVp with an exposure time of 0.08 sec. The X-ray positive is then used to identify the band structure.

II.2 STABLE ISOTOPE MEASUREMENTS

II.2.a Sampling and pretreatment

For stable isotope measurements, a thin strip (~1cm × 1cm) was sliced from the coral section used for X-radiography. This strip was taken along the central growth axis (Fig 2.2). Sampling for mass spectrometric measurements of oxygen and carbon isotopes using dental drill is a popular technique since the pioneering work of Emiliani (1956) However there is some concern about possible isotopic exchange between the metastable aragonite and atmospheric gases and moisture, by the heat produced at the point of drilling (Aharon 1991). Leder *et al.* (1991) claim that such isotopic fractionation is produced only at high pressure and/or if dull drill bits are used. In this work, initially we used slow speed drilling and in the later phases the subsampling was done using a needle file. The use of a needle file enables high resolution sampling and may minimize problems of fractionation as the heat generation is less compared to that in drilling. The sampling resolution varies from 0.5 to 1.5 mm depending on the nature and texture of the sample. About 1 mg $CaCO_3$ powder was filed out for mass spectrometric measurements. The $CaCO_3$ samples were stored in labelled glass thimbles and covered with parafilm till they were subjected to mass spectrometric analysis.

The sample pretreatment is an important prerequisite for the mass spectrometric measurements of carbon and oxygen isotopes. This is because coral powder may contain a small amount of organic matter, which needs to be removed to avoid possible exchange of carbon and oxygen isotopes with the CO_2 evolved from the carbonate during acid treatment. Towards this, prior to acidification, the samples were roasted under vacuum at 350 °C for one hour to remove any volatile material that might be present.

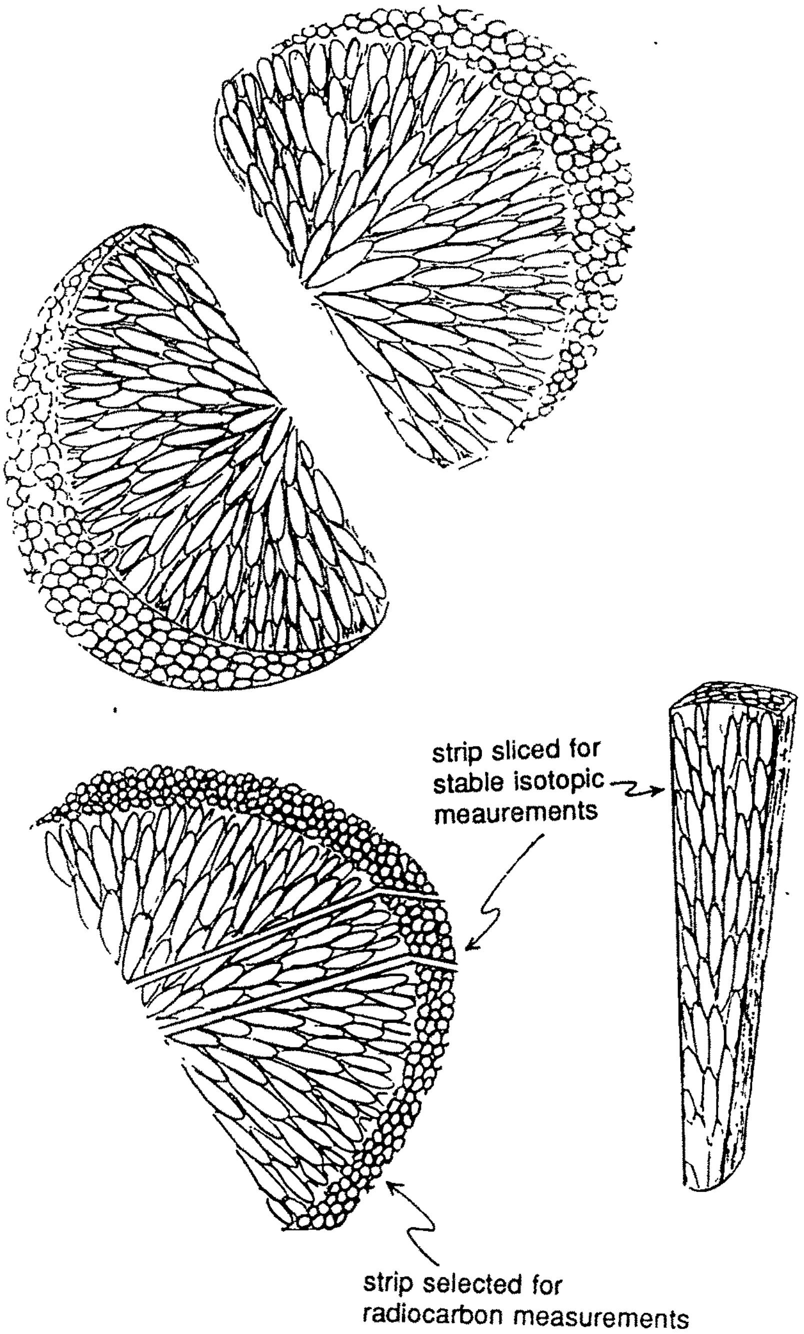

Fig 2.2 Coral sectioning and sampling for X-radiography and tracer measurements

II.2.b Mass spectrometric measurements:

Stable isotopic measurements were performed on two corals from the Covered island (KV-1, KV-2), one coral (Am) and one giant clam (GC) from the Amini island, one coral each from the Gulf of Kutch (GK) and the Stanley Reef (SR).

Measurements of stable isotopic ratios of oxygen and carbon were carried out using a VG Micromass 602D mass spectrometer. The measurements were made relative to an internal standard, the foram standard CO_2 (FS). This standard was prepared by reacting a large quantity of foraminifera separated from the Arabian sea sediments with H_3PO_4(100%) at room temperature (Sarkar, 1989). The FS was stored in a large volume (5 l) flask connected to the reference side of the mass spectrometer. To check the constancy of the isotopic composition of FS with time and the reproducibility of the isotopic measurements, two other standards, Makrana Marble (MMB) and Z-Carrara (ZC) whose δ values are well established wrt PDB (*op cit.*), were also run routinely. Z-Carrara standard was provided by Prof N J Shackleton and was run only during the early phase of this work as its availability was limited. However measurements on MMB were made throughout this work and its long term average δ values showed very good agreement with those measured at the Godwin Laboratory, Cambridge, UK. Table (II.1) lists the δ values of MMB and Z-Carrara (wrt PDB) run during this thesis work. The * marked numbers are the delta values of MMB and ZC analyzed in Godwin Laboratory, Cambridge UK (quoted by Sarkar, 1989) and n stands for number of measurements.

Fig 2.3 shows the delta values of ZC and MMB measured during the course of this work. For all standards and samples isobaric interferences were corrected using the equation of Craig (1957) and the δ values are reported relative to PDB.

During Aug '91 the mass spectrometer was updated to triple collector system (903). The same reference gas (FS) was continued for oxygen and carbon isotopic measurements and found to give consistent and reproducible results.

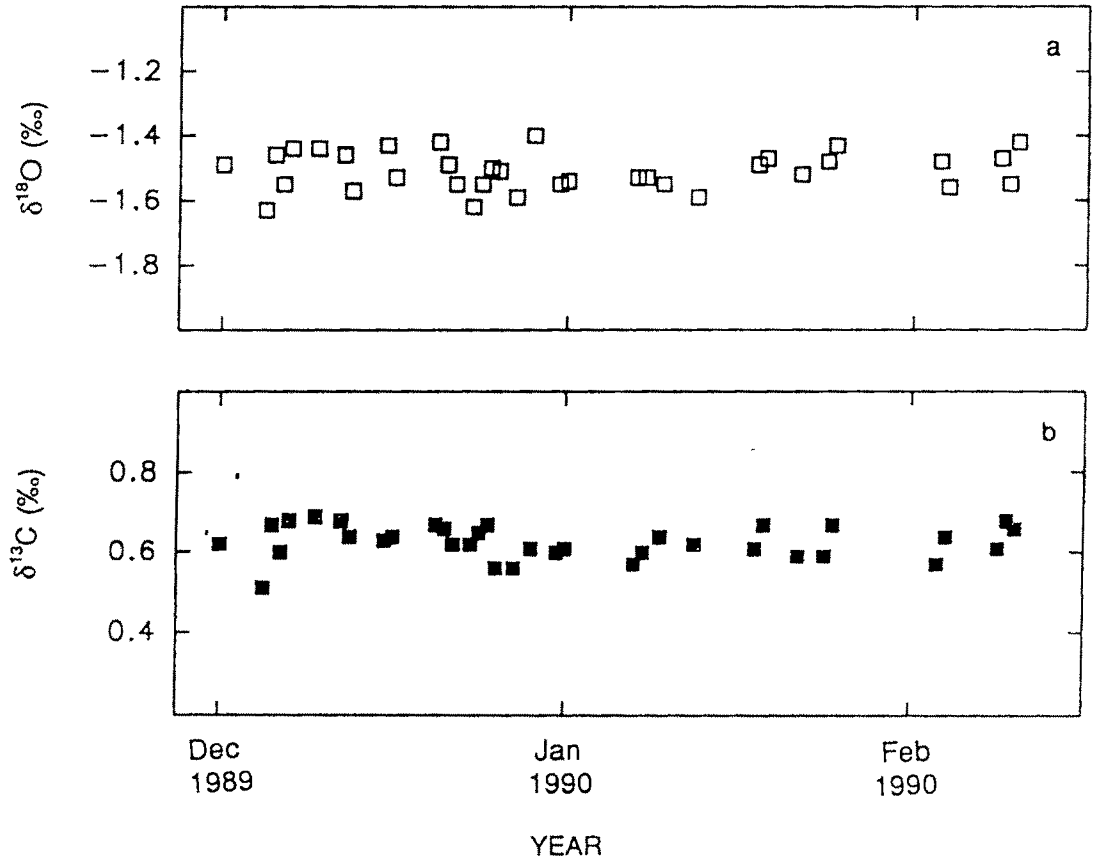

Fig 2.3a Oxygen (a) and carbon (b) isotopic ratios of Z-Carrara (relative to machine standard). The reproducibility over the period of analysis is ±0.06‰ and ±0.04‰ for $\delta^{18}O$ and $\delta^{13}C$ respectively.

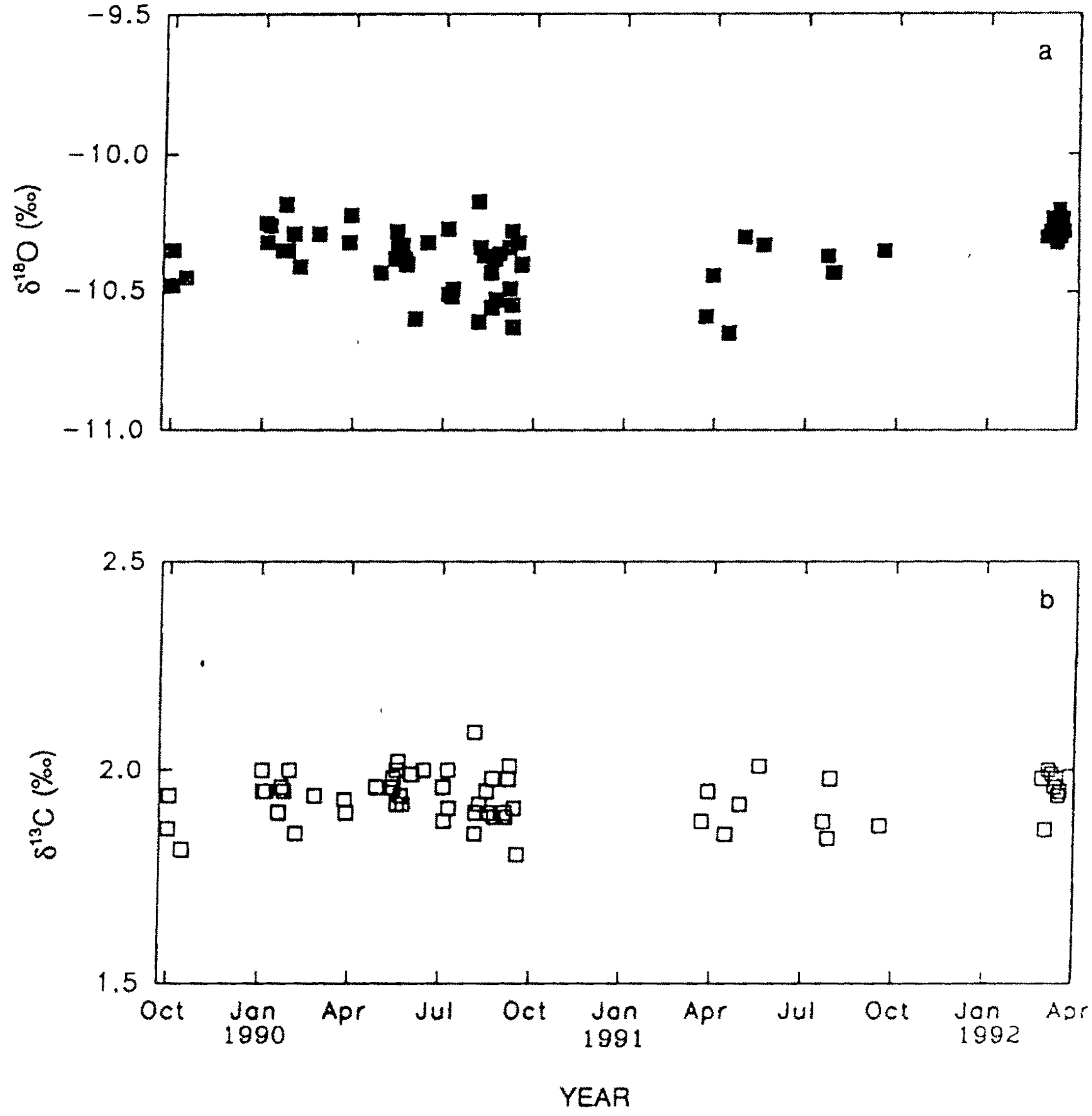

Fig 2.3b Oxygen (a) and carbon (b) isotopic ratios of Makrana marble (relative to machine standard). The reproducibility of measurements over a two year period is ±0.13‰ and ±0.07‰ for $\delta^{18}O$ and $\delta^{13}C$ respectively.

Table II.1 Isotopic analysis of Z-Carrara and Makrana Marble standards

	n	$\delta^{18}O_{PDB}$ (‰)	$\delta^{13}C_{PDB}$ (‰)
Z-Carrara	37	-1.78±0.04	2.09±0.06
	-	-1.79*	2.18*
MMB	80	-10.61±0.13	3.88±0.07
	18	-10.5±0.2*	3.9±0.1*

* Analyses done in Cambridge, U.K.

For the triple collector system, a different set of Craig equations was used (Gat & Gonfiantini 1981). Internal laboratory standard (MMB) and several sample replicates were run routinely. For each sample the purity of CO_2 in the mass spectrometer was also ascertained from the peaks at various masses. If there was any spurious peak, such as in mass 43 due to water contamination during the extraction of CO_2 (Sarkar 1989) in the sample, the run was rejected. Such occurrences, however, were very rare. All δ values are reported with respect to PDB. Errors (0.1‰) given are one standard deviation obtained for the working standard.

II.3 RADIOCARBON MEASUREMENTS

II.3.a Sample preparation

(i) *Corals*

Annual bands from the Gulf of Kutch coral sample (GK) and tree ring samples from a teak tree (ThTR) were analyzed for radiocarbon.

For radiocarbon analysis a ~1cm thick section along the growth axis of the coral was cut (Fig 2.2). From this slice depending on the growth rate, single or multiple annual bands were cut using a small diamond wheel (~1" diameter and < 1mm thickness) operated by an electric hand drill under a distilled water jet. The cut bands were dried by heating overnight at 80-90°C, and powdered using a Tima mill. About 15g of this coral powder was used for radiocarbon measurements.

(ii) *Tree Rings*

Radiocarbon measurements in tree rings were made to determine the time variation in atmospheric ^{14}C activity near the coral site. The tree ring samples were taken from a teak tree (*Tectona grandis*) which grew at Thane (19°14'N, 73°24'E), near Bombay (Fig 2.1a). The chronology of the tree was established by Pant & Borgaonkar(1983). Hydrogen isotopic studies of this species of tree from this region were earlier made by Ramesh *et al.* (1989) to determine the relation between the isotope ratios and meteorological parameters. Annual rings were separated by chiseling from a ~2cm thick cross section of the tree stem. The individual rings were powdered using a Wiley mill. This powder was then treated by soaking in acetone for ~3 hrs with intermittent ultrasonic agitation. This was followed by 6-8 hrs soaking in 5% NaOH and finally in 1% HCl for ~ 6 hrs to remove the resinous and oily substances (Cain & Suess 1976). The sample was thoroughly washed with distilled water, dried and used for radiocarbon measurements. Following this pretreatment about 6g of the cleaned wood powder was used for benzene synthesis.

II.3.b Benzene synthesis

Benzene was prepared from the coral and tree ring samples using a TASK Benzene Synthesizer following the methodology available in literature (Noakes *et al.* 1965, Gupta & Polach 1985). A brief description follows:

First, carbon dioxide is produced from the samples either by acid hydrolysis (in case of coral) or by dry combustion in an oxygen environment (tree ring samples). From some of the coral CO_2 samples, an aliquot was taken for $\delta^{13}C$ measurements. The results showed that the $\delta^{13}C$ in this CO_2 was same within the experimental uncertainty to that of the $\delta^{13}C$ averaged over the portion of the band used for radiocarbon measurement. The remaining CO_2 is then converted to Li_2C_2 which is hydrolyzed to form C_2H_2 (to minimize tritium contamination, the water used for the hydrolysis was obtained by double distilling ground water collected from a 200 m deep well. This ground water is reported to have a ^{14}C age of ~8200 years, Bhushan *et al.* 1993). The acetylene is polymerized using alumina coated vanadium catalyst to produce benzene. The benzene is then transferred to a 7ml glass scintillation vial and weighed. Typically, ~1.5g (for coral) and ~2g (for wood samples) of benzene were obtained. Overall yield of benzene was typically 70%. To the sample benzene, scintillator grade non radioactive benzene was added by weight to make the volume to 3ml (2.637g) which is the standard volume used for counting. To this 0.5 ml of scintillator cocktail [42g/l PPO(diphenyloxazole) + 0.7 g/l POPOP (2, 2'-p-phenylene-bis-5 phenyl oxazole) in benzene] was added. The sample cocktail was mixed well and counted for ^{14}C activity.

NBS oxalic acid was run 3 to 4 times a year to check the reproducibility and for Δ^{14}C calculations. CO_2 was liberated from the oxalic acid by $KMnO_4$ oxidation as well as by dry combustion. The CO_2 was converted to benzene as described above. We have run both NBS oxalic acid-I and II standards during the course of this work. The details of procedure and assay are given in Bhushan *et al.* (1993).

II.3.c Radiocarbon counting

The benzene samples were counted in a Packard Tri-Carb Liquid Scintillator Analyzer, model 2250CA. This spectrometer operates in two modes, normal mode and low level count mode (LLCM). In LLCM, the background is reduced internally by electronic background discriminator using Three-Dimensional Spectrum Analysis (Kessler 1989).

More recently, in addition to electronic background reduction, Packard Instruments have introduced a scintillator sleeving (a low level PICO-XL vial holder) for the counting vial which further reduces the background drastically (~ 40%) with a marginal reduction in ^{14}C counting efficiency (~5%). We operated the counter in LLCM mode with the sleeving.

The β particle of ^{14}C atom has a maximum energy of 156 keV. If there is tritium (0 - 18.6 keV) contamination in the benzene sample, it would affect the ^{14}C count rate. The tritium interference can be minimized by selecting an energy window for ^{14}C counting where contribution from tritium would be minimal and at the same time have high efficiency and low background for ^{14}C. The Packard counter provides a built-in mechanism to select and optimize an energy region for ^{14}C counting. Using the LLCM, the optimized region selected for ^{14}C in our counter was 11 to 98 keV. In addition, the instrument has a three channel provision to measure the count rates. We had chosen the following energy intervals for the three channels.

Channel A	0.0 - 18.6 keV
Channel B	11 - 98 keV
Channel C	0.0 - 156 keV

Channel A is the energy window which would predominantly record tritium counts if any, from the sample. The higher energy betas (with energy 11-18.6 keV) from tritium fall in the ^{14}C window, Channel B. Comparison of count-rates in Channel A of scintillation grade benzene and the marble blanks shows that they are same within experimental uncertainties. This suggests that there is no discernable tritium contamination during benzene synthesis.

II.3.d ^{14}C blanks and standards

To ascertain the background count-rates for ^{14}C, several blanks were run using marble from quarries of Rajasthan belonging to Raialo formation of the Precambrian Period. Fig 2.4 shows the variations in the count-rates of the ^{14}C blanks over a period of ~2 years.

From this figure it is seen that the background (or blank) count-rates are quite consistent (except in four cases which had count-rates ~25% excess over the long term trend) and center around a value ~1.15 cpm. The reason for the high count-rate in these four blank runs is not clear. The average of all numbers gives a background 1.193 ± 0.115 cpm. Rejecting the counts beyond $\pm 2\sigma$ of the mean and recalculation yields an average background count-rate of 1.154 ± 0.008 cpm. This value has been taken as background activity and was used for all calculations. The background count-rate is typically 10% of coral and tree ring sample count-rate. In this context it must be

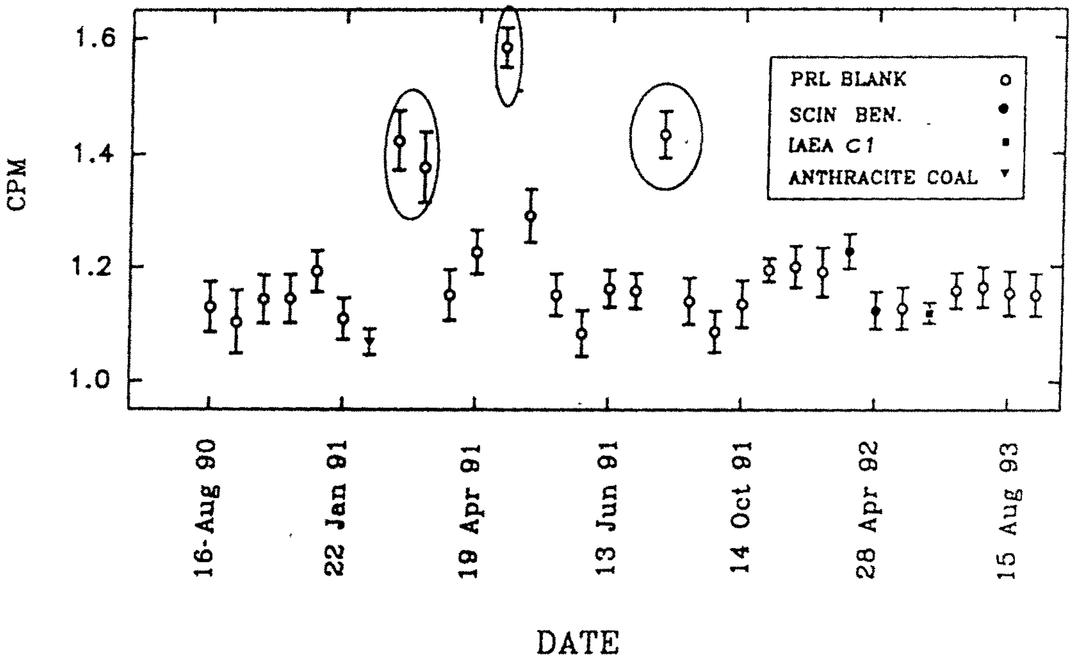

Fig 2.4 ^{14}C blank (background) count-rates of marble and other ^{14}C free materials over a ~2 year period. The measured ^{14}C blank count-rate excluding the four deviant (circled) points is 1.154±0.008 cpm. Error bar = ±2σ.

mentioned here that in the early phase of this work, scintillator grade commercially available benzene was also counted after mixing with scintillator cocktail. The count-rate of this commercial benzene was nearly identical to that of the marble blank. Analysis of IAEA carbonate sample (IAEA Quality Assurance Materials, sample C1, 1991), whose reported percent modern activity is ~ 0 also yielded a count-rate of 1.121±0.018, nearly identical to the mean background count-rate. Benzene synthesized from anthracite coal also gave similar count-rates (Fig 2.4)

NBS-Oxalic acid standard (SRM 4990C) was run 3-4 times per year. Table II.2 gives the net count-rates of the NBS Oxalic acid standard (cpm/3 ml benzene) during 1991 and 1992 when most of the measurements for this thesis work were made. (One of the oxalic acid runs (marked * in the table below) had a count-rate of 28.816±0.103 which was significantly higher (>2σ) than the mean of the all other measurements. For calculation of mean oxalic acid count-rate this run was excluded).

Table II.2 Net count-rates of NBS oxalic acid standard II-4990C

Date	Count-rate(cpm)
16.02.91	28.052±0.127
10.04.91	28.816±0.103*
07.06.91	27.762±0.102
16.10.91	28.077±0.078
07.04.92	28.064±0.087
05.05.92	27.748±0.091
08.12.92	27.760±0.115
Mean	27.911±0.069

Fig 2.5 shows the pictorial representation of the data in Table II.2. The mean net oxalic acid count-rate calculated for the period (1991-1992) is 27.911±0.069 cpm (excluding

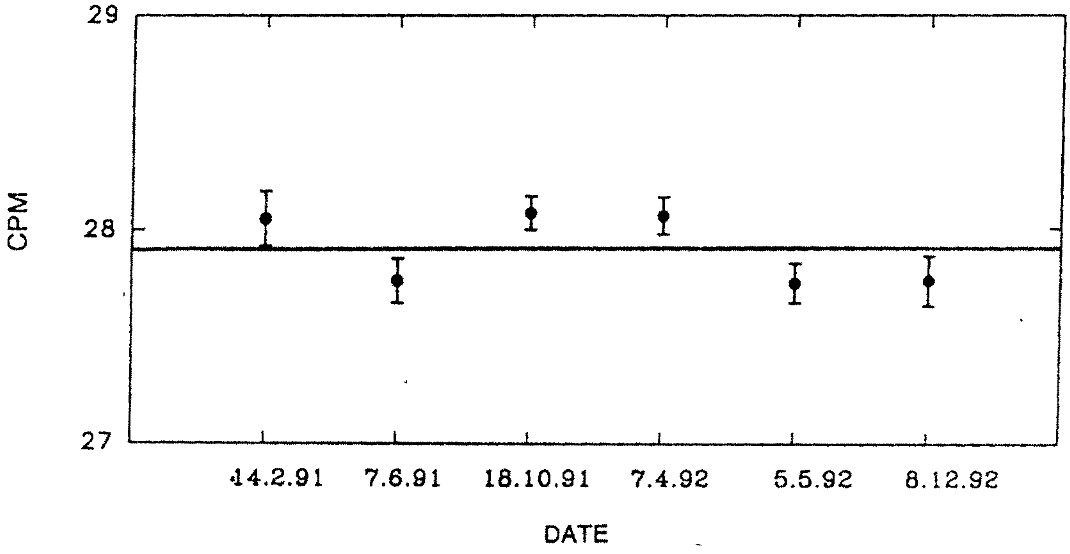

Fig 2.5 Count-rates of NBS Oxalic acid II standard (4990C) during 1991-1992. Error bars are ±1σ. The line drawn is the mean count-rate during the course of this work (27.911±0.069 cpm).

the high count-rate sample; error is on the mean). This value was used for $\Delta^{14}C$ calculations for all coral and tree ring samples.

II.3.e Reporting of radiocarbon activity

For reporting of ^{14}C activity we follow the notations described by Stuiver and Polach (1977).

$$\delta^{14}C(‰)=[(A_S e^{\lambda(y-x)}/A_{abs}) \ -1] \ 1000 \qquad (2.1)$$

and

$$\Delta^{14}C(‰)=[(A_{SN} e^{\lambda(y-x)}/A_{abs}) \ -1] \ 1000 \qquad (2.2)$$

where $\delta^{14}C$ is defined as the relative difference between the absolute international standard (A_{abs}) and the sample activity (A_S) corrected for age, and $\Delta^{14}C$ is the ratio of sample and standard activity normalized to $\delta^{13}C$=-25‰. (A_{SN}= normalized sample activity, y=the year of measurement and x is the year of growth).

II.3.f Intercalibration and repeat measurements

To check the reproducibility, four repeat measurements of a coral powder (Am, from Amini island, Lakshadweep) was run over a period of about two years. The replicate measurements (n=4) gave a mean value of $\Delta^{14}C$=50±11. The large error is because one of the samples deviated considerably from the other three. This sample was also analyzed at Birbal Sahni Institute of Palaeobotany, Lucknow using a gas proportional counter which yielded a value of $\Delta^{14}C$=69±6. Further checks on our measurements were made by analyzing IAEA Quality Assurance Materials (carbonates and cellulose) for their ^{14}C activities. The results (Bhushan *et al.* 1993) are in good agreement with the values published by IAEA.

II.4 CADMIUM MEASUREMENTS

Cadmium measurements were made in a coral (KV-1, *P. compressa*) from the Kavaratti island. This coral had a life span of ~5 years, with an average band width of ~23 mm as determined by X-radiography. This coral was chosen for Cd measurements as it had a high growth rate, which allows subsampling over time intervals of a few months whereby it is possible to look for signatures of seasonal upwelling.

II.4.a Sample cleaning

For Cd measurement ~0.5g of coral sample of 2-3 mm thick was chipped. These chips were thoroughly cleaned through a series of oxidative and reductive reaction sequences as suggested by Shen and Boyle(1988). First the coral chips were placed in distilled water in 20 ml glass vials and were subjected to ultrasonic agitation for 10 min. This was followed by treatment in 0.16N HNO_3 in ultrasonic bath for 3 min and then H_2O rinse. Next the samples were cleaned in a mixture containing equal parts of 30% H_2O_2 and 0.2 N NaOH to hydrolyze and oxidize organic coatings. After this the samples were kept in a boiling water bath for 20 min with repeated ultrasonic agitation. During this treatment, they were rinsed twice with distilled water. The next step was to remove oxide coatings with reducing agents (a mixture of 1 part hydrazine, 6 part conc. NH_4OH, and 3 parts 0.3M solution of citric acid in 7N NH_4OH) on a hot water bath with intermittent ultrasonic agitation. The cleaned samples were rinsed thoroughly with distilled water. In the final step, samples were again treated twice with 0.16N HNO_3 in ultrasonic bath followed by distilled water rinse each time. The samples were then dried under a laminar flow bench at temperature ~80°C.

II.4.b Coprecipitation of Cd with APDC and atomic absorption analysis

The concentration of Cd in corals are too low (~5 nmol Cd/mol Ca) to be measured directly in a coral solution. Thus it needs to be pre-concentrated, which was done as follows. An accurately known amount (typically 0.25 g) of cleaned coral sample was dissolved with

4N HNO_3 and made to 10 ml. 1 ml aliquots of this solution were then transferred to four precleaned 15 ml polypropylene tubes and volume made to ~ 6ml. In three of these four aliquots a known amount of Cd (0.5, 1, 1.5 ng respectively) was added. $CoCl_2$ solution (containing ~ 0.1 mg Co) was added to each tube and pH of solution was adjusted to 4.5±0.5 with ammonium acetate buffer. Cobalt was precipitated as dithiocarbamate by adding a known amount of 1% solution of APDC. The polypropylene tubes containing precipitates were allowed to stand for 3-4 hours, at the end of which the precipitates were centrifuged (at 11,500 rpm) for 5 min. The Co-APDC precipitate was washed twice with distilled water and centrifuged each time to remove the Ca occluded in the precipitate. The precipitate was then transferred to a 5ml conical teflon cup using ~3ml of 8N HNO_3 and warmed at ~70 °C (under a laminar flow bench) to oxidize the organic matter. The residue was dissolved in 20μl of 4N HNO_3 and diluted with distilled water to a final volume 1020 μl.

Table II.3 Reproducibility of blank and Cd standard absorbance

Date	Blank (Abs)	Stand. (Abs)
12.01.93	0.003	0.012
21.01.93	0.004	0.013
24.01.93	0.003	0.012
25.01.93	0.007	0.011
28.01.93	0.002	0.014
30.01.93	0.002	0.011
01.02.93	0.002	0.012
09.02.93	0.01*	0.012
11.02.93	0.006	0.015
13.02.93	0.003	0.012

16.02.93	0.005	0.015
mean	0.0037±0.0017	0.013±0.0014

(* this number was excluded for calculating the mean)

The reagents used throughout the coprecipitation were precleaned as suggested (*op cit.*). Along with each batch of samples reagent blanks and standards were also run.

The Cd measurement in the samples were made using an atomic absorption spectrophotometer (Perkin Elmer model 4000) attached to an HGA 500 graphite furnace. All analyses were performed employing the continuum background correction. Each sample and standard was measured 2-3 times with 20 µl injection. Table (II.3) gives the results of the replicate measurements of blank and a laboratory standard run during the sample measurements. This standard was a $CaCO_3$ solution made by dissolving 10g of coral sample in dilute nitric acid.

Inspite of the variability in the blank absorbance we see that the standard's absorbance is consistent (11% variability) which indicates that the occasional high blanks are due to some contamination. Rejecting the high blank (abs 0.01) we get the mean blank absorbance as ~ 0.004 (typical sample absorbance without Cd spiking is 0.018).

II.4.c Measurement of Cd concentration

The Cd concentration in the coral sample was determined by standard addition method (Beaty 1978). Blank was also ascertained in a similar way. Since the samples and blanks were made to identical volumes, the blank absorbance (0.0037) was subtracted from that of the sample. The Fig 2.6 shows a typical plot between the conc. of Cd added and the net sample absorbance. The regression line gives an intercept (x_m) corresponding to the sample concentration in ng. This is then normalized to the known weight of $CaCO_3$ initially taken to express sample concentration in units of nmol Cd/mol Ca.

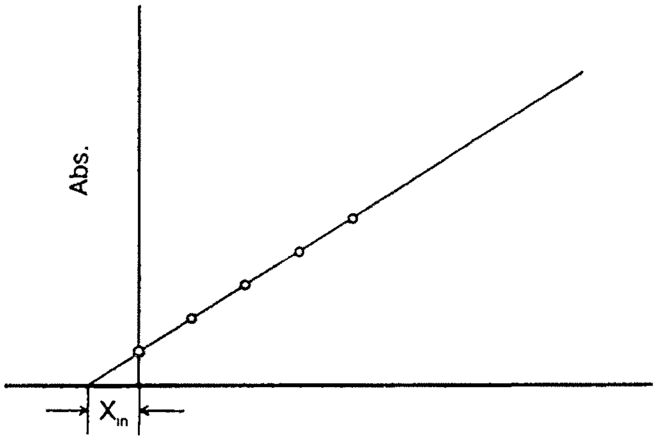

Fig 2.6 Determination of cadmium concentration by the standard addition method

In order to check the reproducibility on Cd concentration a coral sample was run three times. Following table (Table II.4) shows the results of these runs.

Table II.4 Reproducibility of Cd measurements in "spiked" coral solution

Date	conc.(nmol Cd/mol Ca)
20.01.93	10.7±1.65
23.01.93	11.1±1.34
25.01.93	12.3±0.82

II.5 SUMMARY

As discussed above, time series measurements of stable isotopes of oxygen, carbon and radiocarbon, were made in corals and tree rings. Table II.5 summarizes the various measurements made on different samples in this work.

Table II.5 Summary of measurements made in corals and tree rings in this work

Sample Code	Genus/species	Lifespan (year)	Tracers measured
Lakshadweep			
KV-1	*Porites compressa*	5	$\delta^{18}O$,$\delta^{13}C$, Cd
KV-2	*Porites compressa*	25	$\delta^{18}O$, $\delta^{13}C$
Am	*Porites compressa*	4	$\delta^{18}O$
GC	*Tridacna maximus*	4	$\delta^{18}O$
Gulf of Kutch			
GK	*Favia speciosa*	40	$\delta^{18}O$, $\delta^{13}C$ $\Delta^{14}C$
Stanley Reef			
SR	*Porites lutea*	18	$\delta^{18}O$, $\delta^{13}C$ Density*
Thane (tree)			
ThTR	*Tectona grandis*	20	$\Delta^{14}C$

*Density measurements were made by Dr J M Lough of the Australian Institute of Marine Science, Townsville, Australia