Chapter-4

Methodology

Methodology

Expeditions have been carried out to perform reef drilling for various purposes like testing of Darwin's subsidence theory to understand the origin of coral reefs **(Darwin, 1842; Bonney, 1904)**, to test atomic weapons during World war -II etc. Initial drilling operations involved use of cumbersome drilling equipments. The invention of small and light weight rigs made the reef drilling studies feasible in terms of transportation, money and time consumption therefore, it widen the scope of reef drilling studies. Then after a number of drilling exercises have been carried out for research purposes like petrological, chronological and paleo-environmental studies of the reef.

Rees *et al*. (2005), obtained reef cores using a **hydraulic rotary drill with diamond and tungsten drill heads** (5 cm diametre for Rodrigues and 3.5 cm diametre for Lizard Island and Mac Gillivray Reef cores) powered by a petrol generator. Gherardi and Bosence (2005) performed the **core drilling technique using a pneumatic** core cutter (model CP-315-B, Chicago Pneumatics) and a core barrel (measuring 3 inch in diameter and 1 m in length) for the collection of reef cores. The drilling machine was powered by a 10.5-HP diesel engine and a 20-cfm (cubic feet per minute) air compressor. The transportation of the equipment and crew were carried out in two inflatable boats.

Paleontologists studied fossil corals in detail long before biologists started to look carefully at their living descendants. Dating is possible through radioactive isotopes of uranium, thorium (Amin, 1970) and carbon (Agrawal *et al.*, 1965; Gupta, 1973). In the present study age estimation was carried out through radio **carbon dating method (Liquid scintillation counter).**

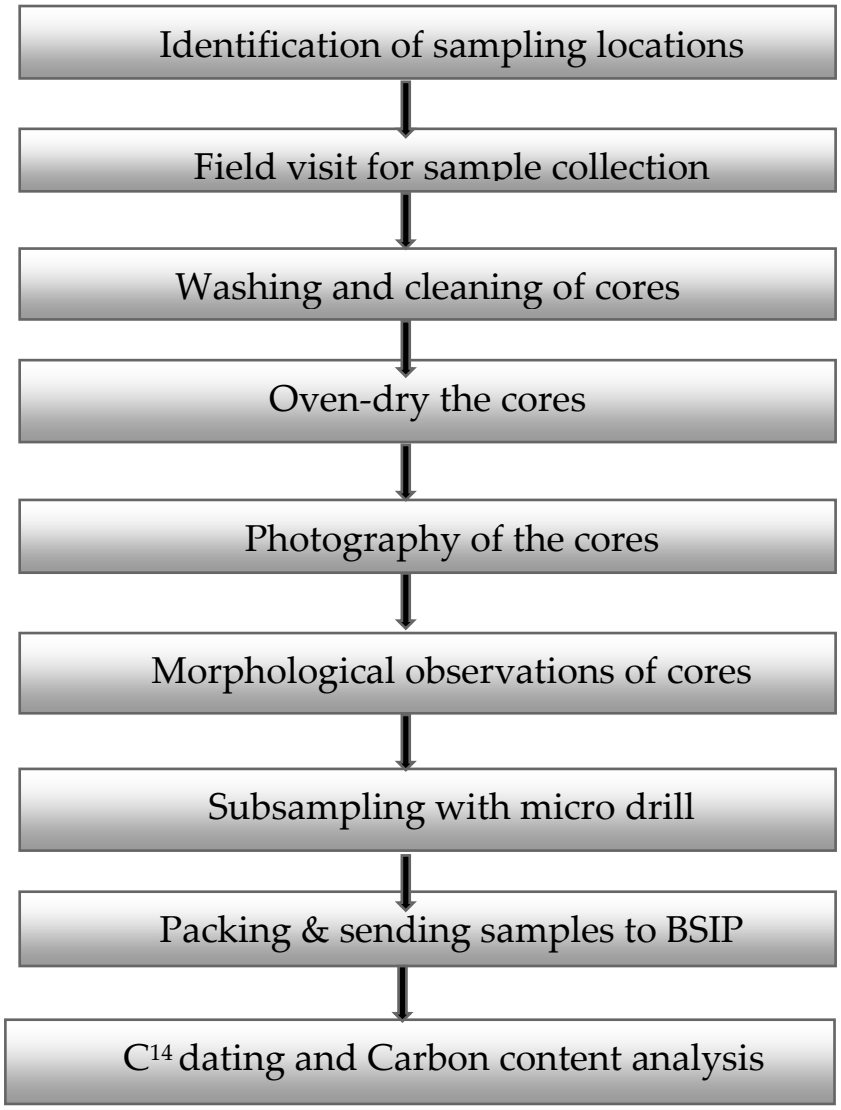
4.1 Step wise Methodology

For the determination of the Carbon Storage Potential of the reefs of Gujarat, the method has been broadly divided into two parts viz.

4.1.1 Field expedition: It involved Site selection, Sample collection labelling and packing

4.1.2 Laboratory analysis: It involved washing and cleaning of cores, morphological study and scientific photography, subsampling, sample packing and C^{14} analysis

Step wise Methodology



1) Identification of sampling locations

The first step for sample collection requires field survey for suitable site selection on the reefs of various islands. A total of 10 locations including submerged reefs, Islands and coastal areas were surveyed for appropriate site selection, namely Poshitra, Okha, Paga, Boria, Ajad, Narara, Goose, Kalubhar, Dedeka-Mundeka, Pirotan.

Figure 4.1 Sample collection

Study locations selected:

1. Boria
2. Ajad
3. Kalubhar
4. Narara
5. Goose
6. Dedeka-Mundeka

2) Field visit for sample collection

As per the discussion with Scientist, BSIP, major criteria for site selection are:

- The drilling should be parallel to the growth axis of coral
- Compact or well indurated site was selected(applied after first collection)
- Depth should be 1 meter
- Accessibility in terms of boat and generator placement

This procedure includes following steps:

- The first step on the reef was to survey the site and fix sampling point by cutting the reef portion using hacksaw to ensure its biological (Coral) origin.
- Equipment preparation: Carrying the drilling machine, generator and packing material to the fixed points.
- The collection of dead coral core was achieved by generator operated diamond core drilling machine- model-212. It had the barrel diameter of 6 mm and barrel length 1 m.
- Labelling and packing of the cores: Each piece of drilled core recovered was labelled to record orientation and position within the entire core section.
- After the completion of first drilling procedure, the researchers again surveyed the reef for second point selection. Meanwhile rest of the people wound up the equipments and took them to the small boat and got ready to take the boat at the second selected point. The duration of this procedure differed site wise.

Plate: 4.1- Field equipments: (a&b) Diamond core driller and (c) Diesel operated generator

Plate:4.2- Field Procedures: a-measuring the drilled hole; b-partially drilled core; c-Drilling going on at very uneven and difficult reef surface; d-Field difficulty- Boatmen partially in water, holding the boat against high tide waters of the GoK.

3) Washing and cleaning of cores

The cores were washed properly using tap water to remove debris to avoid contaminations in laboratory analysis. The cores were not continuous structures but were broken in several pieces/segments during the drilling procedure. Hence, this step was carried out carefully so that the sequence and position of the core segments doesn't get disturbed. Then the external core surface was dried up using tissue paper.

4) Oven-dry the cores

Then these clean cores were kept for oven drying. The duration and temperature of this process was not available in peer-reviewed literatures, therefore, on the trial and error basis, the cores were kept in oven till complete drying. On an average, the cores took about 40 hours at 50°C for complete drying.

5) Photography of the cores

Before preparing the samples for cutting and laboratory analysis, it is required to have a photographic documentation of the core length, segments and the occurrence of any other peculiar characters with reference to length. For this, the cores were placed on black paper and photographed.

Figure 4.2 Core Photography

6) Morphological observations of cores

The morphological study of the cores was performed to extract out lithological descriptions from the cores involves the details of growth axis and layers in the core as well as its analysis and possible interpretation like genus identification or at least genus differentiation based on growth band variations, sedimentation analysis, presence of bores etc.

Figure 4.3 Morphological Study

7) Details of core petrology:

The types of Facies have been identified for each core based on ocular observations. The classification of Facies, the growth band pattern and identification of biogenic origin of the cores were followed after Grossman and Fletcher's petrology study of reef core and the study of late Holocene reef growth and relative sea-level changes in Atol das Rocas, by Gherardi and Bosence (Grossman and Fletcher, 2004 and Gherardi and Bosence, 2005).

8) Subsampling with micro drill

The subsampling was carried out to collect sample powder from different length of the collected cores *i.e.,* i) Point-1: bottom of the core, ii) Point-2: bottom + 25 cm upward. The samples were directly obtained by the micro driller. Then the semi- powdered material was ground to fine powder using the mortar-pestle.

Figure 4.4 Subsampling by micro driller

Rectification: In case of modern bottom date, the other end (Top) and 25 cm downward from it was preferred.

9) Sample packing and sending to BSIP

The prepared samples were weighed 40 gm, then the samples were packed in the air-tight and labelled containers and sent to the BSIP for $C^{14}$and carbon content analysis.

Figure 4.5 Sample preparation

Figure 4.6 Sample packing

10) C^{14} dating and Carbon content analysis

The age determination was carried out in collaboration with Birbal Sahani Institute of Paleobotany, Lucknow. The standard method for calcareous sediment was followed viz. treatment of the sample with hydrochloric acid and purifying the resulting carbon dioxide. This gas was passed through molten lithium in a reaction vessel at 750°C (temperature of the vessel). This was followed by raising the temperature to 900°C and then hydrolysis using demineralised water after cooling. The resulting acetylene was collected at liquid nitrogen temperature after going through multiple purification stages. This was followed by conversion to benzene slowly using vanadium catalyst as the trimerisation goes on overnight. The catalyst was heated and the benzene was collected at liquid nitrogen temperature. After aeration and some time-gap for radon decay, the benzene was carefully weighed and transferred to a vial and put in the counter after adding the known amount of scintillator (Butyl PBD).

Acetylene formation:

$$2CO_2 + 8\ Li \rightarrow 2\ C + 4\ Li_2O$$
$$2CO_2 + 10\ Li \rightarrow Li_2C_2 + 4Li_2O$$
$$Li_2C_2 + 2\ H_2O \rightarrow C_2H_2 + 2\ Li\ OH$$
$$2Li + 2\ H_2O \rightarrow 2\ Li\ OH + H_2$$

Benzene formation:

$$3C_2H_2 + V_2O_5 \rightarrow C_6H_6$$

The counting was done in liquid Scintillation Counter Quantulus 1220 in beta mode. It continues for 24 cycles. The corrections for background and spectral quench parameter were made. The latter is needed because the benzene produced may not be pure and the other hydrocarbons may influence the counting. The production rate variations, reservoir corrections etc. were taken care of by calibration procedure based on the work of Stuiver *et al*. (1998) and its version of 2004 carried out. The ages so resulting are expressed in Calendric Years BP.

Coral Bleaching

The study of coral bleaching was carried out covering two distinct seasons *viz.*, summer and post monsoon from 2010 to 2014. The survey was conducted in the intertidal areas of the sites during low tides. The reef area was surveyed by Rapid Ecological Assessment (REA) through ocular observations using belt transects of 20x1 meter (English *et al.*, 1997). The coordinates were recorded with E-trex Garmin hand held GPS navigator. The observations include the record of affected scleractinian species and other associated invertebrate fauna. The SST data have been derived from the www.oceanmotion.org and www.esrl.noaa.gov for the annual and monthly mean SSTs of the GoK. In order to draw inferences regarding the impact of thermal anomalies on corals, the SST data of last twenty years have been compiled and compared using the Sigma Plot 10.0 technical graphing program.

4.2 Post Sampling

The data collected from field as well as laboratory procedures were processed further in the following sequence:

The data entry was carried out in MS. Excel 2010, in order to bring forth the further analysis. Particularly, the quadrate data for the benthic community coverage and transect data was transferred and basic analysis and Histogram preparation was carried out. Proportion contributed by each benthic community at the site selected for drilling was derived in the form of % covered by that perticular community. This calculation was repeated for all the Study locations.

Statistics

t -Test: The student's t-test was performed between the coral bleaching percentage of every 2 consecutive years.

The calculations/ formula used in order to derive core recovery are as follows (Grossman and Fletcher, 2004):

$$\textbf{Core recovery} = \frac{l_{\text{sum of pieces}}}{l_{\text{tot core run}}} \times 100$$

$l_{\text{sum of pieces}}$ = Sum of length of core pieces

$l_{\text{tot core run}}$ = Total length of core run/drilling column length

Radio Carbon Ages

The radio carbon ages were found in terms of BC or AD, which was converted in to (before Present) BP by adding (BC) or subtracting (AD) 2014.

Age BP=Radiocarbon age (BC) + 2014

Age BP= Radiocarbon age (AD) - 2014