MATERIALS AND METHODS

STUDY AREA

Gujarat

The Coast line of Gujarat, more than 1600 km long and longest amongst all the coastal states of India has three distinct zones the Gulf of Kachchh, Saurashtra coast, and Gulf of Khambhat extending towards south Gujarat (Fig. 2). In terms of coral reef distribution, the Gulf of Kachchh is the northern-most reef of the Indian subcontinent. The region has arid and dry climate with high rate of evaporation. In addition, due to the shape of the gulf it has one of the highest tidal amplitudes that result in negative water balance (Satyanarayana, Ch. and Ramakrishna, 2009). Surface currents along with the sediments are high in the Southern part of Gulf, wherein 42 islands with rich coral reefs and mangrove ecosystems are present. The islands have rocky, sandy, and muddy intertidal zones masked by water that tend to open during the low tide phase.

Marine National Park and Sanctuary (MNP&S), Jamnagar

The southern Gulf of Kachchh (Fig. 3) is declared as a protected area by Ministry of Environment, Forest and Climate Change (MoEFCC) Government of India for conservation. This protected area, established in 1980 was designated as the first Marine National Park and Sanctuary (MNP&S) of India comprising an area of 457.92 sq km from Navlakhi to Okha. The MNP&S contributes its major role in harbouring the major macrobenthic flora and fauna. The Protected area was established for the conservation of its abundant and rich reef ecosystems. These reefs had suffered large scale quarrying of coral blocks by construction industries before the declaration of the Marine National Park.

Two Coastal reefs from MNP&S at Narara and Poshitra (Fig. 4) were selected for the present study to evaluate status of family Faviidae and fauna associated with it.

NARARA

The Narara reef (Fig. 5), Narara Tapu or Narara Bet lies between 22°25.8'N to 22°28.3'N and 69°42.1’E to 69°40.7'E. The reef formation of Narara is now joined to the mainland because of land filling and is accessible by walking or vehicle. This island has rich coral diversity and also supports good patches of mangroves. Narara reef is also one of the famous tourist attractions and a site for Nature Education Camps organized by MNP & S authorities annually to generate awareness on marine life among the local school students of adjoining areas.

POSHITRA

It is also a coastal reef connected to the mainland where the coastline has a small embayment lying close to Laku point (Fig. 6). This area contains eulittoral fringing reefs about 100 meters wide. In this reef during ebb tide several intertidal pools are formed which have rocks covered by barnacles and oysters. This gives the reef a rugged appearance that needs care while treading through them because of the knife-edged topography. These rock pools present in tiers clearly show variation to coral distribution and diversity depending on the exposure to the tides. Though comparatively few tourists visit this place to see marine life, Educational camps are organized here also to create awareness among the public. Fishermen visit this place occasionally to catch crabs and reef fishes.

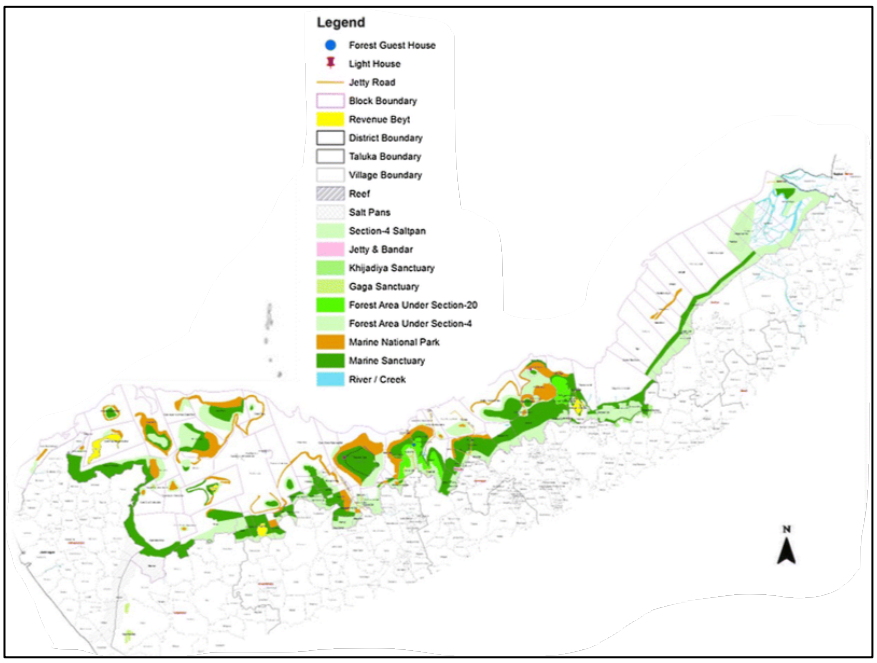

FIGURE 1 MARINE NATIONAL PARK AND SANCTUARY, GUJARAT (MOFCC, IRDAE, NEW DELHI)

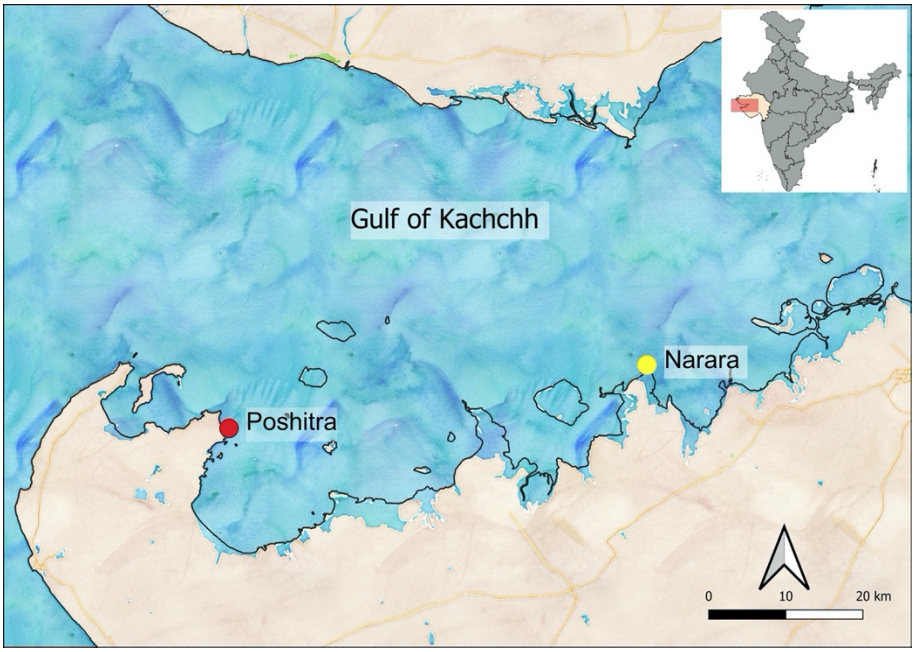

FIGURE 2 LOCATION OF NARARA AND POSHITRA COASTAL REEFS IN GULF OF KACHCHH

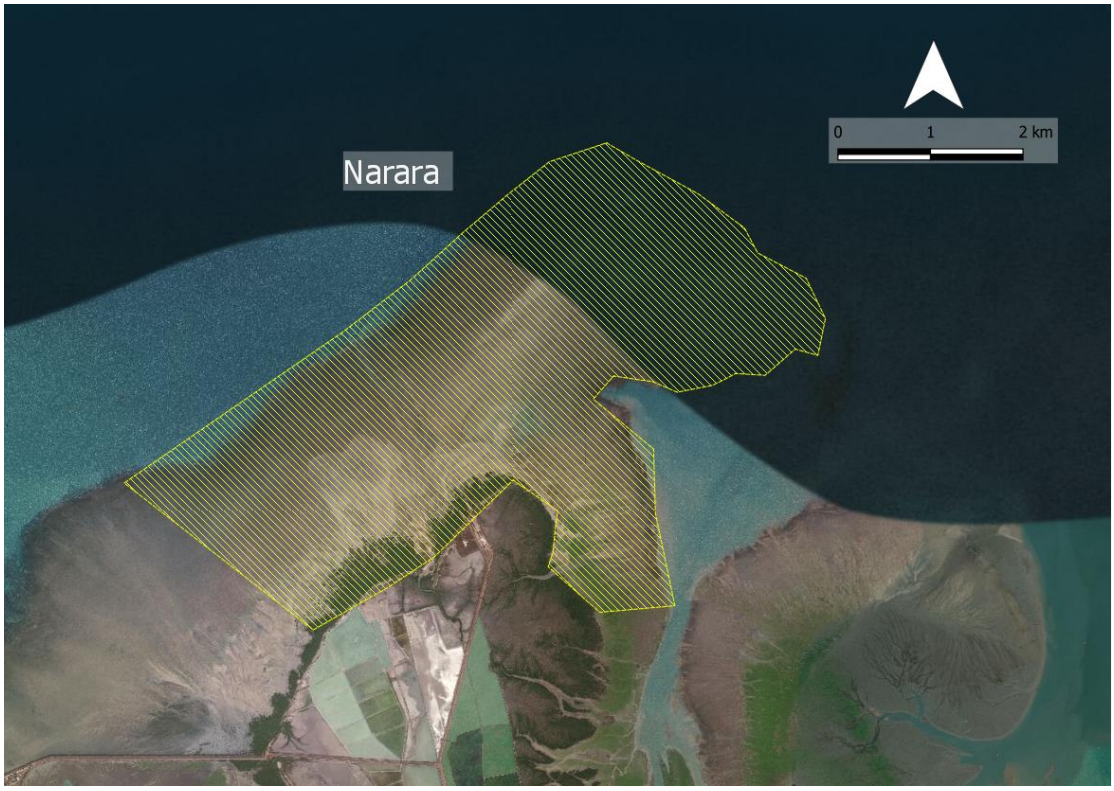

FIGURE 3 AREA COVERED ON NARARA COASTAL REEF

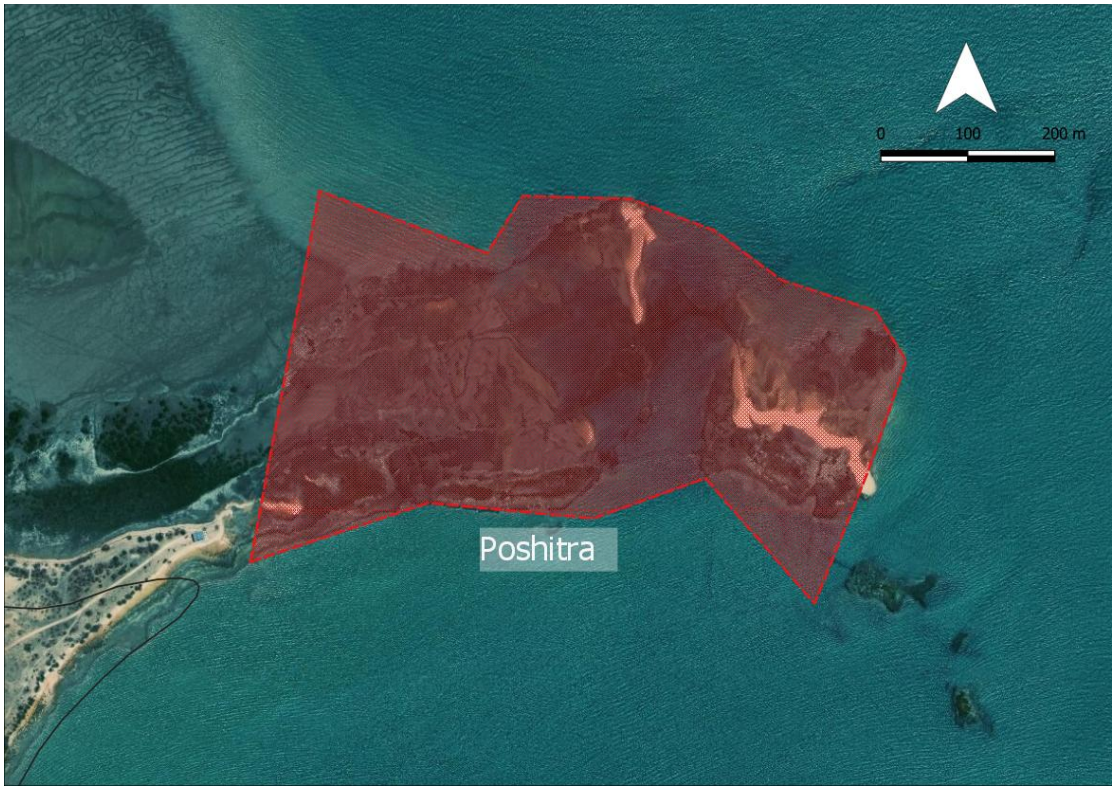

FIGURE 4 AREA COVERED ON POSHITRA COASTAL REEF STUDY SITE

RECONNAISSANCE SURVEY:

Reconnaissance surveys (Fig. 7) were conducted at Narara and Poshitra coastal reefs and the probable areas were plotted on the digital map using the QGIS software. On initiation of sampling, this plotting was used at the reefs to lay the transects, quadrats, and finalizing the sampling sites for the collection of water samples. The survey was planned based on the secondary data available for both (Narara and Poshitra coastal reef) the selected reefs in the Marine National Park, Southern Gulf of Kachchh. The data collection was done by walking on the reefs and not by the SCUBA. The intertidal reefs in the Gulf of Kachchh are muddy and become turbid immediately if walked through.

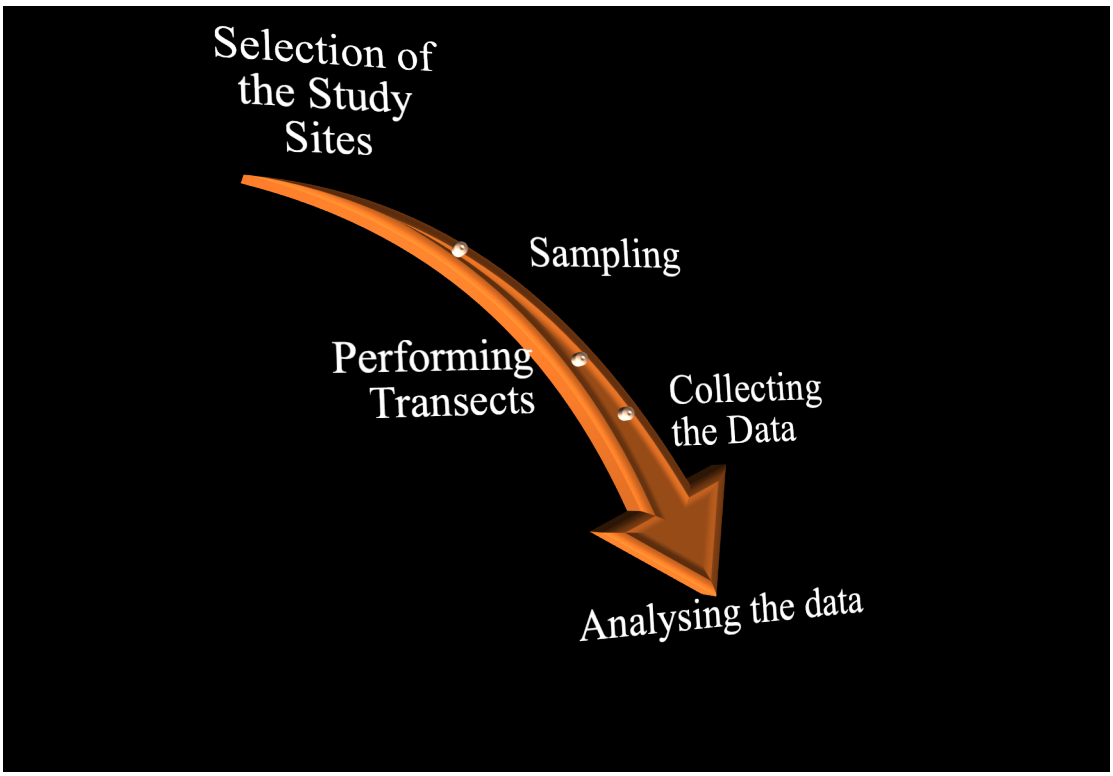

FIGURE 5 FLOW CHART OF WORK PLAN

DATA COLLECTION

The Gulf of Kachchh with its unique entrance to the intertidal zone gives easy access to the selected reefs. During the tide-ebb hours, the reefs here are exposed to a vast extent and hence can be explored for long duration. The total effort given for data collection at each reef was 12 visits with three hours spent on each reef by four people which included myself, two volunteers and a field assistant. These amounted to 144 hours spent separately for two reefs by four individuals totalling to 288 hours.

LINE INTERCEPT TRANSECT

The Line Intercept Transect [LIT] was originally developed to study terrestrial plant ecology. Reef ecologists Loya (1978) and Marsh et al (1984) subsequently adapted this method to study reef ecology. This method is used to estimate the cover of flora and fauna that is encountered within the specified zone by estimating a small area or a location within the length of a specified line that is intercepted. LIT is used to evaluate the total percentage count of diversity that is to be studied. The use of LIT has been ranging from large-scale- spatial problems to morphological comparisons of coral communities and anthropological disturbances as well. A standard procedure to use LIT was performed using a 20-meter long fibre-glass tape. Random Transects of 10 meters were laid parallel to the coast line. After marking the transect a quadrat was laid within the transect line to find out the fauna encountered. Here, quadrates were placed with a distance of 2 meters from the former and latter one (Fig. 8). The next transect was laid after a space of 5 meters to avoid the clatter of the previously laid transects. This standardization helped in acquiring the unbiased form of data from the transects conducted.

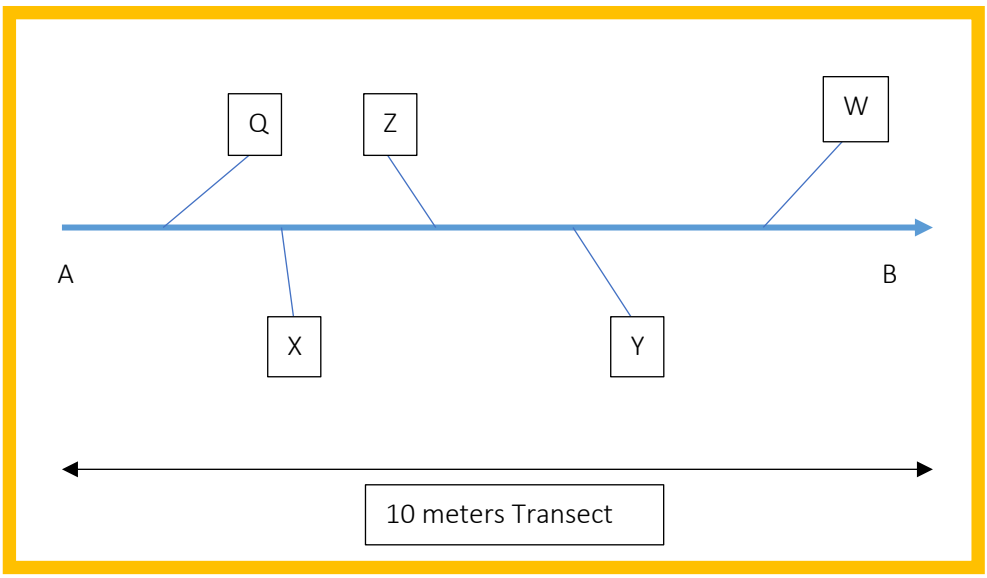

FIGURE 6 A 10-METER TRANSECT EXAMPLE

POINT INTERCEPT TRANSECT

The Point Intercept Method [PIT] is used to regularly monitor live corals and their condition. It is a simple and quick way to assess the associated macrobenthos on the reef systems (Wilkinson, 2002). General reef management conditions could be studied through this method. However, this method also helps in determining the sessile benthic community that is living on the sea bottom floor or on the reefs. A transect of 10 meters was placed parallel to the seashore by fiber-glass tape (Fig. 9). In each transect, the number of corals and the fauna associated was noted. All the transects were performed parallel to the sea to ensure the reef biota is attained in the Point Intercept Transect line.

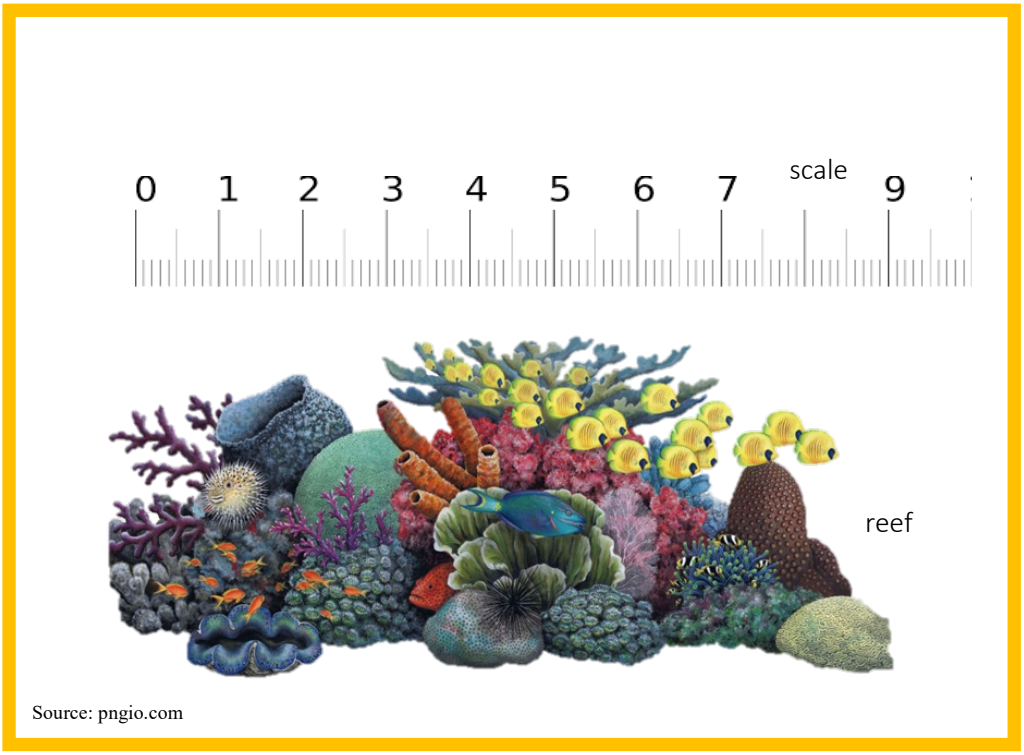

FIGURE 7 POINT INTERCEPT TRANSECT METHOD ON THE REEF

QUADRATS

To collect the data a Quadrat of 1x1 meter size, further divided by 100 small grids of 10 cms each was developed (Fig. 10). Such divisions assist in collecting the data from small coral covered tidal pools. The 1x1 meter size quadrat with grids is strategic for the Gulf of Kachchh area as the visibility remains clear in the quadrat.

As per Ohlhorst (1988) Quadrat refers to a squared sampling area within which the fauna falling into its grid boundary is counted. Quadrat can also be used to estimate the percentage cover of individuals or various species of corals to obtain their density, abundance, diversity, and the macrobenthic assemblages. Generally, one-meter quadrats are used for reef monitoring but it may depend on the size of the organism/s of interest. The quadrat is placed far apart so that the boundaries do not overlap. The placement of the quadrat varies from permanent to random as per the reef monitoring objectives. They can also be sampled with different transect methods. The sampling area being smaller in a quadrat, the identification

of corals and various macrobenthic fauna along with other details on various characteristics of substratum can be obtained comprehensively (Arthur, 2000).

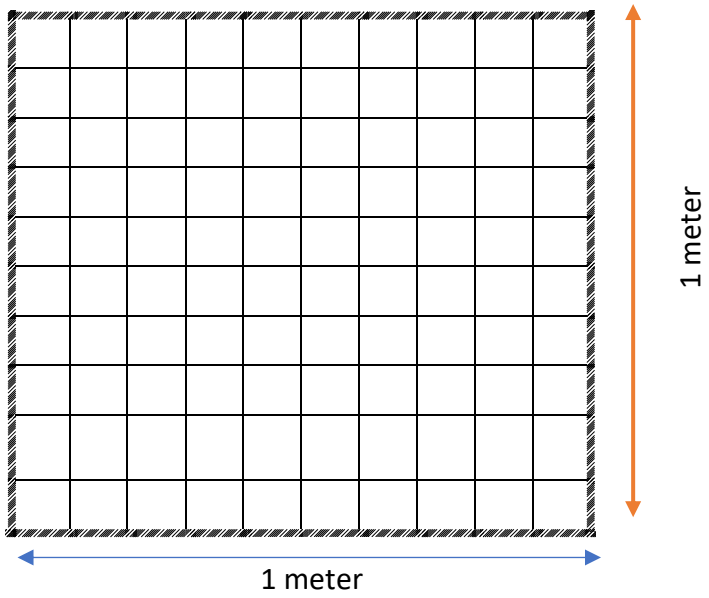

FIGURE 8 1X1 METER QUADRAT HAVING 100 SMALL GRIDS

Techniques for analysis of coral reef communities are derived from concepts used by plant ecologists for the study of terrestrial vegetation (Loya 1978; Scheer 1984). It is important to note at the outset that plant ecology texts (Kershaw 1964; Southwood and Henderson, 2000), as well as standard texts on statistical methods, all emphasize the necessity of random sampling and replication.

The only randomization can furnish unbiased estimates of variance and of the population mean, and it is required for the application of most statistical testing (Green 1979). For example, all ANOVAs require that sampling of individuals be at random (Sokal and Rohlf 1981). On an operational level, the hazards of non-random sampling are that human choice is often biased particularly with respect of variation, resulting in sets of samples that are prejudiced and not representative (Sokal and Rohlf 1981).

Random sampling requires that each unit or member of the population has an equal and independent chance of being selected (Zar 1999). Randomness is ensured by choosing each member from tables of random numbers or by a computer program that generates such a table. In a coral reef context, randomly positioned transects over a reef or randomly located quadrats and points along such a transect satisfy the requirements. In the reef ecosystem use of qudrat sampling also helps in collecting information for substrate quality.

The following substrate characteristics were calculated by the Percentage area covered using grids in the quadrat:

1.Total Percentage of live corals:

Number of grids occupied by Faviids along with associate life forms including other coral species within a quadrat.

2. Bleached coral cover

Number of grids covered by Bleached corals in the quadrat

3. Algal cover

Grids covered by algae associated with Faviids within the quadrat

4. Mud

Number of Grids covered by mud (on the corals and around them)

5. Sand

Number of Grids occupied by sand within the quadrat

6. Rock and gravel

Number of Grids occupied by rock and gravels in the quadrat around the corals. These structures favour the settlement of coral larvae and their growth. Whereas silt, sand, and mud do not allow larval settlement thereby resulting in the confinement of their growth (Lee et al 2009).

MACROBENTHIC ASSOCIATED FAUNA

The macrobenthic fauna associated with corals play important role in composition and association in the reef ecology. The associated macrobenthic fauna included in present study are viz. Porifera, Zoantharians, Cnidaraians, Gastropods, Crustacea, Nudibranchs, Echinoderms, and Pisces. They are identified by various standard books /keys for identification. These include Veron (2000), Satyanarayana Ch. and Ramakrishna (2009) and Apte (2012). In addition, several Websites eg. Sealifebase, WORMS, marinespecies.org, marine species identification.com and several research articles published are used and occasionally experts were also contacted for the identification of species.

The macrobenthic associated fauna was reckoned through the point count method and through the quadrat grid method.

INSTRUMENTS USED

Nikon Coolpix AW120, Bearing Compass Sunto, Quadrat made from

1x1 meter PVC pipe with nylon string fixed into the pipe on two opposite sides, fiber-glass Tape 20 meters, 11-inch forceps, rubber gloves, pH meter, Aquasol Digital- multiparameter AM-AL-01 [pH, TDS, Salinity, Temperature].

The diversity of the species was studied while walking on the transects.

- The data collected for 12 visits from each reef was used to analyse Frequency Distribution Graph using single factor ANOVA for the live coral cover of lower strata using R-stat programming.

- Relative abundance (RA) of the species was calculated as

$$RA = \frac{total\ no.of\ species\ in\ community}{total\ community} \times 100$$

- The species diversity index of macrobenthic fauna and Mean, Standard deviation, average, Percentage cover of all the benthic community, Dominance, Simpsons, Margalef, Equitability J, were performed using PAST version 4.03 Software.
- To find out correlation between various physicochemical parameters Pearson's correlation Index was calculated using R-stat programming.

PHYSICOCHEMICAL PARAMETERS

To assess physicochemical parameters of sea water the water samples from the reefs were collected from random locations from the intertidal pools during the low tide. Temperature, Dissolved Oxygen, pH, Salinity, Nitrates, Nitrites and Phosphates were considered for the present study.

The chemical components of Dissolved Oxygen (DO), Nitrates, Nitrites, and Phosphates were measured with the help of water testing kit (Aquasol).

TEMPERATURE

Temperature influences physicochemical parameters of water and in turn flora and fauna of aquatic ecosystem. Hence, the temperature of sea water was measured at site itself. A calibrated thermometer was allowed to stand in the seawater for two minutes and the reading was recorded.

Equipment used: Certified thermometer having mercury thread: 0-50°C with 0.1° accuracy.

PH

pH is one of the important components in sea water. Depending upon its acidity and alkalinity values the reef fauna exists. The study on ocean acidification suggests the pH as an important factor influencing the coral bleaching and climate change. Hence pH was an important component to be incorporated for the study.

To determine the pH of seawater the electrodes of a pH meter duly calibrated at the desired pH range were introduced in the seawater sample and pH was recorded.

Equipment used (Aquasol): Portable pH meter with glass calomel electrode: Accuracy 0.1.

The device was calibrated with buffer before every field visit.

SALINITY

The sea water is not uniformly saline in the world oceans. There is mixing of water from several rivers and estuarine which regulates the salinity of the sea. The ocean currents also carry water from one place to other. The Salinity was measure by Aquasol multiparameter instrument.

DISSOLVED OXYGEN (DO)

Dissolved oxygen in water gives an idea about productivity of aquatic ecosystem. Hence, DO was measured in sea water sample using kit (Aquaol).

Directions for use: The DO is fixed before testing. DO test bottle were rinsed 2-3 times with sample water. While collecting samples the bottles were filled till the water over flows with sample water and then stoppered to ensure that no air bubbles are trapped inside. 10 drops of DO 1 reagent was then added in to the samples followed by DO 2. Mixed well. After a minute brown precipitate were formed started setting down. After firmly stoppering the contents of bottles were shaken thoroughly. The bottles were then kept at a safe place for maximum of 20 minutes. 10 drops of DO 3 were added, the stopper was replaced and the bottles were shaken vigorously till the precipitate dissolved. This sample was used for testing.

DO determination:

Take 10 ml of sample in the test jar.

Add 4 drops of DO 4 and mix well.

Now drops wise add DO 5, counting the number of drops while mixing until the blue colour disappears.

Calculations:

DO ppm= 0.5 x (Number of drops of DO 5)

NITRATE

Nitrated is one of the important nutrients in an aquatic ecosystem which influences the productivity hence, considered in present study.

5 ml of sample was taken in a test tube and one spoonful of HNT1, was added. The mixture was shaken well and Kept aside for 10 minutes, while shaking intermittently. To this, three drops of HNT2 were added, mixed well and again kept aside for three minutes, while shaking intermittently. Then one spoonful of HNT3 was added and shaken well. After waiting for 5 minutes to allow maximum colour development, the mixture was diluted to 25 ml mark with DM water. The content was transferred to a small comparator tube provided and the ppm Nitrate were read as given in the chart.

NITRITE

Nitrite is also one of the important nutrients of aquatic ecosystem hence included in the present study.

In a 10 ml of the water sample to be tested in the test jar 2 drops of NTH1 were added and mixed well. Then NTH2 were added dropwise counting the number of drops while mixing until pale blue or bluish-green colour appeared.

Calculations:

- Nitrite ppm as $NaNO_2$ = 5 x (Number of drops of NTH2)

PHOSPHATE

With Nitrate and Nitrite, Phosphate is also an important nutrient in aquatic ecosystem. Increase in the levels of these nutrients can lead to eutrophication of an aquatic ecosystem.

In a 10 ml of filtered water sample to be tested in the test jar 2 drops of SQ1 solution were added and mixed well. pH of the sample was adjusted between 2 to 3 by dropwise addition of SQ2 using pH paper. One spoonful

of SQ3 powder(provided) was added to the sample and mixed well. The sample turns yellow. Now drop wise SQ4 was added, counting the number of drops while mixing, until the colour changed from yellow to reddish-orange (S).

Repeated the procedure given above with the raw water to makeup water blank. The number of drops added are noted as (B).

Calculations:

Phosphate as ppm HEDP= 5 x (S-B)

= 5 x (Number of drops of SQ 4 for sample) – (Number of drops SQ 4 for blank)

LIMITATIONS

During the study, there were several limitations of visiting the site and working on the reef area. The study conducted so far was from the month of October to June only as all the National Parks are closed from 15th June to 15th October every year in the Indian Sub-Continent.

PERMISSIONS

The study was conducted in the Marine National Park, Jamnagar Gujarat. Necessary permissions were taken from the Principal Chief Conservator of Forest [PCCF] Gujarat Forest Department [Ref. No. WLP/28/C/126-27/2015-16.] and Gujarat Biodiversity Board [Ref. No. GBB/form-1/T-3b/1630/2015-16].