3 MATERIAL AND METHOD

3.1 Study Areas

As described earlier, the Saurashtra coast has one of the largest continental shelf in India with salient diverse geo-morphological and hydrological features leading to different environmental variables in limited spatial zone. The rocky intertidal zones in these coastal areas were considered for this study.

For the present study the investigation was carried out at four different sites i.e. Navapara (20°56'26.7"N 70°18'23.3"E), Aadri(20°57'35.1"N 70°16'43.0"E), Vadodra dodiya (20°58'47"N 70°15'07"E) and Chorwad (21°00'02.6"N 70°13'34.6"E) along the south Saurashtra coast(**Plate 3.1**).

As stated in section literature review, ample of literature is available on the benthic diversity study of south Saurashtra region including Veraval but scanty or no data is available on the shores of Navapara, Aadri, Vadodra dodiya and Chorwad of the south Saurashtra coastline, which were the selected areas of observation in this study. This situation needs a systematic overall backup study to be acquainted with their present status.

However, in absence of sufficient data, this area was considered as the study site for this PhD work.

All sites except Chorwad are in Gir Somnath district and Chorwad is located in district Junagadh of Gujarat State, India. These all sites were located between two fishing centers Veraval and Mangrol.

All selected areas are located at the southern part of the Saurashtra Coast. The total length of individual shoreline is approximately between 1.5 to 2.5 km. Selected areas between Veraval to Mangrol mainly consists of rocky shore with a few sandy patches. The intertidal zone at selected areas varies from 60 to 70 m. Anthropogenic actions were seen more or less at all selected sampling sites. Due to presence of a Temple in Aadri, it was seen in rise in the anthropogenic activities. Chorwad beach is famous for holiday camps. It was once a home to the royal family of the Nawab of Junagadh. Apart from this, Chorwad is also a visitor's paradise for having the beach which later opens into a rocky intertidal area making this area anthropogenically more disturbed. Recently, Vadodra dodiya, Aadri and Navapara were also flashed out in news because of inhabitancy of lions near the shore area.

3.2 Time Span

This research work was carried out from August 2014 to February 2016. The wide investigations were done in the month of August 2014 onwards. Investigations were completed and rocky intertidal zones were acknowledged and sampling areas were fixed for research work within a period of some months. Next to that consistent once-a-month visit to the selected study sites were started and it was for about one year. Throughout this period the macro benthos diversity of intertidal zone was studied. The population ecological data were collected and recorded from the December 2014. Monthly data collection was done for population ecology from December 2014 to November 2015. The data were collected till the month of November 2015 after that, till February 2016, visits were completed to the selected sites at a monthly interval with an objective of producing a database on the available macro benthos and the data was obtained corresponding with that of the forgoing year to cross check the

correctness of the study. The months of December 2014 to November 2015 were allocated in four seasons. viz., winter, summer, monsoon, and post monsoon.

All selected study sites were regularly surveyed at fixed period for the duration of the lowest tides in the course of the study. All intertidal macro benthos (flora and fauna) observed were noted correctly, recognized and classified scientifically and a checklist was arranged and confirmed by various bulletin boards and online forums (research publication, thesis) with the help of internet.

Water samples were collected from the intertidal zone of all selected sampling sites along the south Saurashtra coast of India from the duration of December 2014 to November 2015 for the seasonal as well as between site variations of the sea water quality.

3.3 Methodological Approach and Study Groups

The study was performed to find out the temporal as well as spatial variants of intertidal macro benthic populations of selected key species and also the quality of sea water along the coastline of Saurashtra region. For this study total, four sampling sites were selected from Saurashtra coastline.

3.4 Zonation

The intertidal zone (the area between high and low tides) is a harsh and unforgiving habitat, subjected to the rigors of both the sea and the land. It has four distinct physical subdivisions based on the amount of exposure each gets, the spray zone and the high, middle and lower intertidal zones. Each subzone has a typical and different biological community.

An attractive phenomenon acknowledged as zonation occurs throughout the intertidal area. Intertidal zonation is the upright banding of the organisms, which occurs in part from many complex biological and physical factors which disturb the marine flora and fauna. The zonation of organisms is a mirror image of their reaction to both biological and physical factor (Parulekar, 1982; Mettam, 1994). Ecological characteristics like waves, cyclic tidal actions and stress by temperature contribute to formation of this phenomenon. In addition, the intertidal zone is also acknowledged as the littoral zone. In marine environment, intertidal zone is the part which is exposed to the air at the time of low tide and submerged during high tide only, i.e. the area to be found between the lower and higher tidal marks. A rocky intertidal region can be visibly separated into four zones known as the supra tide, high tide, mid tide and low tide zone which are also known as spray, upper littoral, middle littoral and lower littoral zone respectively. (**Plate-3.2)**

3.4.1 Spray Zone

The zone beyond the spring high-tide line full of water only at the time of storms is termed as Spray Zone; it is relatively dry and lightly populated. Although there is an extreme environment due to fluctuations in moisture, temperature and salinity, there are certain flora and fauna found in this zone. This zone is possibly more a part of the terrestrial than the sea. It is underwater only during rare, very high tides or severe storms but it is commonly sprayed by splashing waves and wind-blown spray.

3.4.2 Upper Intertidal Zone

The upper intertidal zone remains underwater for the period during high tide only. This zone is covered by the highest tides, and spends maximum of its time as

terrestrial habitat. As the tide goes back; this zone is exposed to air for longer duration. In this zone, the water is not largely available to sustain population, although some organisms do survive in this tide zone. Barnacles, chitons, crabs, green algae, limpets etc. are the dominant organisms in this sub region. Rock pools are also present in this zone. Life is more abundant in this zone as compared to the spray zone.

3.4.3 Mid Intertidal Zone

The mid intertidal zone remains underwater for almost equal periods in each tide cycle. The wave is more extreme as compared to the high tide and spray zones. Surroundings of mid intertidal zone contain a variety of different organisms when compared to the splash or high tide zone. This zone has higher populations of marine vegetation and fauna such as anemones, barnacles, crabs, algae, *Onchidium* and sponges. Due to such an abundant population, life in this zone is extremely differentiated in comparison to the high tide zone and spray zones.

3.4.4 Low Intertidal Zone

This intertidal zone is generally underwater and open at the time of low tide and during extremely lowest tides open for a longer period. Area covered by low intertidal zones is extremely crowded with marine life. The most remarkable difference of this sub region is much more marine vegetation, especially seaweeds from the other three sub region. Reason of more species richness in this zone is the organisms are underwater maximum of the time with compare to other intertidal zone and due to this more interaction takes place. Life of flora and fauna in this region is generally not acclimatised for the periods of long water unavailability or even high temperature. Flora and fauna such as seaweed, crabs, algae, sea stars, sea urchins, shrimp, snails

and sponges are very largely observed in this zone. As the water is available in plenty with compare to other zone, marine flora can develop better in sizes. In this zone, water is as enough as to allow required light to reach the flora which is useful to permit the photosynthesis activity, and also the salinity is at almost normal levels.

3.5 The Selection of Key Species

Selection of key species was carefully done on the basis of their existence and large quantity in the selected study area. These organisms were selected because they were the most noticeable and also their availability on intertidal zone throughout the year, species selected were described as a non-migrant (Inter coast) also, and therefore these species selection would make sure a long duration research on a similar aspect. It was also found that this species were found dominant when compared to the available other species in all the three zones of intertidal area of selected sampling site.

During the period of the survey it was observed that some molluscs species were most abundant in the selected rocky intertidal zone, hence for the studies of population ecology following molluscs species were selected.

Phylum: Mollusca.

Class: Gastropoda.

Order: Archeogastropoda.

(1) Family: Patllidae: *Cellana radiata.***(Plate 3.3)**

Order: Mesogastropoda.

(2) Family: Terebridae: *Conus miliaris.* **(Plate 3.3)**

Order: Systellommatophora.

(3) Family: Siphonariidae: *Onchidium verriculatum.* **(Plate 3.3)**

3.6 Sampling Methods Followed

3.6.1 Sampling for Macro Fauna Diversity Studies

Before conducting a quantitative assessment of macro fauna at each selected sampling site, an assessment was completed to select the proper study sites to work at. These sites were examined on a regular monthly basis and all the macro fauna were documented at the primary phase of this study, Taking photographs of the animal species and identified with the help of identification keys, collected works on coastal areas in the form of books, research papers, use of internet and also from Ph.D. theses of students especially who worked on coastal areas of Gujarat.

The study was done in a non-destructive way; so that habitats of selected coast were not disturbed unless and until additional examination was necessary to bring together the sample for lab investigations. As soon as the organisms were identified, no further collection was done for this purpose during the continuous surveys and only record of the encountered species was made.

All the selected sampling sites were surveyed regularly during the low or lowest tides throughout the study. As stated before, all the macro fauna encountered at intertidal zone were identified and recorded and also classified systematically and prepare a checklist. It was confirmed by authentic museums worldwide (through their websites

and other standard available identification keys) and by different bulletin boards and online forums with the use of the internet.

3.6.2 Sampling for Macro flora Diversity Studies

Intertidal macro floral diversity was carried out in a similar method like the intertidal macro fauna. Method of extensive photography was employed and identified with same manner as stated above in section 3.6.2.

3.6.3 Sampling for Seawater Quality

In this study, water samples were collected from all the selected sampling sites once in a month. Sample collection location in each sampling sites were fixed. For analysis of water sample parameters, samples were collected from the surface directly in amber coloured glass bottles (BOD bottle-approximately 300 ml capacity) for dissolved oxygen and biochemical oxygen demand. For other selected parameters, seawater samples were collected and stored in sterile polyethylene bottles and transported with ice box (whenever required) for analysis to the laboratory. Glassware and equipment used for analysis were washed to avoid contamination before analysis.

3.6.4 Methods for Seawater Analysis

Analysis of sea water quality was done as per standard methods of Trivedi and Goel, 1986; APHA, 1995 for selected parameter.

Temperature and pH were immediately measured after water collection with the help of the electronic thermometer and portable digital pH meter respectively and recorded

the final result. pH defines the medium of samples such as alkalinity and acidity. pH meter was used after calibration using standard pH buffer.

Salinity is defined as the total amount of the dissolved inorganic solids in water, it is expressed as parts per thousand (ppt, ‰). Refractometer was used for measuring of salinity in samples of sea water

Using gravimetric method, total solids (TS) and total dissolved solids (TDS) were determined. TS represents a water portion which are not lost even after evaporation of the unfiltered known volume of water sample, while TDS are excess left after evaporating the filtered sample through standard filter. Unit of TS and TDS expressed as a gram per litre (gram/litre).

Dissolved Oxygen (DO) was analysed in the water samples by Winkler's method. The unit of expression for dissolved oxygen is milligram per liter (mg/l).

3.6.5 Sampling for Population Dynamics Studies

3.6.5.1 Transect

The sampling for both flora and fauna, one of the most recognized method is "transect" sampling method. Transect method (Misra, 1968) was used for study of structural attributes of the intertidal fauna.

Transect methods such as Belt and foot were employed for generating the data on this study. Foot transect method was primarily used for generating the population database. The foot transect method took the maximum available ground into consideration.

3.6.5.2 Movement for sampling

At all the intertidal zone of selected sampling sites, direction was followed in criss-cross manner to cover the maximum exposed area. Visits were made in every month during the low tides. Sampling sites were started visiting during the start of the low tide and it was completed to finish two sites within the time of approximately 3 to 4 hours.

3.6.5.3 Size Quadrate and number

For sampling, quadrates are used in almost all subdivisions of ecology and there are many methods available (Greig-Smith, 1983). Size of quadrates was 0.25 m^2 used to follow a slanting direction and covered maximum area on the intertidal belt of all selected sampling sites. At all selected sampling site, frequency of quadrates was fixed as per overall length of selected intertidal zone. Quadrates were used ten times to be laid at each of the selected sampling site during the course of the study period. As stated earlier, sampling was started at the start time of the lowest tide and was completed to finish the two sites in the specified time of about four hours.

3.7 Population Dynamics

Among the attributes of ecological, monthly variations in the density, abundance and frequency of selected prominent species in each selected sampling site of study were calculated and recorded (Misra, 1968).

Ecological attributes were calculated and recorded as per following mention formula.

(1) Density =

$$\frac{\text{Number of species recorded from all the quadrates}}{\text{Total number of Quadrates}}$$

(2) Abundance =

$$\frac{\text{Total number of species recorded.}}{\text{Total number of Quadrates where the species recorded}}$$

(3) Frequency (%) =

$$\frac{\text{Number of Quadrates where the species recorded.}}{\text{Total number of quadrates}} \times 100$$

3.8 Anthropogenic Impact

To study this parameter, survey observation method was done as par Misra (2004).Thus, in this study the numerous anthropogenic effects on open shores and the role of macro-invertebrate on the shores were confirmed by field experiments.

The selected study sites were identified by frequently visit of each site in each month and extensive field study of sampling sites were regularly carried out. Numerous anthropogenic actions like industry, fisheries, port activity, tourism, sewage and many other disposal wastes were noted. Other than this furthermore all the selected sites are also centre point of social settlement by people very near about the coastal area. Stress of anthropogenic activity is the answer of biological entity (individual, population, community etc.) to an anthropogenic disturbance.

The area of coastal is polluted by the water pollution most of the time, which is directly or indirectly produced by anthropoid and industrialized settlement near the

coastal area as well as natural process. These are the terrifically affected the community of intertidal area. In this study, seawater quality parameters were used to describe the pollution in coastal area.

3.9 Statistical Analysis

Statistical analysis was done for the overall acceptability of the calculated monthly data of population ecology and data of water analysis. The main aim of statistical analysis was to find out whether there was any significant variance in the population of the selected key species as well to know the significant variation of water quality amongst the selected sampling sites. It was used as a display to monitor the intertidal health on the Southern Saurashtra Coastline. For statistical analysis, ANOVA (analysis of Variance) was the convenient test to apply for refereeing the variability in the population dynamics of selected sites.

The obtained data were offered by month as well as season for the seasonal approach such as winter, summer, monsoon and post-monsoon. After that data were calculated for mean and SD (standard deviation). All the data were calculated automatically using Microsoft Office Excel software to maintain the accuracy of the calculation. Sigma plot software was used for comparison of significance of spatial and temporal variations.