Materials and Methods

Study area

Selection of study sites

Gujarat state is a western proximity of India, contains major portion of Indian coastline. Out of 1650 km long coastline of Gujarat, Saurashtra coast occupies 850 km long stretch. The entire coastline of the Saurashtra was surveyed prior to selection of the study sites. During the survey both biological and physical characteristic of each sites were noted down. After the reconnaissance survey, four different study sites Viz. Veraval (20^0 54' 37" N, 70^0 21' 04" E), Sutrapada (20^0 49' 53" N, 70^0 29' 17" E), Dhamlej (20^0 46' 29" N, 70^0 36' 19" E) and Kodinar (20^0 45' 29" N, 70^0 39' 39" E) were selected for the present study (Fig. 5).

All the four sites are located in the newly formed Gir Somnath district (Formerly part of Junagadh district). Few characteristic were taken into the consideration for the selection of the study sites like habitat diversity, anthropogenic pressure and accessibility of the study sites. The habitat in all the four sites is more or less similar which gives better platform for the comparison of the occurrence of the macro faunal community. The intertidal area of all the four sites varies in term of exposure in which maximum exposure is observed at Dhamlej (150 meters) during low tide while minimum exposure is observed at Veraval (50 meters). The intertidal zone is rocky in nature with upper intertidal zone made up of sandy shore. The width of the sandy shore is more or less similar at all the sites (20) meters. The rocky shore of all the study sites is covered by tide pools. The size, depth and density of tide pools vary at different sites.

Veraval: Veraval is located on the south west coast of Saurashtra 15 km away from Somnath. Somnath is a famous religious place for Hindus as it is one of the important sites amongst the 12 shivlingas of lord shiva. Veraval is very important for the

commercial fisheries point of view as it is one of the largest fish landing centres of India. The coastal areas receive sewage pollution from the domestic households as well as industrial pollution from the small to large scale industries located on the coastal belt. The sampling sites selected for the present study is located behind the Veraval Fisheries Collage near the light house. The study site is facing high level of anthropogenic pressure as local people visit the site for various purposes like relaxing, jogging and fishing.In the present study total 2 km stretch of shore line was surveyed for the sampling. The exposure area of the intertidal zone varies from 30 meter to 50 meter during the low tide. The intertidal area is flat in nature with lower intertidal zone ends with steep vertical slope towards sub tidal zone. The upper intertidal zone is made up of sandy shores. The middle and lower intertidal zone covered with tide pool and deep crevices in which shallow tide pools are present in the mid intertidal zone while deep tide pools with small opening are available in the lower intertidal zone. The rocky shore line is interrupted by large and deep channels and boulders (Fig. 6a).

Sutrapada: Sutrapada is located 20 km south of Veraval. Sutarapada is a small town but it is very famous for the production of $NaHCO_3$ by Gujarat Heavy Chemical Limited. Gujarat stands at first position for the production of $NaHCO_2$ in India and almost entire lot of the $NaHCO_3$ is produced by Gujarat Heavy Chemical Limited located in Sutrapada. The coastal areas of the Sutrapada receive particulate laiden effluent released by the factory. The coastal areas of the site face moderate level of anthropogenic activities. The site selected for the present study is located near the local fishing harbour. In the presentstudy, total 2 km long stretch was surveyed for the sampling. The exposure area of the intertidal zone varies from 100 meter to 120 meter during the low tide. The intertidal area is rocky in nature with upper intertidal zone made up of sandy shore. The slope of the sandy shore is comparatively more than other study sites. The middle and lower intertidal zone is comparatively flat in nature. The mid intertidal zone is covered by shallow and large tide pool in the lower part of mid intertidal zone some large tide pools contains large boulders of hard corals. The lower intertidal zones is covered by deep tide pools and

deep inter connected channels and crevices. These deep tide pools remain filled with water during low tide (Fig. 6b).

Dhamlej: Dhamlej is located 30km south of Veraval. Dhamlej is a small village of fisherman community. The coastal areas of the village are mostly utilized by the local people for fishing activity and very low level of anthropogenic activities is observed in the study site. The site selected for the survey is located near the village fishing harbour. The intertidal zone is rocky in nature with upper zone made up of sandy shore. The slope of the sandy shore is lowest as compare to the other study sites. The site selected for the present study is located near the local fishing harbour. In the present study, total 2 km long stretch was surveyed for the sampling. The exposure area of the intertidal zone varies from 110 meter to 130 meter during the low tide. The mid and lower intertidal zone is flat in nature. In the mid intertidal zone very few shallow tide pools were observed. The mid intertidal zone is mostly covered by small channels and crevices. The lower intertidal zone is covered by shallow and large tide pools (Fig. 6c).

Kodinar: Kodinar is located 45 km south of Veraval. Kodinar is a town famous for the Ambuja Cement Factory. The site selected for the survey in present study is located in the Mul Dwarka village which is situated 3 km away from the Kodinar town. The study sites start near the Ambuja jetty terminal. In the present study, total 2 km long stretch was surveyed for the sampling. The 2 km stretch is divided into two parts because the central part of the stretch only gets exposed during minus tides. The exposure area of the intertidal zone varies from 100 meter to 120 meter during the low tide. The upper intertidal zone is made up of sandy shore with moderate slope. The upper part of mid intertidal zone is covered by large shallow tide pools which remains filled with water during low tide while the lower part is covered by shallow rock crevices. The lower intertidal zone is covered by large shallow tide pools. Few large tide pools also contain large boulders of hard corals. The anthropogenic pressure on the present study site is very less as compared to other study sites (Fig. 6d).

Time Span: The present study was carried out from May, 2011 to August, 2014. The reconnaissance survey was carried out in March and April, 2011 and site selection was done. The brachyuran crab collection and identification was carried out at every site from May, 2011 to February, 2012. The study of zonation pattern and macro faunal community was carried out from March 2012 to March 2013. To study the seasonal variations, monthly visits were carried out to the each study site for the quantification of macro fauna using quadrate sampling method. The study of habitat preference and distribution pattern of brachyuran crabs in different zones of intertidal area was carried out from March 2013 to March 2014 using belt transect method. In this particular study, field visits were done with an interval of two months. The study of burrow architecture of brachyuran crab *Ocypode ceratophthalma* was carried out from April, 2014 to July, 2014.

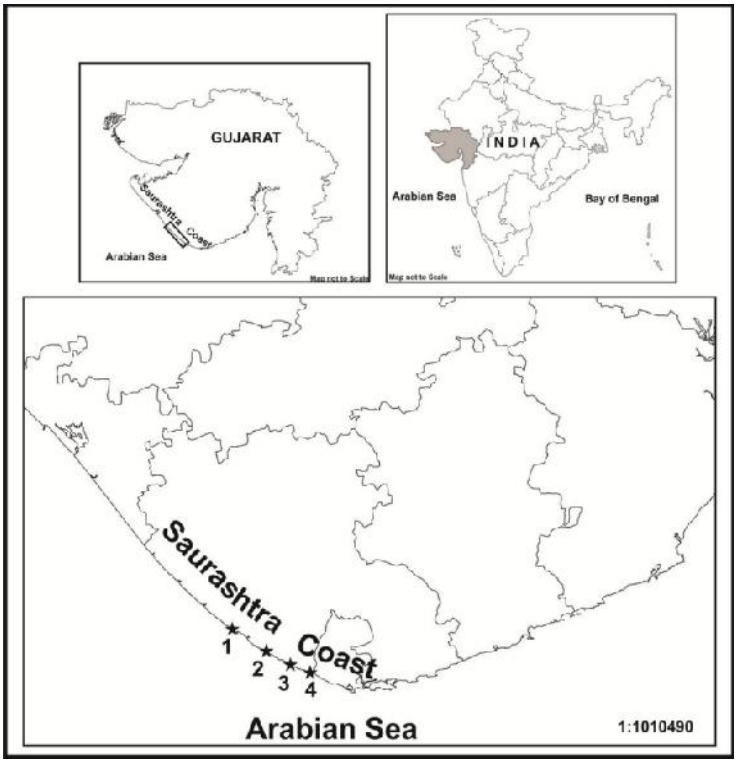

Figure 5: Map of study area, (1) Veraval, (2) Sutrapada (3) Dhamlej (4) Kodinar

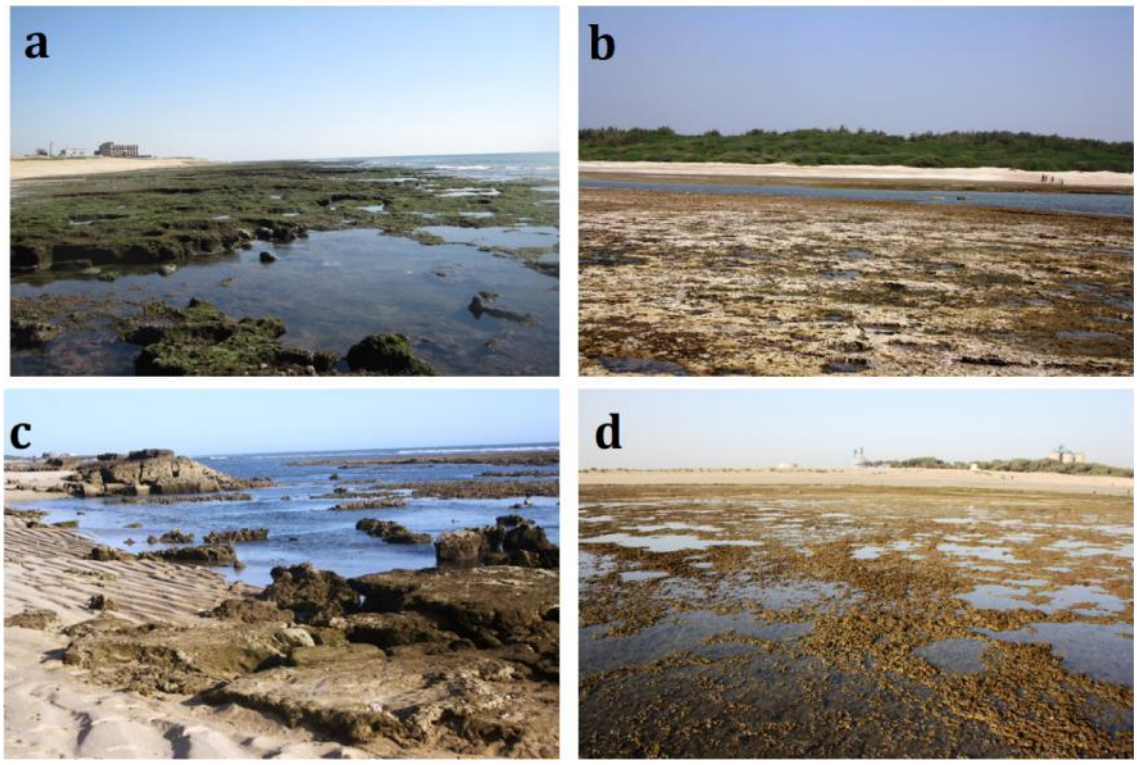

Figure 6: Habitat of study area (1) Veraval (2) Sutrapada (3) Dhamlej (4) Kodinar

Sampling Methodology

Abiotic Study:

Sea water analysis

Monthly data was collected for three basic abiotic parameters like pH, salinity and surface temperature for sea water. The sea water accumulated in the tide pools was used for the testing. The water was tested using digital calibrated instruments. The monthly data collected for each sites was compiled for different seasons to see the seasonal fluctuations in the abiotic parameters.

Sediment sampling and analysis

Different methods were adopted for the collection of sediment based on the type of the analysis and study component.

Scooping: Scooping is the best method to study the upper sediment layer. The upper surface of the sediment was scooped out using shovel after removing the upper one or two cm layer.

The pour water salinity, sediment pH was measured for the collected sediment using digital instruments. The sediment surface temperature was also measured using digital thermometer in which the steel probe was inserted into the sediment up to 2 cm depth and temperature was measured. Monthly data was collected for the sediment abiotic parameter and compiled for different seasons. All the abiotic parameters of sediments were measured at the interval of 25 cm in the burrow architecture study of *Ocypode ceratophthalma.*

Burrow cast

The structure of barrows varies in different macro faunal group and burrow casting method is the best method to study the burrow architecture of burrowing form of macro fauna. In the present study, burrow casting method was employed to study the burrow morphology of brachyuran crab *Ocypode ceretophthalma*. The Unsaturated resin mixed with catalyst was poured into the crab burrow until the burrows were totally filled. The resin was allowed to get solidified for one hour after that the burrows were excavated carefully and the cast brought to the laboratory. Different morphological measurements were recorded for each burrow cast which includes burrow opening diameter, total length, total depth, inclination angle etc.

Biotic Study

Macro faunal Study: The macro fauna residing in the intertidal zone were collected and photographed properly for the identification process. The macro faunal samples were brought to the laboratory and identified up to species level using different identification keys, books, research papers etc. Once the species was identified no further collection was done only if some new species encountered, the specimen was collected and identified.

Brachyuran crab collection and Taxonomical identification

Hand picking method was adopted for the collection of brachyuran crab specimen. Sometimes menthol crystals or alcohol was poured into the burrows and rock crevices for specimen collection. Bait method was adopted in which visceral part of molluscan species was tied with the string and then it was hanged in the deep tide pools. Whenever the crab catches the visceral part, it was pooled out using the string and the specimen was collected. After the collection, the crabs were narcotized using methanol, stored in icebox and brought to the laboratory. All the specimen were sorted out according to their sex. Detail morphometry of each specimen was carried out using calibrated instruments and different morphological characters were measured like carapace length, carapace width, frontal length, cheliped length etc. The specimens were identified to the species level using different identification keys and monograms (Chhapgar, 1957; Sethuramalingam and Ajmal Khan, 1991; Jeyabaskaran, et al., 2000). For further confirmation of species, all the specimens were examined and compared with the photographs and identification information available on Marine Species Identification Portal website (www. speciesidentification.org) and NIO marine fauna information website (Jeyabaskaran and Wafar, 2002). The classification of brachyuran crabs was adopted from WoRMS website (www. marinespecies.org).

Specimen preservation and Deposition

Specimen were preserved using two different methods *Viz.* wet and dry preservation. In wet preservation, Specimen were preserved in 10% formalin. Some specimens were also preserved in 90% alcohol for molecular phylogenetics work. In dry preservation, mostly the male specimen was scarified. All the visceral mass of the animal was scooped out and all the body parts were oven dried for 24 hours at normal temperature. Varnish was applied on the dried body parts of the specimen

and after that all the body parts were glued on the transparent acrylic sheet using strong adhesive material (Fig. 6).

All the specimens of identified species were deposited into the Zoology Museum, Department of Zoology, Faculty of Science, The M. S. University of Baroda, Vadodara, Gujarat, India. Specific deposition code (e.g. MSU-ZL/AR-CR/1: *Scylla serrata*) were allotted to the each identified species. Specific label describing information about species code, scientific name, family, collection site, date and collectors name were applied on the bottles containing specimen of the particular species (Figure 2).

Figure 7: Dry and wet preservation method for brachyuran crabs

Quantification of macro fauna

Line transect method: Line transect method was adopted for the survey of zonation pattern and habitat characterization. Total 10 line transect were laid parallel to the shore line randomly at each site. The line transect were intercepted by 0.25 m^2 quadrate for the quantification of the macrofauna residing in different zones of the intertidal area. Total 6 quadrates were laid randomly on each line transects. Monthly surveys were carried out for the quantification of macrofauna to see the seasonal variation in three different ecological attributes like density, abundance and frequency of occurrence.

Belt transect method: Belt transect method was adopted to study the distribution pattern of brachyuran crab in the intertidal zone of different study sites. The belt transect were laid parallel to the shore line from upper to lower zone. The width of the transect (1 meter) were constant while the length of transect varied with the tidal exposure at different study sites. On the sighting of brachyuran crab in the transect area various observations were recorded which includes crab species, site, time, zone and micro habitat. Only presence/absence data was collected for different crab species for each study site.

Quadrate method: Quadrate sampling method was adopted to study the seasonal variation in the burrowing pattern and population of brachyuran crab *Ocypode ceretophthalma*. Total 50 quadrates ($1m^2$) were laid randomly on the sandy shore of each study sites monthly. Data was quantified for three different ecological attributes which includes density, abundance and frequency of occurrence. In the quadrate area different observation were recorded which includes total number of burrows, burrow opening diameter of selected burrows etc. The monthly data was compiled for different seasons.

Analysis of data

The seasonal quantified data of various macro fauna residing in different zones of intertidal area of different study sites were analysed using three different ecological attributes listed below.

$$\textbf{1. Density} = \frac{\text{Total number of individuals recorded from all the sample plots}}{\text{Total number of sample plot studied}}$$

$$\textbf{2. Abundance} = \frac{\text{Total number of individuals recorded}}{\text{Total number of sample plot where the individuals occurred}}$$

$$\textbf{2. Frequency (\%)} = \frac{\text{Total number of plot where species occurred X 100}}{\text{Total number of sample plots}}$$

Statistical analysis of data

The data collected using different sampling method was analysed using various statistical method. The quantified seasonal data of various macro fauna occurring at different study sites was presented in the form of mean ± standard deviation. Analysis of variance (ANOVA) is a handy statistical tool to know the variation between mean values of the parameter. Two way ANOVA without replication was employed to know the variation in the mean values of studied ecological attributes quantified for different macro fauna between different seasons and study sites. Regression analysis was employed to find out the relationship between various abiotic factors and mean abundance of the different macro faunal species. The Pearson correlation coefficient was calculated to establish the relationship between body sizes of brachyuran crab with different morphological parameters of the burrow cast. The mean values of various morphological characters of burrow cast were also compared using paired t test.

The Bray Curtis similarity index was calculated to know the similarity in brachyuran crab species composition between different study sites and different zones of intertidal area. The kite diagram was prepared to know the distribution pattern of various macrofauna in different zones of intertidal area. All the statistical analyses were carried out using software like Microsoft EXCEL and PAST.