2.1 STUDY AREA

The study focused on the mudflats of Kamboi (22° 12' 59.1444'' N, 72° 36' 54.7992'' E), situated in the upper reaches of the Gulf (Fig. 2.1). Kamboi, strategically located at the mouth of an estuary heavily influenced by marine forces, was chosen as the primary research site. Kamboi represents a broad estuarine opening into the Gulf of Khambhat and features extensive tidal mudflats (Fig. 2a). Within Kamboi, there is a distinct estuarine intertidal area with discernible high and low tide lines. The Upper Intertidal Zone (Zone I & II) consists of mangrove plantation and other vegetation in smaller patch with elevated mudflats, Middle Intertidal Zone (Zone III & IV) is comprising of elevated mudflats, soup-mud and lower margin having sandy composition, while the Lower Intertidal Zone (LIZ) forms a flat plain with fine sandy composition. These two areas are separated by a runnel system and small tidal pools (Fig. 2.2b-e). The hydrodynamics of the Gulf of Khambhat and the geomorphology of the Mahi estuary play a crucial role in shaping its sedimentological and water quality characteristics. The substantial sediment deposition on the banks and erosion on the opposite bank contribute to the creation of geologically significant zones.

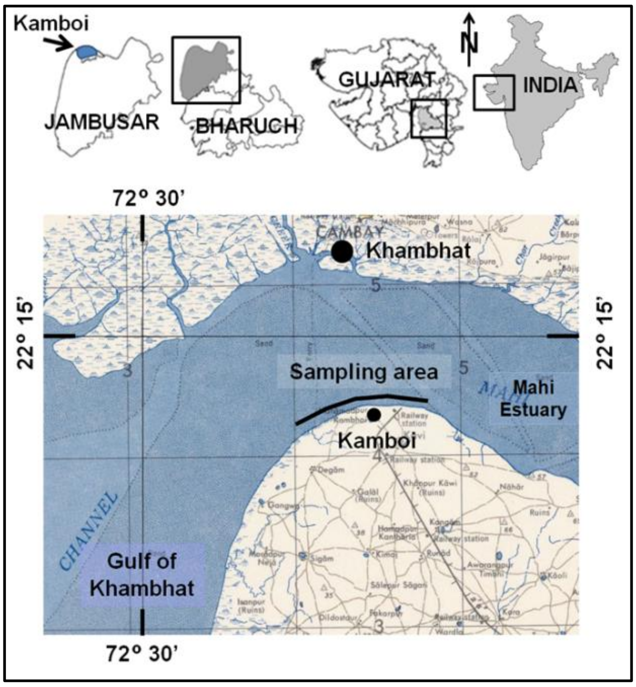

Figure 2.1: Location of the study site: Kamboi Coast, Gulf of Khambhat, Gujarat, India

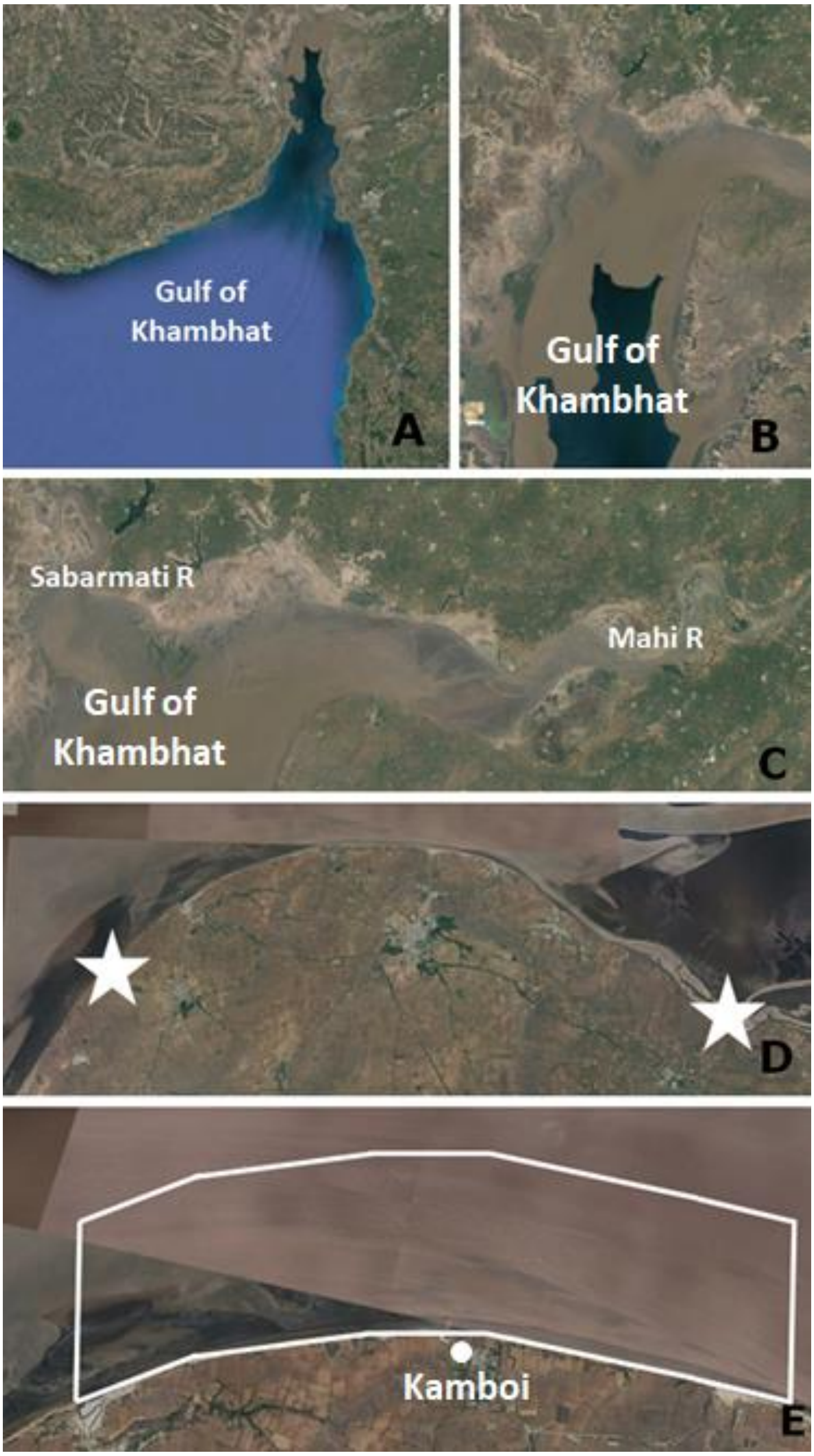

Figure 2.2: Descriptive map of study area. A & B: Entire region of Gulf of Khambhat showing its peculiar structural features, C: Apical most northern region of the gulf showing the mouth of Mahi and Sabarmati Rivers, D: The entire survey area from coastal village Dahegam (Left star) to Nahar (Right star), E: the area along coast of Kamboi which was actually studied for the present research

2.2 METHODOLOGY

2.2.1 Habitat Characterization

To comprehend an ecosystem variability through visual evaluation and approachability, the study area initially investigated. After that, PVC pipe coring was adopted for collection of sediment samples from the Zone I, Zone II, Zone III, and Zone IV. The core sample was extracted with care and securely sealed on both ends using PVC caps in the field prior to its transportation to the laboratory. Sediment samples were brought to the lab, where they were air dried and then crushed into powder using a mortar and pestle to break up any clumps and prepare the sediment for further examination. Then after sediment grain size analysis was carried out using sieving technique. In the wet sieving procedure, the sediment samples that had been dried were treated with a solution of Hydrogen Peroxide. The samples were then agitated for a duration of five minutes in order to facilitate the release of silt and clay particles. Subsequently, the specimen underwent a rinsing process employing distilled water, which was passed through a series of sieves with varying mesh sizes as. The sieves used had pore size 2 mm (for gravel), 2.0-1.0 mm (for very coarse sand), 1.0-0.5 mm (for coarse sand), 0.5-0.25 mm (for medium sand), 0.25-0.125 mm (for fine sand), 0.125-0.075 mm (for very fine sand), 0.075–0.020 mm (for silt + clay), and particles smaller than 0.020–0.002 mm were classified as clay.

2.2.2 Diversity of Brachyuran Crabs

To collect brachyuran crab species, extensive field surveys were conducted from January 2021 to October 2022, utilizing the hand-picking method for sampling. During fieldwork, the visible characteristics of the specimens were recorded, and photographs were taken to document their coloration. Subsequently, the collected specimens were preserved in 70% ethanol and transported to the Marine Biodiversity and Ecology Laboratory (MBEL) at the Department of Zoology, The Maharaja Sayajirao University of Baroda (MSU). Upon arrival at the laboratory, all specimens were segregated based on their sex. They were then identified at the species level by comparing their morphological features with available illustrative keys, research papers, and monographs. To further confirm their species classification, all specimens were examined and compared with images and identification characteristics available on the Marine Species Identification Portal Website (http://species-identification.org/). The most recent classification of crabs was adopted from the World Register of Marine Species (WoRMS) website (www.marinespecies.org). Additionally, morphometric measurements, including carapace length, carapace width, frontal length, cheliped length, etc., were measured for the collected specimens.

2.2.3 Distribution Pattern of Brachyuran Crabs

From April 2021 to June 2021, initial field surveys were conducted to assess the distribution of six brachyuran crab species - *Austruca sindensis* (Alcock, 1900); *Ilyoplax sayajiraoi* JN Trivedi, Soni, DJ Trivedi & Vachhrajani, 2015; *Dotilla blanfordi* Alcock, 1900; *Macrophthalmus* (*Macrophthalmus*) *sulcatus* H. Milne Edwards, 1852; *Eurycarcinus orientalis* A. Milne-Edwards, 1867; *Scylla serrata* (Forskål, 1775) at the study site. To examine the seasonal variations in the distribution pattern of *A. sindensis, I. sayajiraoi* and *D. blanfordi* crabs, monthly surveys were carried out during low tide from October 2021 to September 2022. For data collection, three transects were laid perpendicular to the shore and spaced 100 meters apart. Along each transect, 0.25 square meter quadrates were placed at 5-meter intervals for the entire length of the transect. The number of burrows belonging to different brachyuran crab species within the quadrate area was counted. The quantified monthly data from the quadrates for burrows were aggregated into different seasons, including winter (November to February), summer (March to June), and monsoon (July to October). This categorization was based on the relatively stable climatic conditions in the study area during consecutive months (Pandya and Vachhrajani, 2010).

The quantitative seasonal burrow data was then analyzed for three ecological attributes: density, abundance, and frequency using the following formulas.

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

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

$$\text{Frequency (\%)} = \frac{\text{Number of Quadrates where the species recorded x 100}}{\text{Total number of quadrates}}$$

2.2.4 Burrow architecture in Austruca sindensis (Alcock, 1900) *and Ilyoplax sayajiraoi* JN Trivedi, Soni, DJ Trivedi & Vachhrajani, 2015

The study examined the variation in burrow architecture with respect to the season for two crab species, *A. sindensis* and *I. sayajiraoi*. Burrow casting was conducted between March 2021 and January 2022 for *A. sindensis*, while burrows were cast during the pre-monsoon and post-monsoon seasons, *i.e.*, March to May 2021 and September to November 2021, for *I. sayajiraoi*. The study was conducted about one hour after the commencement of low tide when the burrowing activities of both species were at their peak. For *A. sindensis*, burrows were selected from two different zones of the Upper Intertidal Zone *i.e.*, Zone I & II. For *I. sayajiraoi*, burrows were randomly selected from the study site, and the Burrow Opening Diameter (BOD) was measured using digital Vernier Calipers (Yuri YUR01 Digital Vernier Caliper 0-150 mm).

A mixture of unsaturated resin, cobalt, and catalyst in a 3:1:1 ratio was poured into the selected burrows until they were completely filled. Any crabs that emerged from the burrow during this process were captured and preserved in 70% alcohol. After two hours, the solidified burrow casts were carefully excavated, labelled, and transported to the laboratory for further analysis. In addition to casting, the study also measured the variation in burrow temperature along the depth of the burrows using a digital thermometer (Eurolab ST9269B, ± 0.1°C). Temperature measurements were taken at 5 cm intervals up to a depth of

25 centimetres. Beyond this depth, measurements could not be recorded due to the limited length of the digital thermometer's probe.

In the laboratory, the burrow casts were of *A. sindensis* and *I. sayajiraoi* were separated and categorized based on their shapes, and various parameters related to burrow morphology were recorded. These parameters included the burrow Inclination Angle (IA), Total Depth of the Burrow (TDB), Total Length of the Burrow (TLB), and Burrow Volume (BV) for both *A. sindensis* and *I. sayajiraoi*. The BV was determined by weighing the burrow with an accuracy of ±1 gram and dividing it by the density of the unsaturated resin (0.96 grams per cubic centimetre) as per Trivedi and Vachhrajani (2016). These calculations were conducted using Microsoft Excel 365 on Windows 11. In the laboratory, the crabs were classified as male or female, and their carapace length (CL) and width (CW) were measured using a digital Vernier calliper for both studied species.

$$\text{Burrow Volume} = \frac{\text{Weight of the casted burrow}}{\text{Density of the unseturated resin}}$$

Regression analysis was conducted to establish the relationship between the carapace lengths of the crab and various morphological parameters of the burrow cast for both species. However, Principal Component Analysis (PCA) was solely performed for *A. sindensis* since burrows of this specific species were selected from various zones within the study site. In factor analysis, univariate descriptive statistics were utilized. The correlation matrix considered two selected attributes: the 'coefficient' and 'significance level.' The 'factor analysis extraction' attribute displayed an 'unrotated factor' solution and 'scree plot.' The extraction method was based on 'Eigen value > 1.' All the statistical analyses were carried out using the "Statistical Package for the Social Sciences (SPSS version 22) software."

2.2.5 Effect of lunar phases on burrowing activity in Austruca sindensis **(Alcock, 1900),** ***Ilyoplax sayajiraoi in*** **Trivedi, Soni, DJ Trivedi & Vachhrajani, 2015, and** ***Dotilla blanfordi*** **Alcock, 1900**

For the present study, three abundant burrowing crab *A. sindensis* and *I. sayajiraoi*, and *D. blanfordi* were selected. The sampling for the burrow density and its diameter for selected species was conducted for 6h during two full moon spring tides (28 March 2021; 19 November 2021), two new moon spring tides (12 April 2021; 4 December 2021), two waxing moon neap tide (22 March 2021; 11 November 2021), two waning moon neap tide (4 April 2021; 27 November 2021), two waxing crescent moon tide (17 March 2021; 4 November 2021), two waxing gibbous moon tide (26 March 2021; 15 November 2021), two waning crescent moon tide (1 April 2021; 23 November 2021) and two waning gibbous moon tide (8 April 2021; 1 December 2021).

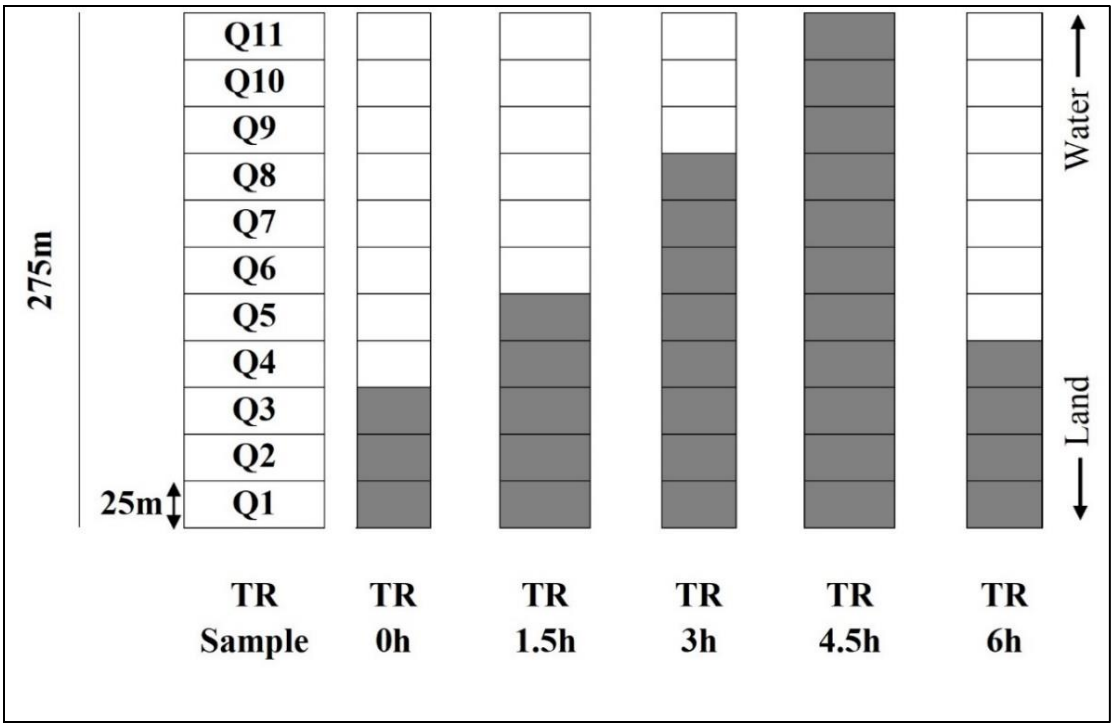

Figure 2.3: Scheme of sampling of counting and measuring of burrows of *Austruca sindensis* (Alcock, 1900), *Ilyoplax sayajiraoi* Trivedi, Soni, Trivedi & Vachhrajani, 2015 and *Dotilla blanfordi* Alcock 1900 during duration of tidal exposure on various lunar phases at Mud-flats of Gulf of Khambhat, Gujarat, India. Q: quadrat, TR: transect

In each six-hour sampling period, samples were taken every one and half hour interval from Zone I to IV. For that, three transects, 100m apart, were demarcated perpendicular to the waterline. Each transect was intercepted with 0.25m^2 quadrates which was laid 25m apart from each other (Fig. 2.3). The burrows inside each quadrate were counted and their diameter was measured using a digital Vernier calliper (Yuri YUR01). Crab density was calculated considering the number of burrows within each quadrat. The physical parameters (temperature and moisture content) were measured concurrently from the place where data for density and diameter were taken. For statistical analysis, the mean ± standard deviation value of the BOD and density of each species from three transects was calculated. All the statistical analysis was performed using Statistical Package for the Social Sciences (version SPSS 22) software.

2.2.6 Foraging range and mud balling pattern in Austruca sindensis **(Alcock, 1900),** ***and Ilyoplax sayajiraoi*** **JN Trivedi, Soni, DJ Trivedi & Vachhrajani, 2015**

The foraging range and pattern of *A. sindensis*, and *I. sayajiraoi* were investigated by capturing scaled photographs. A total of 200 burrows for each species were randomly selected, and their opening diameter was measured using a digital Vernier calliper (Yuri YUR01). Photographs of the chosen burrows for each species were taken, and the total foraging area, total number of mudballs, and distance between mudballs were determined using DIGIMIZER Image Analysis Software. Besides that, resident crabs were collected randomly form the selected burrows for both species and their carapace length was measured using Vernier calliper. Approximately, 10 mudballs for each selected burrow were collected and their diameter were measured using Vernier calliper. Then after mudballs surrounding each selected burrow were collected and their weight was measured using weighing balance. Regression analysis was conducted to examine the relationship between the carapace length with opening diameter, the total foraging area, total number of mudballs, total weight of mudballs, average size of mudballs, and average distance between mudballs.

2.2.7 Bioturbation potential of Austruca sindensis **(Alcock, 1900),** ***and Ilyoplax sayajiraoi*** **JN Trivedi, Soni, DJ Trivedi & Vachhrajani, 2015*:***

The preliminary survey was carried out in December 2021 to check the bioturbation potential of *A. sindensis*, and *I. sayajiraoi*. For the data collection, a total of 50 quadrates were laid randomly from Zone I to III. From each quadrate, a total number of burrows of individual species were counted and their opening diameter were recorded using digital Vernier callipers (Yuri YUR01). After that, the mudballs of the individual burrow of each species were collected and labelled in separate zip-lock bags. The zip-lock bags were brought to the MBEL at MSU and dry weight of the mudballs of each zip-lock bag were measured. Regression analysis was performed in SPSS 22 to check the relationship status of dry weight of mudballs with opening diameter of burrow of each species.