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1. STUDY AREA

1.1 Location

Mahi is one of the major rivers of Gujarat that joins the Arabian Sea at the northern part of the Gulf of Khambhat near Kamboi, Bharuch district, Gujarat (Fig. 1). The estuarine stretch extends up to more than 50 km upstream from Fajalpur/ Vasad to Kamboi/ Khambhat on the south/ north banks. The mid-estuarine region up to Dabka is under extensive tidal influence. The highest high tide influence extends up to Fajalpur/ Vasad (Fig. 2). The hydrodynamics of the Gulf of Khambhat and geomorphology of the Mahi estuary specify it's sedimentological and water quality features. The high sediment input on the banks as well as erosion of the opposite bank subsequently form geologically important zoning. Since Mahi River passes through the golden corridor of chemical industrial zone of Gujarat and the effluent is released into the lower estuarine region, therefore, pollution impact on estuary is envisaged.

1.2 Site Description

The mouth of Mahi River, the Kamboi and Sarod, 13 km upstream to Kamboi has been taken consideration for current study. Kamboi, located at the Mouth of the estuary that shows high marine influence, was selected as a prime site for the study (Fig. 3). Kamboi represents a broad estuarine mouth opening into the gulf of Khambhat and represent tidal mudflats. Kamboi shows distinctive estuarine intertidal area with a high tide line and low tide line. The upper intertidal area is represented by elevated mudflat while the lower intertidal area is a flat plain with fine sandy composition. The two areas are separated by a runnel system and small tidal pools. Sarod, located at the upstream to Kamboi it also shows high Marine influence, represent tidal mudflats. Sarod represents the opening of Effluent channel which receives heavy load of polluted water (Figure 4).

2. ABIOTIC STUDY

2.1. Habitat Survey and Sampling

Study area was initially surveyed for site selection based on high marine influence and long term approachability of an area. The survey also analysed the important topographic features which directly or indirectly affect the animal distribution and can be taken into consideration during the entire study period. Preliminary habitat mapping was done using the topographic features. Sampling design has been done according to lunar cycle and selected days of neap tide and spring tide has been selected for further study.

2.2. Water Sampling and Analysis

Water samples were collected from study sites (Total 4 from downstream sites) using 1 litre polypropylene bottles. Looking to the approachability of an area, samples were taken from bank of the river (knee deep water) or from the main channel using boat whenever possible. In case of downstream sampling (i.e. Kamboi), samples were taken from main channel during low tide and direct incoming tidal water during high tide timings. Sampling from very shallow and churning water was usually avoided as can give altering turbidity level than the routine. Salinity was measured using handheld refractometer (IRMA) with range of 1-100 ppt. Samples were brought to laboratory on the same day and were analyzed for various parameters using standard methods (APHA).

2.3. Sediment Sampling and Analysis

2.3.1. Scooping

This method was employed for superficial (Upper 5-7 cm) sediment layer. The sediments were scooped using hand or shovel after removing upper 1-2cm layer of sediment. The sediments were taken in plastic zip bag and carried to the laboratory for further analysis.

2.3.2. Pipe coring

PVC pipe coring was used to sample the subsurface sediment profile study. For the purpose, PVC pipe of 60 cm (23.63 inch) and 75mm diameter with 1 - 2mm thickness was used for pipe coring. The core was placed straight (right angle) on the sediments and pressed into the bed by hand as much as possible and later by a strokes with wooden piece. After the core was filled by sediments to full extend, a PVC cap was put on the exposed end of core and the pipe was pulled from the sediments with twisting motion. At many instances, due to high compactness of the surrounding sediments the adjacent area to the core was dug out to trace the end of the core. The core was carefully taken out and was sealed on the other end using the PVC cap. Finally, the core was carried to the laboratory for further analysis. The core was later on divided and cut into 3 divisions/depths 0-20 cm, 21-40 cm and 41-60 cm.

After bringing to laboratory the samples were air dried and powdered by applying light weight in order to break the clumps and sediment aggregation. These samples were later used for further analysis

2.3.3. Sediment composition

Sediment sample were dried, powdered and treated with Hydrogen peroxide followed by distilled water and supernatant was drained once and twice to remove the hardness due to salts which causes clumping of the grains. Later on sodium fluoride was added which serves as flocculent and avoids clumping of grains. Mechanical dry sieving method was employed as described by Folk and Ward (1957). Sample was taken after coning and quartering so as to minimize biasness. As differentiation of silt and clay does not make a significant difference in with animal composition, silt/clay was taken as a whole. Sieves with standard ASTM no. 150 (0.106mm), 200 (0.075mm) and 250 (0.063mm) were used looking to the nature of the sediments to find the sand and silt/clay composition. At some

instances wet sieving method was also employed with same sieves in case sample contained high silt and clay.

2.3.4. Water Content

Water content is difference between wet mass and dry mass. Wet weight of 100 ml sample has been noted, than sample has been dries in 60^{o} degree s. temperature for 24 hrs and than dry weight has been noted. The difference between wet mass and dry mass has been calculated for water content (Avnimelech et al., 2002).

Water (%) by mass = (wet mass - dry mass) x 100

2.3.5. Porosity

Porosity is division of volume of water by volume of sample.

Porosity = Water (%) by mass / Total Volume of Sample

2.3.6. Wet bulk density

Wet bulk density is the mass of wet sample in a given wet volume of soil. Wet bulk density = wet weight / wet volume

2.3.7. Dry Bulk density

Dry bulk density is the mass of dry solids in a given bulk volume of soil. Dry bulk density = dry weight / dry volume

2.3.8. Soil organic matter:

Sediment samples were air dried and finely powdered. If needed the sediment were made free from calcretes and pebbles. A required amount of sample was taken from the bulk after an enough mixing taking care of avoiding any biasness. The organic matter analysis was done by acid digestion of samples following titrimetric method commonly known as Walkly black method (1934). Electric conductivity, Ph and available phosphorus were done according standard method given in APHA.

3. BIOTIC STUDY

3.1 Plankton Sampling

3.1.1 Plankton collection and preservation

Plankton samples were collected from surface layer of the water at the depth of 1 mt. The samples were collected in plastic bottles during high tide and low tide from three sites Dabka, Sarod and Kamboi. Samples were fixed at the study site using 5 % formalin.

3.2.2. Plankton Identification

The samples were brought to the laboratory and identified using standard descriptive and illustrative keys (Needham and Needham, 1962; Todd and Laverack, 1991).

3.2 Brachyuran crab sampling

3.2.1. Animal Collection and Preservation

Crabs were either hand picked or were forced to come out of their burrows by pouring menthol crystals or some alcohol in the burrows. In some cases the burrows were dug out and crab was collected.

3.2.2 *Taxonomic Identification*

The detailed identification to species level was done using the morphometry of animal. Important identification marks like appendages, carapace structure, pleopod structure, 3rd maxilliped, chelepeds etc, were studied by separating different parts and drawing them to get the exact picture (Ajmal khan et al., 2005, Ng et al., 2008; NIO database).

3.3 Qualitative and Quantitative Faunal Studies:

3.3.1. Distribution pattern and density of brachyuran crab

In order to reveal the distribution of Crab on the estuarine intertidal area, line transect sampling method was employed. Transect (2 km) covering all specified regions were laid perpendicular to the

shore line from surf zone to lower intertidal area on specific day (8th day, 15th day, 23rd day and 30th day) of month from August 2010 to July 2011. Crab burrows falling on transect were identified and counted to analyze the distribution pattern. Previously used and suggested by Butler and Bird (2007) and Skov and Hartnoll (2001). Distribution pattern of animals is studied with respect to Sediment composition, season, tidal extent and exposure period. During survey presence of *Uca dussumieri, Scylla serrata, Uricarcinus orientalis, Parasesarma pictum Uca annulipes, Ilyoplax sayajiraoi Dotilla sp., Metopograpsus masor* and mud skipper (*Boleophthalmus dussumieri*) were noted and their distribution zone was recorded. Change in distribution pattern of animals during the juvenile recruitment period of perticular species is studied. Five distinct zones were identified according to sediment sorting and dominancy of specific animal, which is surfing zone (Z1), *U. annulipes* dominant zone (Z2), *I. sayajiraoi* dominant zone (Z3), Mud skipper dominant zone (Z4) and *Dotilla sp.* dominanr zone (Z5). 5 quadrates (1 X 1 m) were laid in every zone during 4 selected days of month (8th day, 15th day, 23rd day and 30th day) from August 2010 to July 2011. As crab species like *Macrophthalmus sulcatus* and *Ashtoret lunaris* only active during night time at low tide, night survey were done to observe distribution of this crab.

The study was done to compare difference in activity and density of crab during different lunar cycle phase and season. Quadrate sampling (1 X 1 m) of *U. annulipes*, *I. sayajiraoi* and *Dotilla sp.* were done during low tide with the time lapse of one hour after tide recedes in their respective zones. Five quadrates were laid on each specific zone on transect line and observed 5 times with the time lapse of 1 hours on eight selected days of tidal cycle;4th day, 8th day, 19th day and 23rd day (Neap Tide) while 12th day, 15th day 27th and 30th day of spring tide. Sampling was repeated for 3 lunar cycles on different season from August 2010 to May 2012.

3.3.2. Carapace Width and burrow diameter Study

The relation between crab carapace width (CW) and burrow diameter (BD) was recorded for 100 burrows of *U. annulipes*. In this case measures of burrow diameter and crab CW was obtained using a vernier caliper (±0.05 mm).

3.3.3. Behavioral Studies

Cryptic activities of macrobenthos on exposed intertidal area were studied by their behavioural displays. The track and trail marks, burrowing patterns, feeding and burrowing expels by crabs and other benthic forms are some of the indirect evidence which mark the presence of the species in the ecosystem and provides clues to habitat interactions. Crab behaviour like Cheliped waving, Hiiding, Staring and Fighting was observed for two species of crab namely *I. sayajiraoi* and *U. annulipes*. Chelipeds waving behaviour which shows by both the species of crab its territorial behaviour use to expand their territory to save their burrow from other wandering crab and some times to attract opposite sex. Staring to each other is first phase of fighting behaviour, whenever other crab approach towards burrows owner crab first they rush towards other crab and stop for sometimes and analyse the situation, after that either they hide in burrow or make decision to fight. Hiding behaviour is escaping behaviour, whenever crab threatened from surroundings or to avoid fight with other crab shows this behaviour. Fighting Behaviour is last decision of burrow owner crab, *I. sayajiraoi* fight mostly by their side legs and *U. annulipes* by their Chelipeds.

To understand behaviour in detail, crabs were classified in three classes according to their carapace width size and behaviour recorded. Second, behaviour was recorded in two different time span: early hours of tide recedes (0-2 hrs) and late hours of time recedes (3-4) hours. Time budget of behaviors: The time budget of crab behaviors was observed during daytime low tides for 10 minutes by direct record or by

Videographic evidence (Koga 1995). This study was classified in two level, if the crab is surrounded by other crabs of their own species it is called intraspecific behaviour study and in the intermediate zone where more than one species of crab interact with each other it is called interspecific behaviour study. The differences in the intraspecific behaviour study and interspecific behaviour study was compared.

Dotilla sp. was found in very high number during their recruitment season. Their chimney building activity, feeding activity and different movement depends on microhabitat like ripples formation, water pool, presence of organic matter and other reasons. To understand their movement and activity on specific day 36 quadrate were laid , each with distance of 10 meter in 50 × 50 meter and observe four times with time lapse of 1 hour during low tide. Percentage of water was noted. Crab burrow presence with classification like chimney or without chimney, open burrow or close burrow were noted.

3.3.4. *Photographic and Videographic Documentation*

Many of the species are opportunistic and swift to be collected. Also, their preservation may, in due course, cause loss of original colour and pattern or some morphometric distortions particularly in case of soft bodied animals. This in turn can cause difficulty in their identification later on. Keeping in mind these aspects, photographic documentation of animals and habitat features was done in field using Fujifilm digital camera. Further, the collected specimens were thoroughly cleaned and appropriately photographed in the laboratory. In case of crab Morphometric, photographs of various body parts of the different species were taken using macro/super macro zoom function. Behavioural displays of crabs were documented by taking video recording in field using Sony Handycam without disturbing the animal. The recording from distance was done continuously for Ten minutes and following a break of ten minutes rerecorded for further Ten minutes

for Activity Time Budget and behavioural aspects. Such several bouts of activities were recorded for at least 70 different individuals of a crab.

4. ECOTOXICOLOGICAL STUDY

4.1 Water Sampling and Processing

Water samples were collected from study sites (Total 4 from upstream to Downstream) using 1 lit. Polypropylene bottles. Samples were brought to laboratory and were analysed pollutant specific parameters like inorganic matter like oil & grease, Phenol and heavy metals using standard methods (APHA).

4.2 Sediment Sampling and processing

Sediment samples were collected by pipe coring from two different zone of Sarod and four different zone of Kamboi. After bringing to laboratory the samples were air dried and powdered by applying light weight in order to break the clumps and sediment aggregation. These samples were analysed for pollution specific parameter analysis inorganic matter like oil & grease, Phenol and heavy metals using standard methods (APHA).

4.3 Animal tissue and vegetation Sampling and analysis

Animal tissues were collected from *I. sayajiraoi* and vegetation samples were collected from grass (*Aeluropus lagopoides*) collected from Sarod and Kamboi. Samples were dried and microwave acid digestions were done by using a standardized chemical combination of concentratednitric acid and hydrogen peroxide (Abida et al., 2009; Hsiao-Chien et al., 2009). The heavy metal content of the samples collected were analysed by using Inductively Coupled Plasma –Mass Spectrometer (ICP-MS) (Anderson and Melzer, 2002).

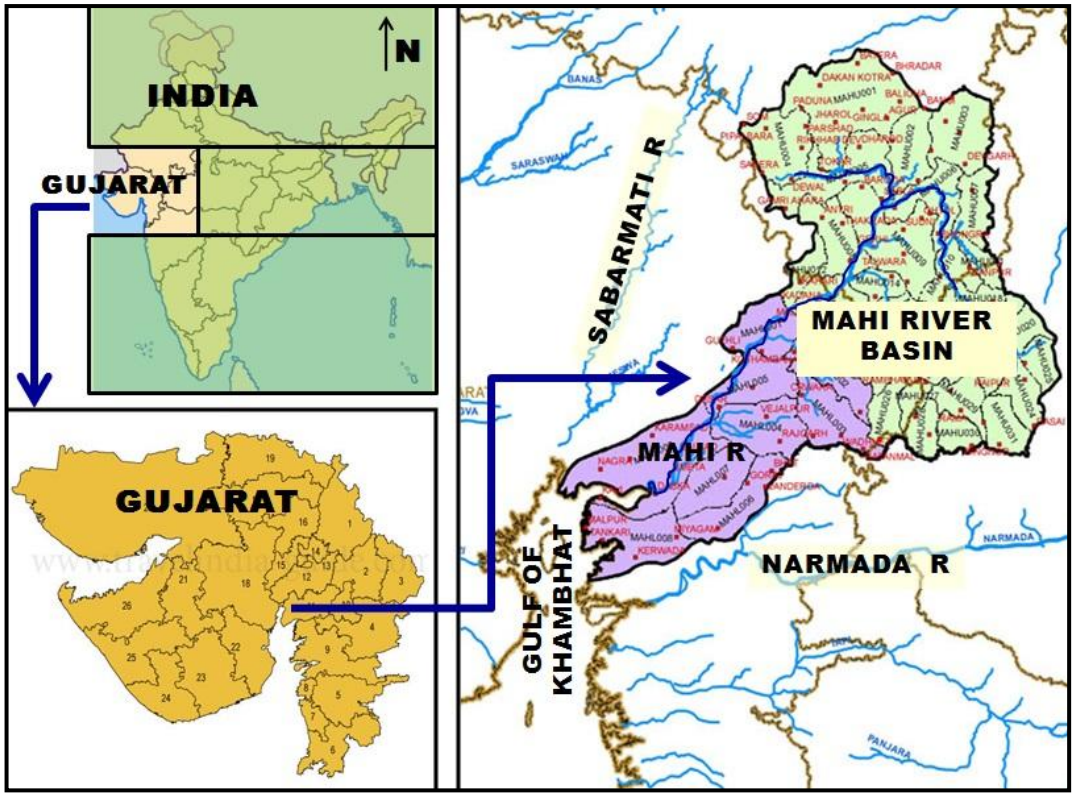

Figure1. Map of location of study area with reference to Mahi River.

Figure 2. Map showing the toposheet details of the study spread over Mahi River estuary from Fajalpur to Kamboi.

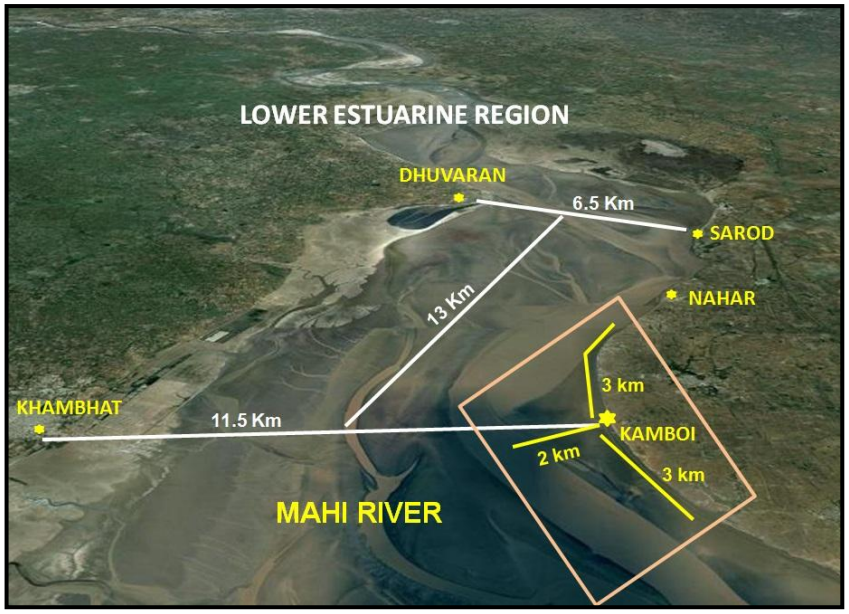

Figure 3. Location of the study area specifically showing region between Sarod and Kamboi, the area where most of the studies were carried out.
(Google earth image)

Figure 4. Showing location of 'J' Point or the site of effluent channel opening in the lowere estuarine region of Mahi River near Sarod village.
(Google earth image)