CHAPTER-III
MATERIALS AND METHODS
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Preliminary survey of all the three rivers was done in the post-monsoon period of 2006 when the water level just started decreasing. During this period the methodology for determining the available habitats and collecting data was developed. Rivers were surveyed and classified into different habitat units based on morphology (Bisson et al., 1982) and finally divided into different study sites each of 100 m length, after studying Survey of India topographic maps, satellite imageries etc., for ease in further analysis with GIS and Remote Sensing. Because some habitat types could not be sampled for habitat of mahseer, we limited our sampling to pool-riffles-cascade types of microhabitat along with cobbles-boulder-bedrock substrate which commonly account for a large portion of each river in the upstream region. As there was some indication during preliminary work we choose 8 numbers of study sites in river Barak, 4 numbers in river Jatinga and 5 numbers in rivers Dhaleswari as mahseer habitats.

We used systematic sampling scheme along particular direction (upstream/downstream) from a particular spot in a particular day. Sampling were done in the pre-monsoon and post monsoon periods of 2007-2008 when the water level remains low, easy to travel remote areas to collect fish, plankton and other habitat inventory data.
3.1. Habitat Inventory Parameters

Habitat inventory parameters were recorded in a standard format (NBFGR, 2002) from each study spot in the field itself. The geographical co-ordinates (Latitude - longitude) and altitude above mean sea level (m.s.l.) of study spots were recorded with the help of Garmin made GPS-60. Habitat features were quantified using transects within each stream reach. Variables measured at each point of a transect were water depth, water velocity and width of the river. In each stream reach within 100 m thalweg length (the longitudinal path following the fastest cross sectional water velocities) habitat such as run, sheet, pools, riffles, cascades and falls were identified. Length, width and depth of each habitat were measured base upon criteria outlined in Armantrout (1990), Kar (2007) and Arunachalam (1999). Habitats were identified and the length width, depth and substrate type of each habitat were measured. Water column velocity was measured with the help of National Instruments Corporation (Roorkee) made Pigmy type of Current meter (rev/40s) and also with scale and stop watch (30 cm/? s) in case of surface water current in certain places, finally the results were converted into m/s after standard chart. Riparian cover was measured using a spherical Densiometer. Gradient was determined by averaging three repeated measurements with Finland made Sunito Clinometer. Instream cover for fish was estimated in the microhabitat area. Instream covers recorded were: overhanging vegetation, turbidity, undercut bank and bottom free big boulders. Measurement of habitat structure was followed using Gorman & Karr (1978) with modification based on the Indian conditions with depth, current and substrates. Habitat area (in square metre) was calculated as the product of length and mean width of the study reach. Habitat area times mean depth was used as indication of habitat volume (in cubic metre).
3.2. Physico-chemical Parameters

Some of the physico-chemical parameters such as air and water temperature, pH, turbidity etc. were measured on the field itself, and water samples were collected in the pre-cleaned bottles to study other parameters like turbidity, pH, dissolved oxygen (DO), free carbon di-oxide (FCO₂), total alkalinity (T.A.), conductivity, phosphate (PO₄), nitrate (NO₃) etc. in the laboratory using instruments and titration methods after APHA (1995, 1998).

In the field temperature was measured with centigrade Mercury Thermometer, pH was measured with the Qualigens made Indikrom pH-Papers of wide range (pH 2.0-10.5), turbidity was measured with standard turbidity tape.

In the laboratory pH was measured with Systronics made digital pH meter Type-335, turbidity was measured with Systronics made digital Nephelo-Turbidity meter Type-131, conductivity was measured with Systronics made D.D.R. Conductivity pH meter Type-335, D.O. was measured with both Systronics made digital Dissolved Oxygen meter Type-312 and Winkler's Iodometric titration method (Welch (2003), FCO₂ and T.A. were also measured with the Titration method of Welch (2003) using Phenolphthalein and Methyl Orange as indicators. Phosphate was measured with Stannus Chloride method (APHA, 1995) and nitrate was measured with the Brucine method (APHA, 1998) using Systronics made Spectrophotometer Type-105.
3.3. Fish assemblage structure

Fishes were extensively sampled at each site on each occasion with the help of local fishermen through experimental fishing using different kinds of mono filamentous gill nets with varying mesh size (10-15 mm) and vertical height (1.0m-1.5m; length 100m-150m), cast nets of different diameter (3.7m and 1.0m) and mesh size, dip nets, drag nets (vertical height 2.0m), triangular scoop nets (vertical height 1.0m) and with hook and line in certain places (where netting is banned). Camouflaging technique was also used to catch the fishes. Fishes were also collected from certain fish landing centres situated around. The weight of the catch, the diameter of the gears used, number and duration of operation were recorded for the calculation of fish yield etc (FAO, 1974). The fish samples were then preserved in 8-10% formalin at the field stations. The big sized specimens were also suitably injected with 10% formalin solution for proper preservation. In the well equipped Aquaculture and Limnology laboratory of Assam University the specimens were morphologically and morphometrically studied and identified from various authoritative sources including Day (1885, 1889), Jayaram (1981, 1991, 1999, 2003), Kar (2007), Kar & Sen (2007), Menon (1974, 1999), Menon et al., (2000), Misra (1976), Roberts (1980, 1994), Rainboth (1985), Sen & Jayaram (1982), Sen (1985), Tilak & Hussain (1974, 1981) and Talwar & Jhingran (1957, 1975, 1977, 1982, 1988, 1991). Some of the specimens were also identified from Zoological Survey of India (ZSI) Shillong. For the morphometric and meristic characters the procedures of Dwivedi and Menezes (1974) was followed. After labelling the photographs of the fishes were taken with the help of both ordinary camera (Kodak, Nikkon, Pentax) and digital camera (Canon Power shot A-530).
Various habitat characteristics, interaction and interviews with local peoples were recorded with Sony digital video camera.

3.4. The definition of measurements

The measurement of various body portions were taken with utmost care. All are straight point to point measurements, taken with fine pointed dividers and recorded. The characters considered important are those shown here under and taken in relation to standard length. The **total length** (TL) is the greatest distance between the most anterior projecting part of the head to the posterior most tip of the caudal fin, where the caudal fin has either of the lobes longer than the other, and the maximum length is taken.

**Standard length** (SL) is the straight distance from the anterior part of the head to the posterior edge of hypural plate i.e. base of the caudal fin. **Head length** (HL) is the straight measurement of the distance from the tip of the snout to the most distance part of opercular membrane. The **head breadth** is measured as the distance in its widest part. **Head depth** is the perpendicular distance measured from the midline at occiput vertically downwards to the ventral surface of the head or the breast. **Eye diameter** is the distance between the anterior and posterior margin of the bony orbit. The **inter-orbital distance** is measured along the dorsal surface between the upper rim of each orbit at the nearest point. The **length of the snout** is the distance from the tip of the snout to anterior margin of orbit. The **depth of the body** is taken as the distance along the vertical line of the fish at its deepest part.
The **pre-pectoral distance** is measured from the tip of the snout to insertion of first ray of pectoral fin. The **pre-dorsal distance** is taken from the tip of the snout to insertion of first ray of dorsal fin. The **pre-pelvic distance** is measured from the tip of the snout to insertion first ray of pelvic fin. The **pre-anal distance** is the distance from tip of the snout to insertion of first ray of anal fin. The **distance between pectoral and pelvic fin** is measured from the base of pectoral to the pelvic fin; that of **pelvic fin and anal fin** is the distance is taken from the base of pelvic fin to base of anal fin. The **heights of the dorsal and anal fin are** measured along its longest branched or unbranched ray. The **base of dorsal and anal fin** is measured as the distance between the first ray to the last ray at the base. The **lengths of the pectoral, pelvic and caudal fins are** measured as the distance along their longest ray from the base. The **distance between pelvic fin and anus** is taken from the insertion of first pelvic ray to anterior margin of anus. The **distance between anus and anal fin** is measured from posterior margin of anus to the insertion point of first anal ray. The **length of caudal peduncle** is the distance between last ray of anal fin and base of caudal fin. The **least depth of caudal peduncle** is measured as the distance at vertical length at its narrowest part.

**Scale counts of lateral line** (L.I.) are between upper angle of the operculum and the level of posterior edge of the hypural plate along the normal course of lateral line in this genus. The **lateral transverse rows of scales** are taken from the origin of dorsal fin vertically downwards to the lateral line. **Scales between lateral line and base of pelvic fin** are counted from below upwards and forwards from the base of pelvic fin to the lateral line. **Pre-dorsal scales** are counted in the median line from the commencement of dorsal fin forward as far they exist. **Circum-peduncular scales** are counted around the least depth of caudal peduncle. For small fishes scales and fin rays are counted
under binocular microscope. The lateral line (L.l.) counts generally referred to the pores on the scales. When the lateral line is incomplete, the un-pored scales in the horizontal row from the end of lateral line to the caudal fin base were counted. The diagnosis and description of species are arranged according to almost same order of sequence, so that comparison may be made easily. The body proportions are expressed in relation to standard length. The values shown outside parenthesis are arithmetic ranges, while arithmetic means are kept inside brackets. The ratio index of each morphometric measurements is calculated as a percentage in the standard length.

3.5. Plankton assemblage structure

Collection, analysis and both qualitative and quantitative estimation of plankton samples of each study sites were done after Battish (1992), Michael & Sharma (1988), and Kar (2007). Approximately, 50 litres of river water was filtered through plankton net (No.30) of bolting silk cloth having 55 mesh/cm² and collected in a plankton tube of 50 ml capacity. The sample was then fixed with 1% formalin and taken to the laboratory. Finally, the identification that is qualitative analysis was done by the Lackeys drop method under a Labomed made binocular light microscope. For quantitative analysis thoroughly mixed sample was taken with graduated dropper to fill the ‘Sedgewick Rafter cell’ having capacity of 1 ml and the number of planktons per ml was estimated.
3.6. Diversity indices of Planktons and Fishes

Measurements of diversity have been of historical significance and their importance still remains today given the obvious declines in habitat quality in almost every ecological system. The Shannon-Wiener Diversity Index is one of the most widely used species diversity indices for examining overall community characteristics comparing two or more distinct habitats. It is derived from a function used in the field of information and has been adapted by ecologists to describe the average degree of uncertainty of predicting the species of an individual picked at random from the community. The uncertainty of occurrence increases both as the number of species increases and as the individuals are distributed more and more evenly among the species already present.

A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simply species richness (i.e., the number of species present); they also take the relative abundances of different species into account. It is not necessary to key all organisms to their specific species nomenclature (i.e. organisms not expediently identified may be assigned numeric values such as species 1, 2, 3). However, in order to derive accurate diversity values, all organisms should be keyed to the lowest possible like taxonomic level.

The Shannon-Weiner Species Diversity Index is calculated by taking the number of each species, the proportion each species is of the total number of individuals, and sums the proportion times the natural log of the proportion for each species. Since this is a negative number, we then take the negative of this sum. The
higher the number, the higher is the species diversity. In the ideal situation, one should compare populations that are the same size in numbers of individuals.

The formula is as follows: \( H = - \sum \pi \ln \pi \)

It combines two quantifiable measures; 1. The **Species Richness** \( S \) (the number of species in the community) 2. **Abundance** \( N \) (is the total number of individuals in the sample). The index is termed \( H = \neg \sum \pi \ln \pi \) with higher values indicating increased diversity. 3. \( \pi = \frac{S}{N} \).

When properly manipulated, it will result in a diversity value (\( H \)) ranging between 0 (indicating low community complexity) and 4 (indicating high community complexity). Diversity indices provide important information about rarity and commonness of species in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure.

3.7. Statistical analysis

The range, average and standard deviation of the ratio index of 26 morphometric characters of the mahseer fishes were estimated with the Microsoft Office Excel’2003. The physico-chemical characteristics of water sample fish and plankton assemblage of different study sites were represented graphically with the help bar diagram, column diagram, line diagram and pi diagram etc. Variations and amplitude of various aquatic parameters at each study spots of rivers were presented with the help of box plots of SPSS. The average value of water chemistry of different
study sites of a river was shown with the help of column icon plot of using Statistica 99\textsuperscript{th} Edition software to compare with that of the other study sites and rivers. Relationship between the fish yield with the different physico-chemical parameters and the percentage of dependence of fish yield on particular physico-chemical parameter in each study site were measured with the help of regression analysis using SPSS 10.0. Significant test results for mean difference of the different physico-chemical parameters between the present study and previous findings of other workers were calculated with the help of independent sample test using SPSS.

3.8. Habitat mapping and spatial analysis

3.8.1. Image pre-processing

Images were processed after importing to ERDAS Imagine 9.1. The images were then geometrically corrected to a master image using ground control points and Survey of India toposheet.

3.8.2. Digital image processing

The digital images of IRS1D - LISS III of different Path-Row and date (Table 3.8.1.) with 3, 2 and 1 bands combination, obtained from the National Remote Sensing Agency were used for the present study. The spatial resolution of this imagery was 28 m (at nadir) with three spectral bands. The Images are rectified with respect to registered SOI topographic maps and Ground Control Points (GCP) collected with GPS during field visits. Then the georeferenced images are mosaiced and subset the area of interest. Nearly 30 topographic maps of the Survey of India (SOI) (Table 3.8.2.) of 1:50
000 scales were used for the present analysis. The false colour composite (FCC) digital image was georeferenced by co-registering the selected ground control points that are predominantly identifiable both from the image and from the topographic maps. Rectification was done so that the spatial co-ordinates correspond to its geographic coordinates. The projection applied was geographic UTM with Spheroid and datum WGS 84, Zone 46 North. It was not possible to trace the boundary of the rivers from the image because of the continuity of the surrounding riparian vegetation and rice fields from the adjoining land areas with the rivers. So, the river boundary extracted from mosaiced topographic maps was considered to be a good representation of the original river area. The river boundary derived from the topographic maps was overlaid on geo referenced satellite image and updated to extract the river area. The subsetted image of the river area was enhanced to produce a crisp image and to reduce the noise and atmospheric attenuation using different (e.g. spatial, spectral, radiometric) enhancement techniques that are available in Geomatica software.

Data presentation in the form of map is very important to perform the spatial analysis and for which uniform scale is very necessary. Survey of India topographical map on 1:50,000 scales were used for base map generation. The entire panning was made and the thematic maps were generated in 1: 50,000 scale. The steps adopted during the analysis are:

1. Visual image interpretation and field verification.
2. Information extraction from the existing maps and toposheet.
3. Attribute information collection from various sources.
4. Primary data collection in the field.
5. Analysis of the samples collected from the field.
6. Feeding the collected data for analysis in the GIS environment.

After collecting data, database for all the themes were transformed into a computer readable digital format, so that the necessary processing and analysis GIS software can be employed.

For georeferencing the satellite images, in each of the selected raw image, at least three points (control points) were selected whose longitude and latitude were known and keyed interactively. On the basis of the longitude and latitude control points, the whole image was transformed into a real world co-ordinate system. Finally all the images were mosaiced to create the total image.

The study area was clipped by using the software for further interpretation. Moreover, all the necessary image enhancements were done in ERDAS Imagine 9.1 platform.

Table 3.8.1. List of Satellite imageries IRS-1D, LISS-III of product code STPC0026J used in the present study.

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Table 3.8.2. List of Survey of India Toposheet of 1: 50,000 Scale used in the present study.

<table>
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<th>Sl. No.</th>
<th>SOI Nos.</th>
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<tr>
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3.8.3. GIS based Data Management and Analysis

The GIS software Geomatica 10.1.0 was used to perform the GIS based study. All the spatial (map data) and non-spatial (attribute data) information were converted to digital form and stored in vector data format. The attribute information was either keyed in or joined to the spatial database. The following steps were followed to perform the GIS analysis:

1. All the required maps were converted to digital form in raster data format by scanning.
2. As the satellite image was georeferenced, the rest of the raw images were georeferenced with reference to the georeferenced image by linking points identifiable in both the raw image/map (source data) and the georeferenced image (target data).
3. Spatial information contained in the georeferenced raster images was extracted layer wise in vector format. For this on screen digitisation was carried out in the Geomatica 10.1.0 platform. During this process, various categories of parameters taken into
consideration were identified by using standard visual image interpretation techniques, which includes the element of image interpretation. After identifying a particular category its boundary was mapped accordingly. If certain categories having similar response and spectral signature appear side by side, it becomes difficult to delineate them separately and in such cases, categories were marked for detailed verification on the ground.

In this process information were saved as separate layers in the respective ‘shape files’. Depending on the nature of information, they were digitised either in points, line or polygon.

4. Field verification of the interpreted categories of various parameters is an important of remote sensing study. The categories that are marked as doubtful on preliminary interpretation were verified and corrected in the field and subsequently updated in the system. Apart from the doubtful area verification, all the classified categories from the image were also visited during field survey. Thus, land use/land cover categories of the study area were verified in the field. For ground verification, the hard copy printout of satellite imagery and topographic map were carried to the field for reference.

5. After necessary correction and field verification, the attribute or non-spatial information was attached. The required fields in the attribute table were created and attribute data were entered. In certain cases, the attribute information present in an external database table was joined with the attribute data with the help of common field.
6. Once the data attachment was complete, the necessary analysis, viewing, printing of maps etc were done in the GIS software. Moreover, during the course of analysis, some new data were also generated, which in turn were used for analysis.

The rectified image and the subsetted topographic maps of the river area were incorporated into Geomatica 10.1 for further analysis because vector layer analysis can be performed in Geomatica 10.1 in an accurate manner. The land use map of the rivers in 1967 was developed based on the classes given in the SOI topographic maps. Since the width of the river fluctuates with the seasons, the rivers were classified under two categories, namely dry water area and the monsoon area of liable to be flooded during the rainy season. Different land and water classes were on-screen digitized to get the exact shape.

3.8.4. Ground truth verification

Detailed ground truth verification was carried out using Garmin GPS-60. The extent and latitude and longitude of the different land and water use classes were verified. The boundary of the riparian vegetation and agriculture field in the land use map was also checked and corrected through GPS readings wherever necessary.
3.9. Software Used

Basically, six software were used for this study viz.,

(a) Microsoft Office 2003 - Used basically for the text typing, graph preparation, calculation and presentation of the research.

(b) Geomatica v 10.1- Used for Image georeferencing, registration, digitisation and map generation etc.

(c) ERDAS Imagine 9.1- Used for Image pre processing and mosaicing etc.

(d) ArcGIS – This was also used to compliment the display and processing of the Data.

(e) SPSS 10.0 - Basically used for the calculation of Correlation, mean difference and generation of box plots.

(f) Statistica 99 – Used for generation of column icon plot etc.