CHAPTER THREE

MATERIALS AND METHODS

CHAPTER -III

MATERIALS AND METHODS

3.1: Description of Study Area:

3.1.1: Description of the Study Site:

Survey Area:

During the Study period, (January 2004 to December 2006) 17 (seventeen) districts (Map No. 1) out of 23 (twenty three) districts of the state of Assam, India, were covered only for the collection and identification of freshwater prawns. The seventeen districts are 1. Dhubri, 2. Kokrajhar, 3. Goalpara, 4. Bongaigaon, 5. Barpeta, 6. Nalbari, 7. Kamrup, 8. Nagaon, 9. Darrang, 10. Lakhimpur, 11. Sonitpur, 12. Dibrugarh, 13. Jorhat, 14. Sibsagar, 15. Tinsukia, 16. Golaghat and 17. Morigaon.

The state Assam is situation in the North Eastern part of India between 24.1^0N and 27.9^0N latitude and 89.8^0 E and 96.1^0E longitudes. Its total area is 78,523 sq. kms, the survey are covered around 58% of the total area of the state of Assam.

The soil in the plains of Assam is alluvial and fertile. The soil in the hilly areas is red in colour.

Assam is full of rivers. Besides Brahmaputra and Barak river, there are many tributaries flowing into it. The Brahmaputra valley is a part of Indogangetic Brahmaputra river system of North-East India. The valley

comprises of 18 (eighteen) out of 23 (twenty three) district and is spread over 72% of total area of the state.

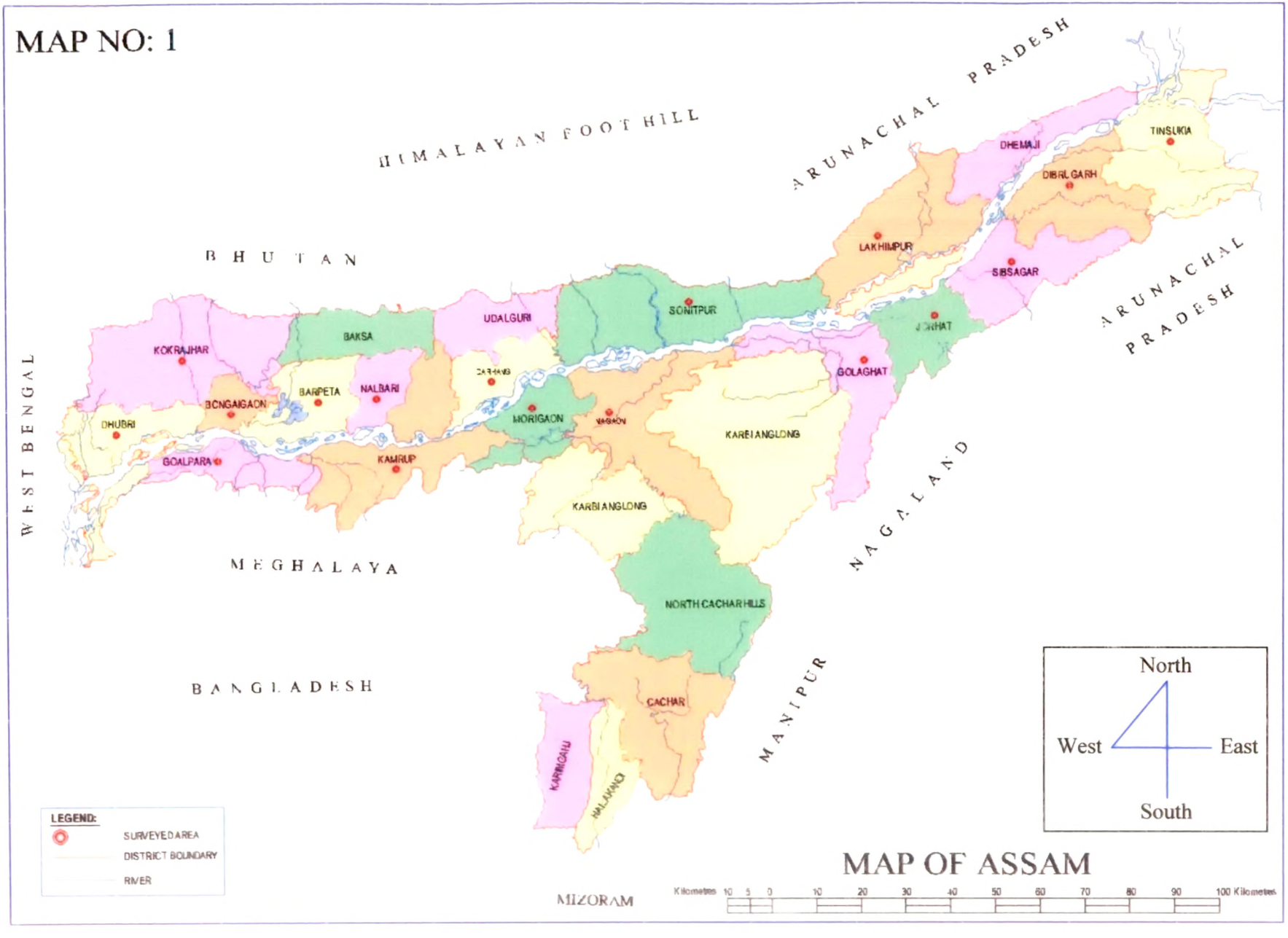
To the south of the North Cachar hilly region is the Barak valley. The river Barak flows through this valley from east to west in a serpentine manner.

Site for Eco-biological study:

Ecological and biological observations were carried out in few neighbouring districts only where freshwater prawn population is found abundant. These districts include – Goalpara, Dhubri, Bongaigaon, Kamrup, Nalbari, Morigaon, Nowgaon and Darrang district. Water samples and soil samples are collected randomly from river, beel, pond, paddy field, and swamps and monthly average value of different parameters are recorded. Similarly aquatic vegetation and plankton, both phyto and zoo are studied in a similar manner. Again the whole study is made in different seasons as mentioned in the study period.

The state has extensive fresh water resource with huge potential for fisheries development. The flood plain lakes are a conspicuous feature of both the Brahmaputra and the Barak Valley in Assam. Three types of beels are found in Assam viz. Oxbow lake type, lake type and true tectonic depressions. Oxbow beels are cut off portions of river meanders or dead rivulet courses. Many of them are connected with the main river through channels. Ox bow beels are relatively morrow, long and either bent or serpentine in shape. Lake

MAP NO: 1

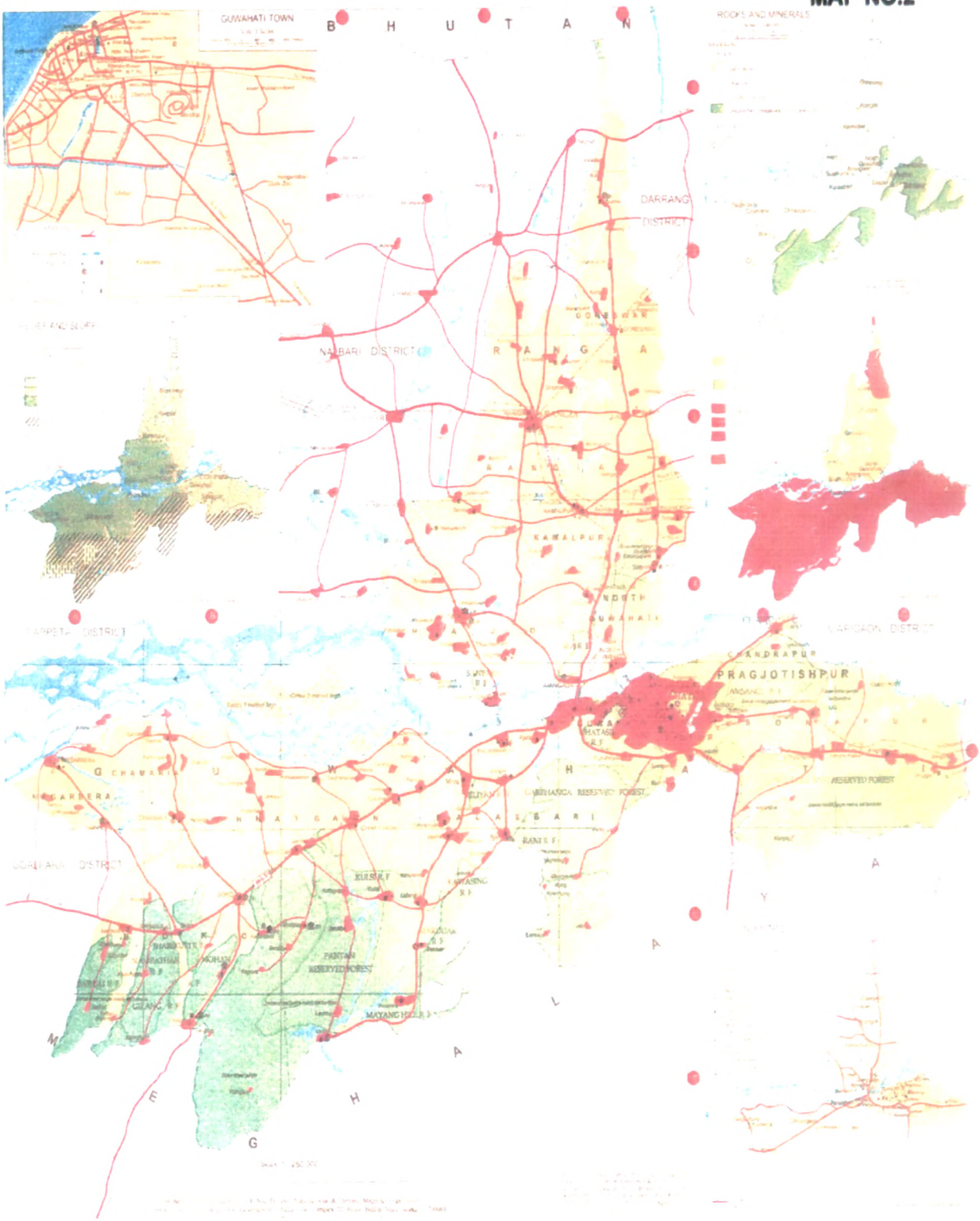

HIMALAYAN FOOT HILL
ARUNACHAL PRADESH
BHUTAN
WEST BENGAL
MEGHALAYA
BANGLADESH
NAGALAND
MANIPUR
MIZORAM
ARUNACHAL PRADESH
TINSUKIA
DHEMAJI
DIBRUGARH
LAKHIMPUR
SIBSAGAR
JORHAT
GOLAGHAT
SONITPUR
UDALGURI
BAKSA
KOKRAJHAR
BARPETA
NALBARI
BONGAIGAON
DHUBRI
GOALPARA
KAMRUP
DARRANG
MORIGAON
NAGAON
KARBI ANGLONG
NORTH CACHAR HILLS
CACHAR
KARIMGANJ
HAILAKANDI
North
West
East
South
LEGEND:
SURVEYED AREA
DISTRICT BOUNDARY
RIVER
MAP OF ASSAM
Kilometres 10 5 0 10 20 30 40 50 60 70 80 90 100 Kilometres

like beels are wide, shallow and irregular in contour. They are also connected to river through channel and receive water from them.

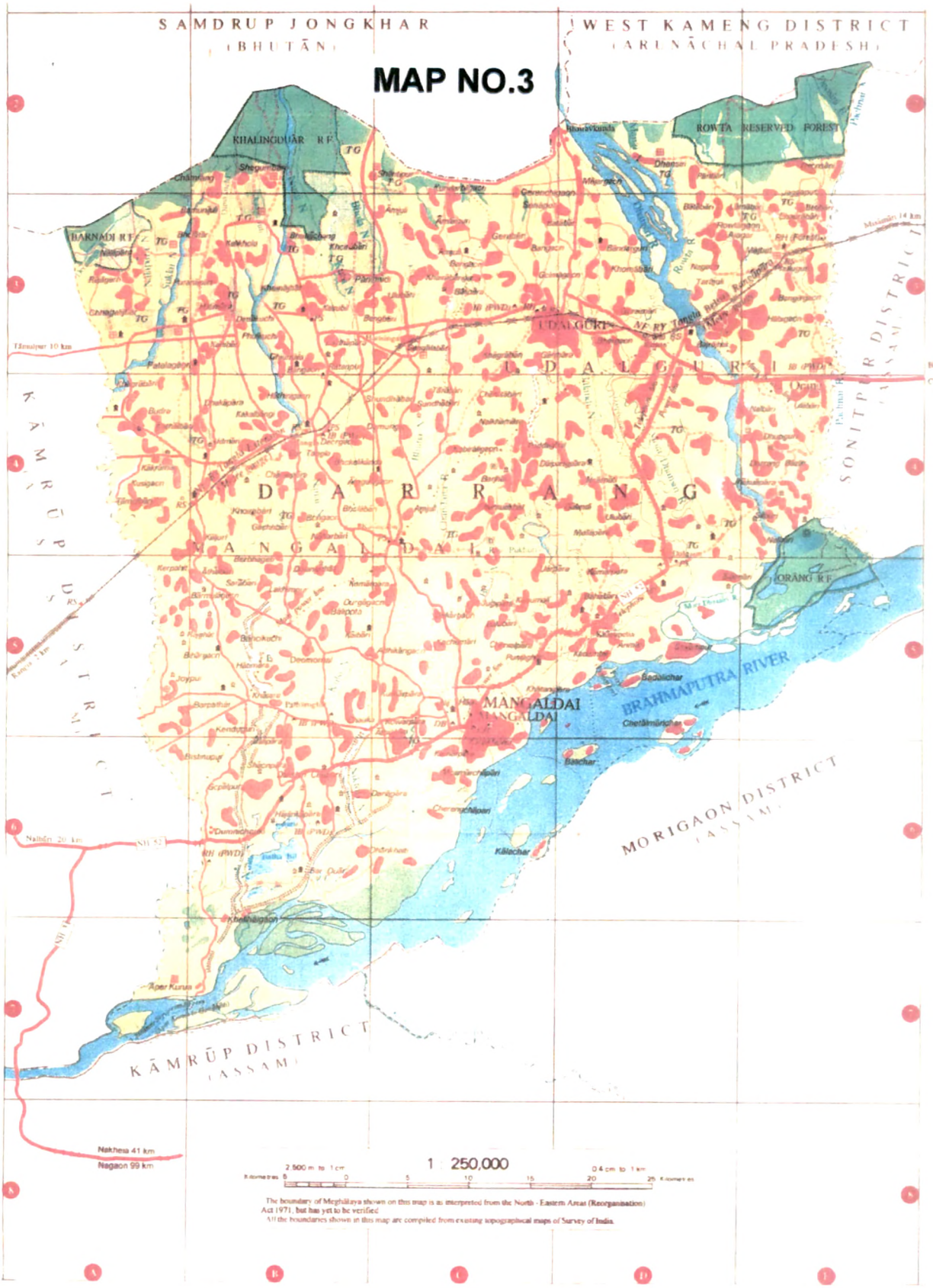
The beels in Brahmaputra basin are situated along the flood plains of rivers Dibru, Buridihing, Dishang, Dikkow, Jhanji, Kokodonga, Hhansiri, Sonai, Kapili, Kallong and Kulsi, Kameng, Dhansiri, Pagladia, Manas, Aai, Champabati and Saralbhanga on the North bank.

District	River Basins
Tinsukia	Lohit, Dibru, Dhala, Dengari, Buridiheng, Dumduma
Dibrugarh	Buridiheng, Dibru, Sensa,
Sibsagar	Dimow, Dishang, Dikhow, Jhanji, Teok, Namdang.
Jorhat	Jhanji, Teok, Dishoi, Kakodonga, Bhogdoi.
Golaghat	Kakodonga, Dhansiri, Diflu, Doiang, Lengtaian, Rengma, Digholi.
Lakhimpur	Subansiri, Kada, Ghagra, Ranga, Dikrong.
Nagaon	Kollong, Sonai, Diju, Na, Kapili.
Morigaon	Kallomg, Sonai.
Sonitpur	Kharoi, Buroi, Borgamg, Bharoli, Jiagabharu, Belshiri, Sipai.
Darrang	Dhansiri, Mora, Nowa, Nalapani, Baranadi
Kamrup	Puthimari, Hajosuta, Baranadi, Digaru, Barapani, Kulsi, Shibang.
Goalpara	Krishna, Balbala, Dudhnai
Nalbari	Pagaldia, Tihu, Baralia
Barpeta	Moramanai, Karokhowa, Kaladiya, Bhelengi.
Kokrajhar	Champabali, Ai, Manas, Saralbhanga, Kanamakar.
Dhubri	Sankhos, Tarang, Gobadhar, Diplai
Bongaigona	Tamranga Beel.

MAP NO.2



Legend : Water bodies



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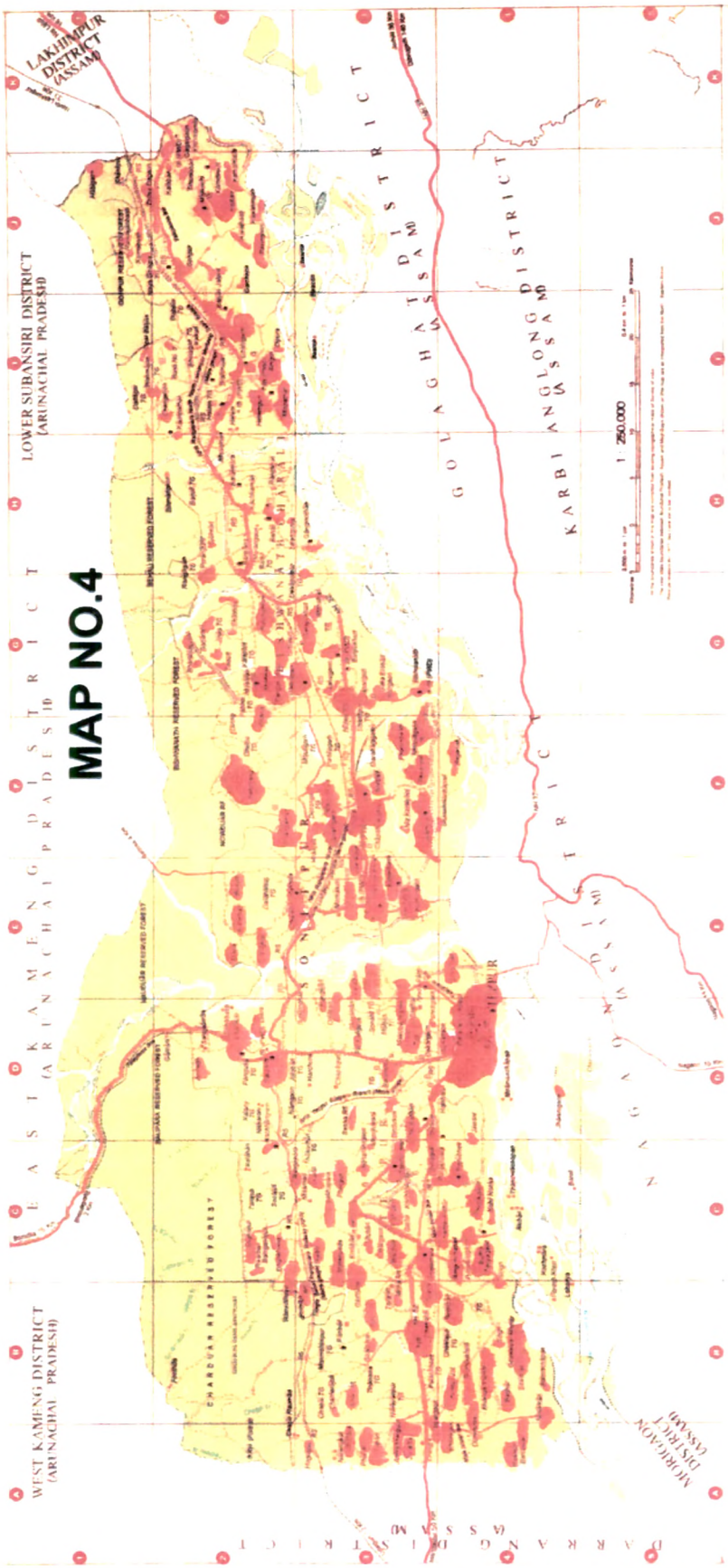
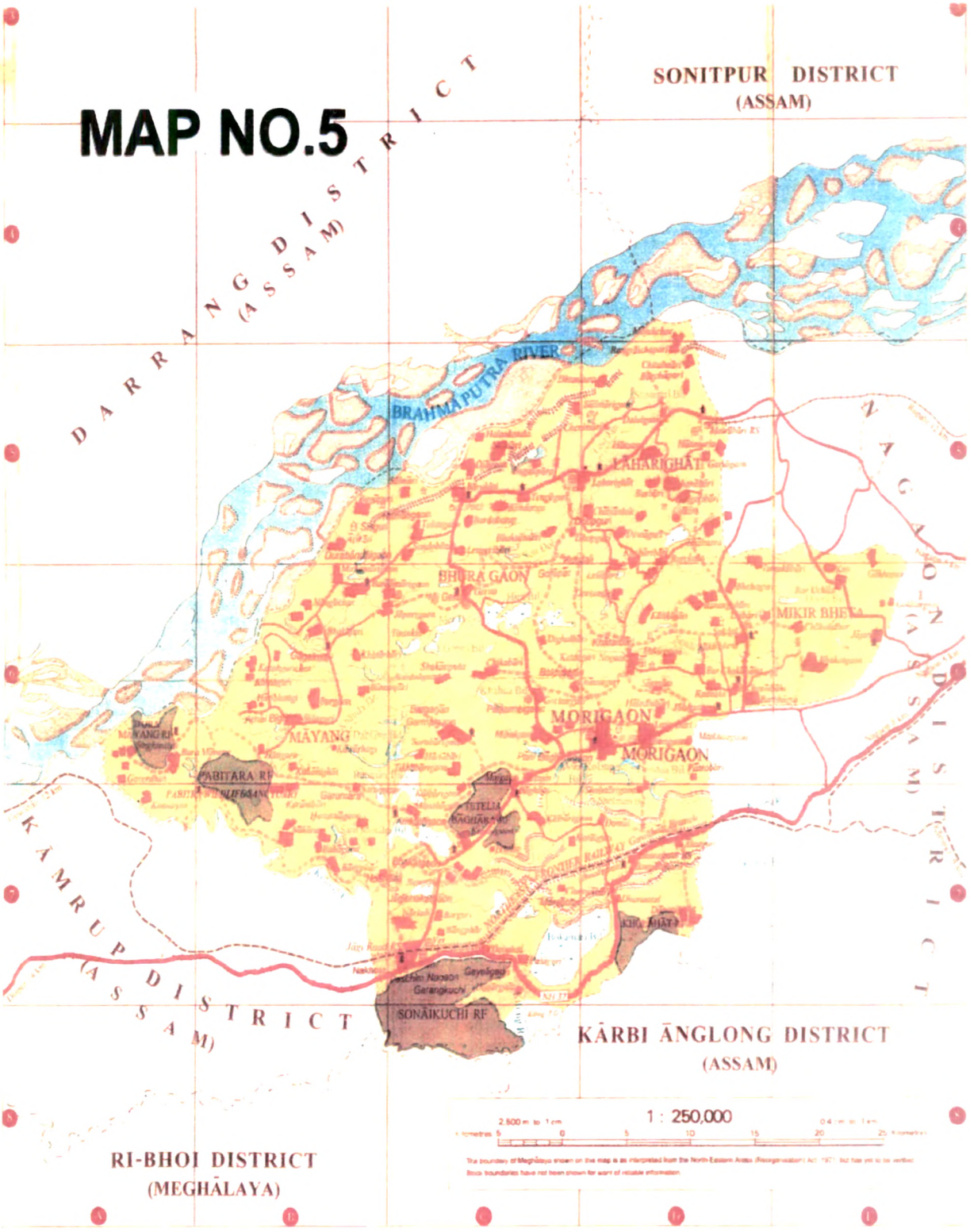
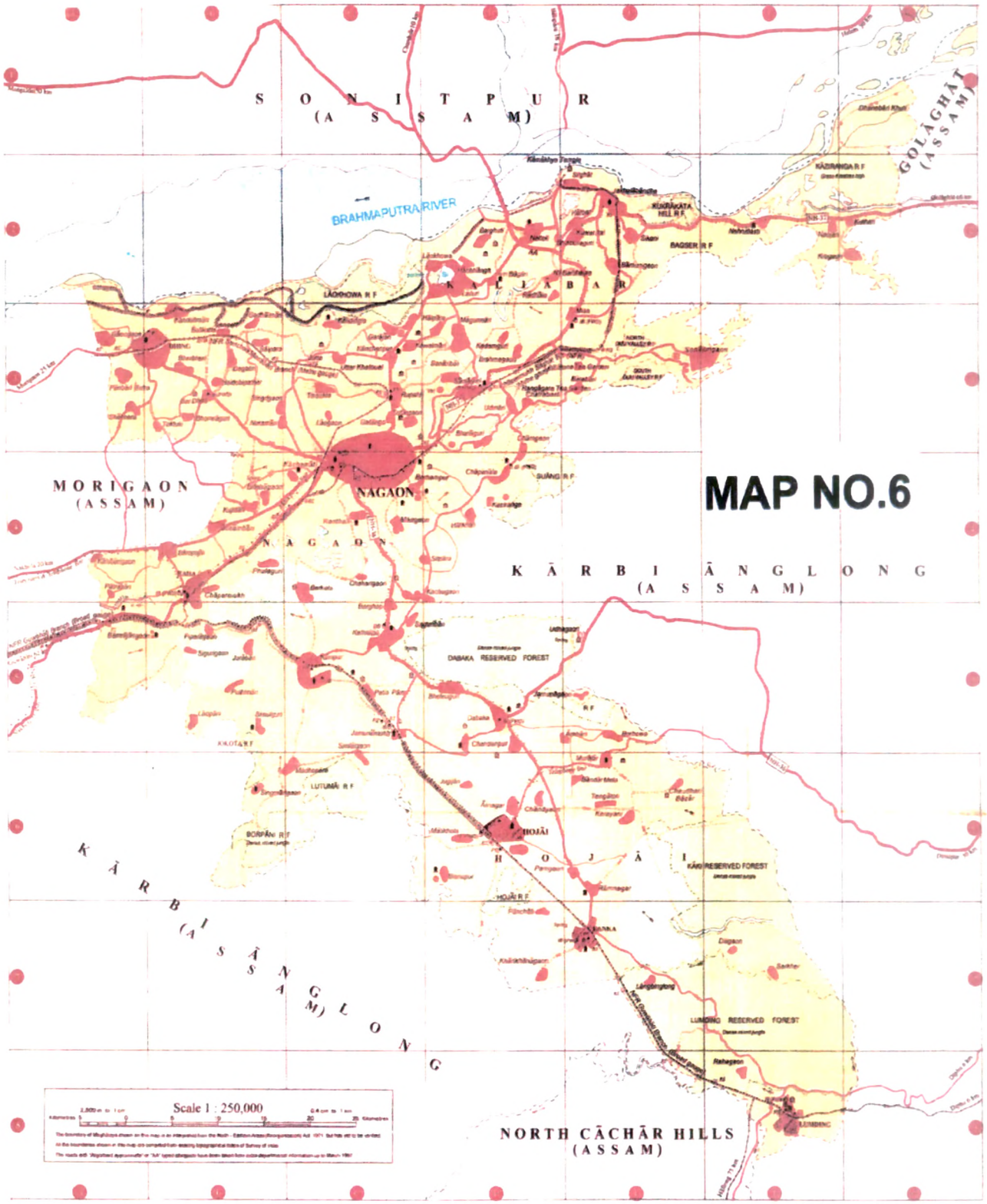


Legend : Water bodies

MAP NO.5

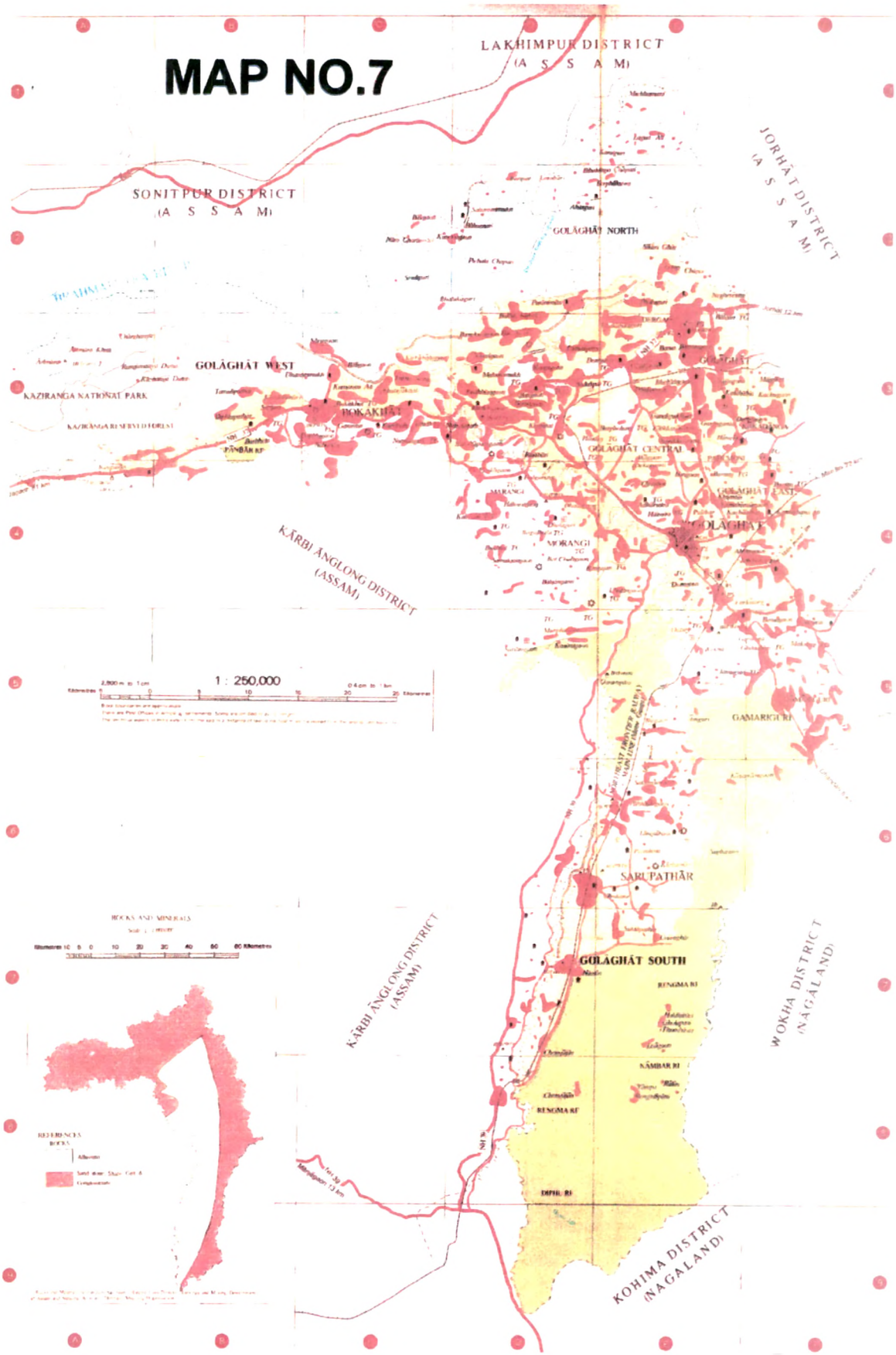


Legend : Water bodies

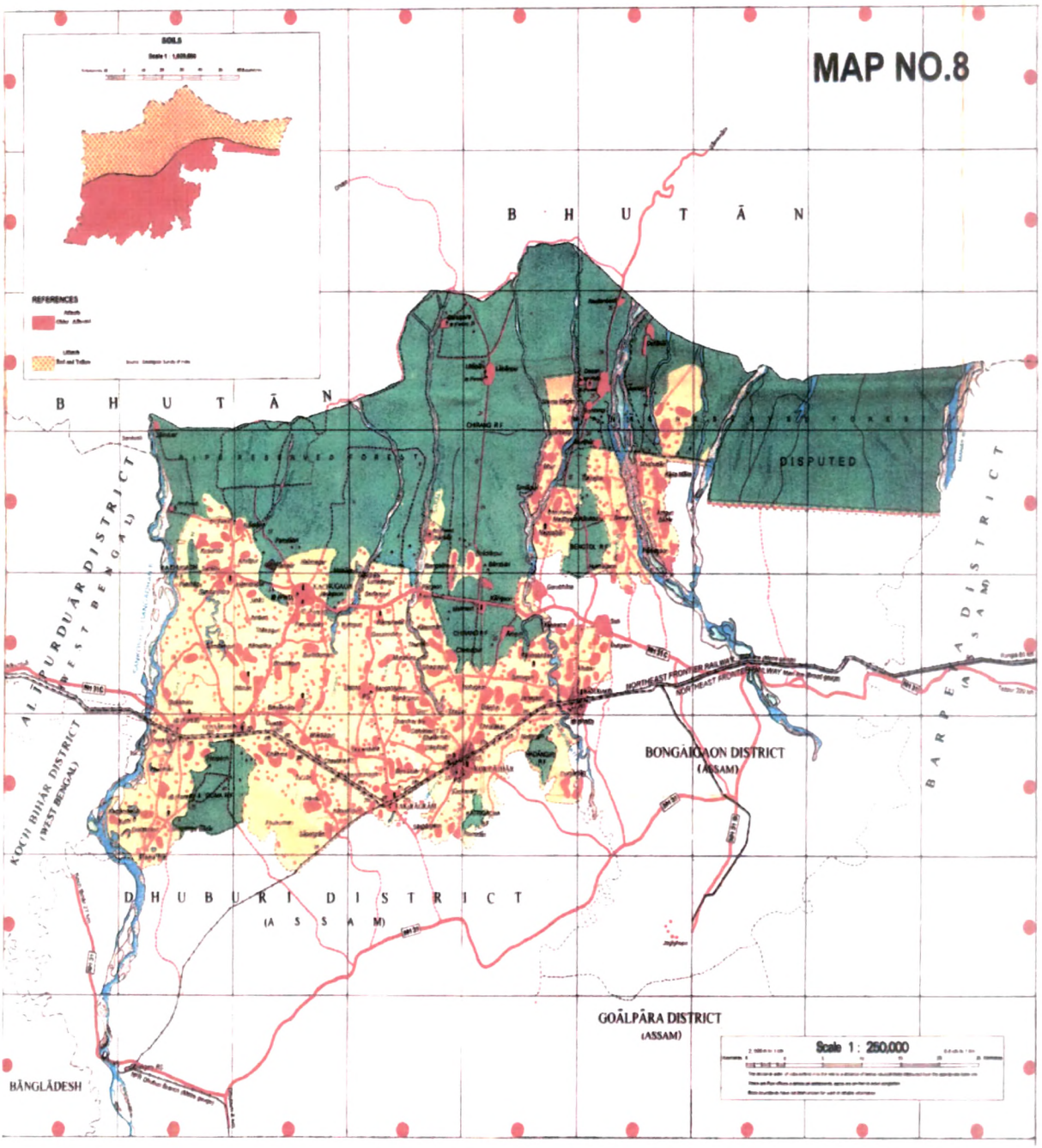


Legend : Water bodies

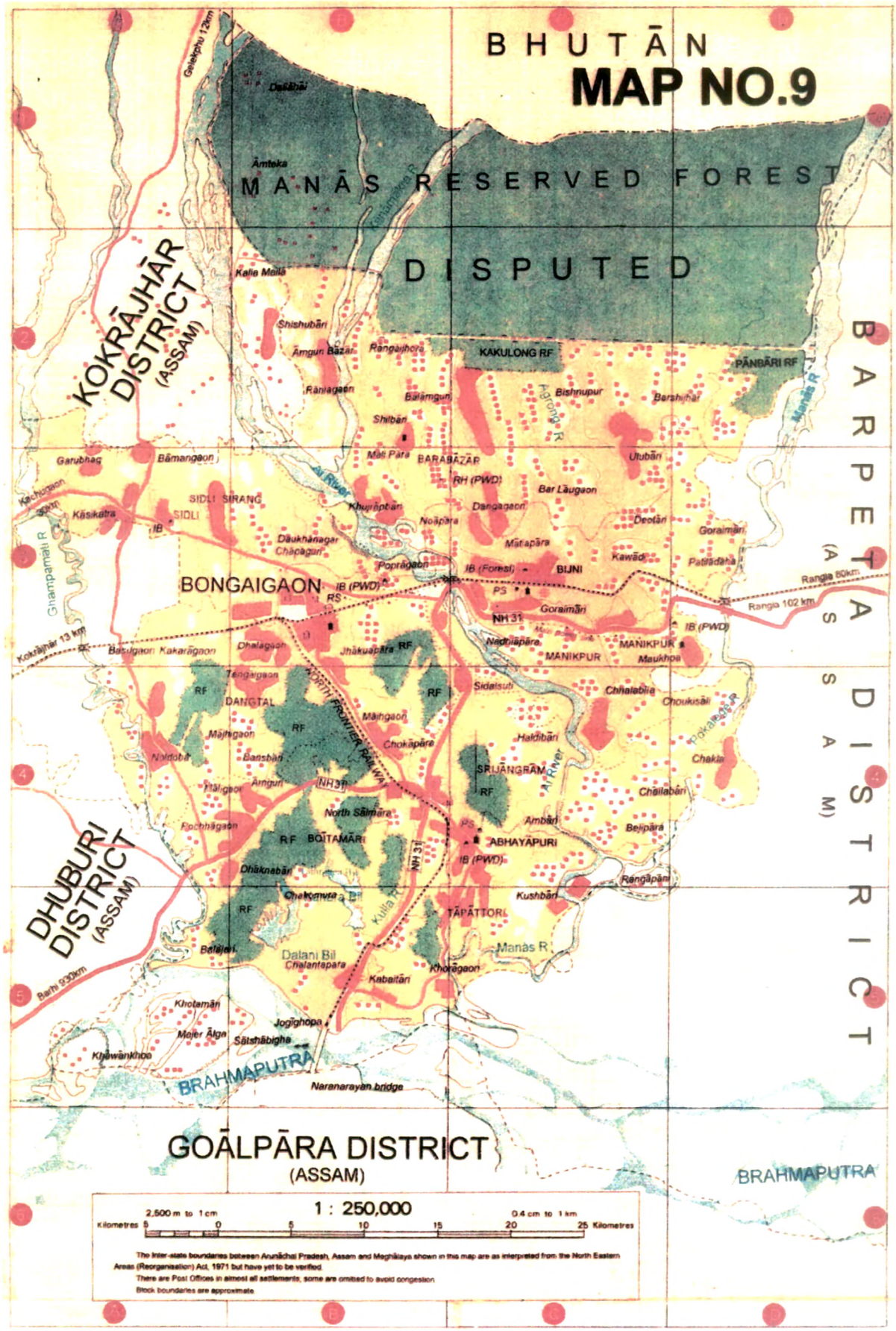
MAP NO.7



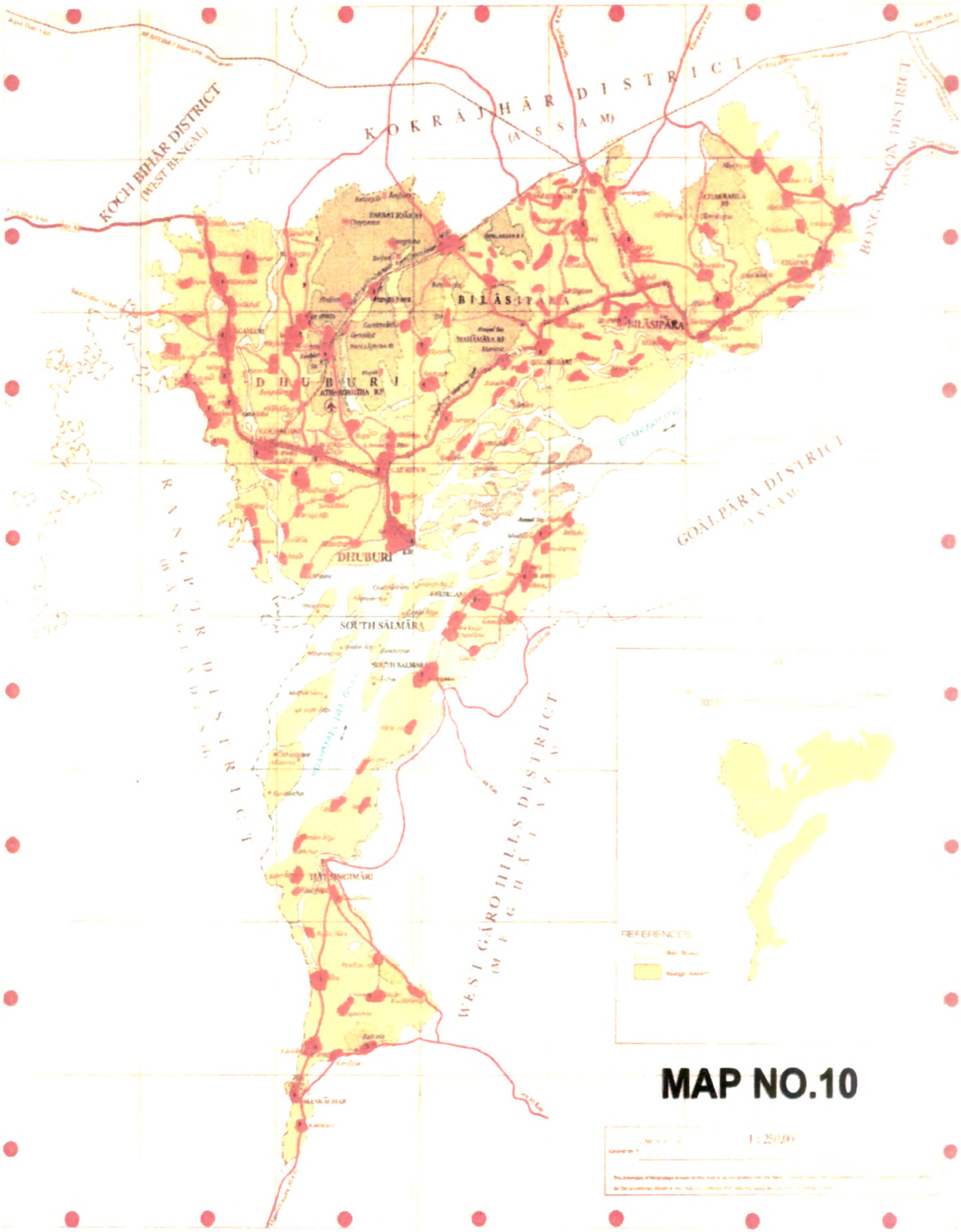
Legend : Water bodies



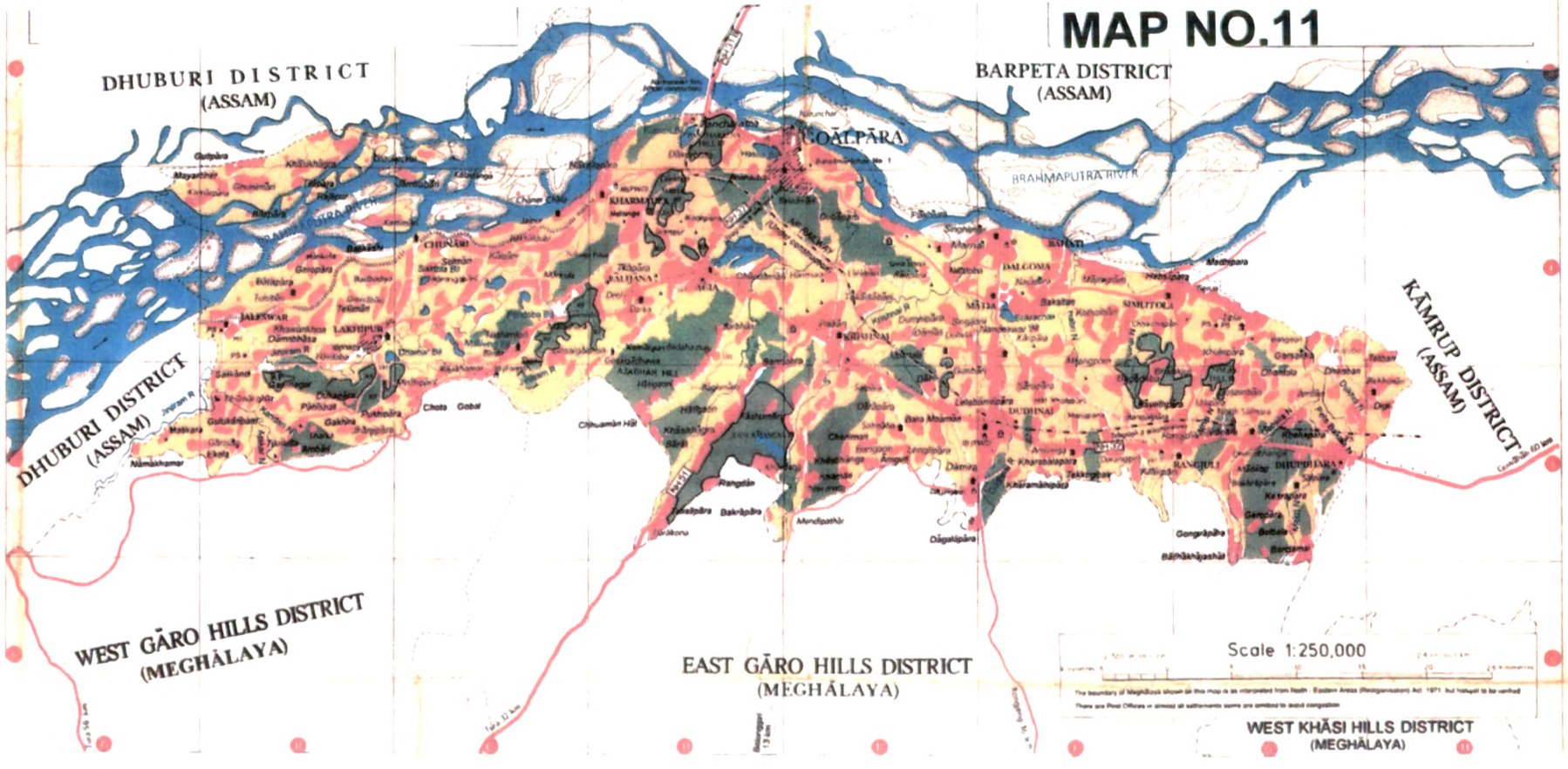
Legend : Water bodies



Legend : Water bodies



Legend : Water bodies



Legend : Water bodies

Name of some beels found in different districts of Assam :

Tinsukia	Motapong, Mota, Udaipur, Rampur.
Dibrugarh	Lomghori, Mer, Dihingerasuti.
Sibsagar	Boka, Borboka, Dikhowmornai.
Jorhat	Gorormaj, Borchala, Gelabeel.
Golaghat	Sankar, Nabeel, Goruchara, Galabeel, Moridisoi, Tinsuki – borbeel, Bortalikhosa.
Lakhimpur	Bilmukh, Morichampora.
Nagaon	Somrajan, Merr, Sibasthan, Samaguri, Lakhanobhanda, Satiyan, Dighalipalati, Brahmamaijan.
Marigaon	Charan, Mori, Bormonoha, Jaluguti, Kasodhara, Deora, Thekera, Udori,
Sonitpur	Dighali, Kharoi, Goroimari
Darramg	Mailhata, Bodhisichi, Gathia Batha, Roumari, Diplingamailata, Gathia.
Kamrup	Mailhata, Bageswari, Rangai, Dora, Selsela, Siligurijan, Deepar beel, Solmari.
Goalpara	Tamranga
Dhubri	Kalidanga, Hakama, Nandini, Harinchora, Barundanga, Bhoispuri, Jogra, Chandakhal.
Barpeta	Sagmara, Kapla, Chotokapla, Tabha, Alpajan.
Nalbari	Barbilla, Botuakamakhya, Ghograjan, Morasulkhowa.
Bongaigaon	Tamranga

Study Period:

From the month of January 2004 to December 2006.

3.1.2: Climatic Condition of the Study Area:

Among the study areas, Deepar Beel is studied in the district of Kamrup at 10 Km South West of Guwahati (Capital of Assam, India). Its area extends $26^0$03'26" to $26^0$09'26" N and $90^0$36'39" to $90^0$41'25"E in the South of mighty Brahmaputra. The beel has an actual perennial water holding area

of about 10.1 sq. km and the total area extends upto 40.1 sq. km during flood. The survey area covered by sixteen districts is belonging to the Brahmaputra valley. So the study areas are completely influenced by the climatic conditions of Brahmaputra valley.

The climate of Brahmaputra valley is highly fluctuating with moderate temperature, high humidity and trace, moderate and heavy showers of rains in addition to periodic wind, storm and thunders. Rainfall is highly seasonal in the area (Kalita and Sarma, 1986) and the high lands occurring here are sometimes subjected to severe drought (Kalita, 1992).

On the basis of variation in temperature, humidity and rainfall, the climate of Assam as well as Northeastern region is grouped in four seasons (Barthakur, 1986)

Premonsoon	:	March, April and May
Monsoon	:	June, July, August and September.
Retreating Monsoon	:	October and November
Winter	:	December, January and February.

Premonsoon (PM):

Premonsoon extends over the months of March, April and May. Clouds produces thunderstorm and also gives rise to intense precipitation in discontinuous and short spell in the form of shower as distinct from continuous rain in the monsoon. The thunder, lightening and low to moderate intense dust storms are also produced in this season.

Season's predominant characteristics:

- Retreat of winter
- Onset of pleasant morning, hot day and finally warm weather.
- Heavy rainfall for short duration.

Monsoon (MO)

Monsoon starts from the month of June and continues upto the month of September. The depression of pressure in the Bay of Bengal produces cyclic storm and circulates it anticlockwise by coriolis forces in the Northern Hemisphere. Heavy rainfall extends to the Southern and Southeastern part of the West Bengal and Assam, when the depression moves from the head of the Bay of Bengal (Kalita, 1992). The rain of the monsoon begins having wide intensity spectra from drizzle to heavy precipitation from few hours to several days. The high intensity of cyclonic storm and rainfall in the Brahmaputra Valley is the gift of Southwest monsoon.

Season's predominant characteristics:

- Rainfall moderate to high intensity.
- Hot highly humid and uncomfortable weather.
- Moderate to devastating flood.

Retreating monsoon (RM):

This season extends over the month of October and November. This is the transitional period between monsoon and winter. Season's predominant characteristics:

- Occasional rainfall.
- Accelerates the initiation of cold.
- Abundance of cirrus and nimbus clouds.
- Cheerful comfortable weather.

Winter (WN):

Winter continues December, January and February. The maximum cold occurs in the late December and early January. Due to dense fog, the sun does not appear in the morning hours and in the day also weak intensity of the Sunlight falls on the earth for which temperature decrease.

Season's predominant characteristics:

- Dominance of Cold.
- Abundance of fog in morning
- Temperature falls down.

Season covered during study period :

- For the collection of prawns all four season (premonsoon, monsoon, retreating monsoon and winter) were covered.
- The ecological and biological observation were conducted in there different seasons of the year, i.e.
- Premonsoon
- Monsoon
- Retreating monsoon.

3.2: Collection and identification of Prawns:

Prawns were collected from the different places of Assam from rivers, beels, swamps, ponds, paddy field, market places and from the fishermen's catch directly. Collections were done by hand picking, nylon net (16 nos. meshes /cm^2) and sometimes fishermen were employed directly in some sites for collection using various fishing gears.

Seventeen districts of Assam (Map No. 1) were taken as survey spot and visited seasonally (first, middle and last part of every season) and the community fishing sites were also examined during January 2004 to December 2006.

The collected samples were packed in airtight, polythene bags and then taken to the laboratory for preservation. They were washed and preserved in 7% formaline for identification.

Identification of prawns were done on the key to identification from Joychandran & Joseph, 1990. for key to identification, observation were made on Rostrum, Rostral Formula, Carapace, Compound eye, Antenna, Antennules, Chelate legs, Non chelate legs, pleopod, telson etc. which is mentioned below –

Key for the field identification:

The sample for the present studies were collected from the various water bodies of Assam and preserved in 8% formaline brought to the laboratory for future identification. The following are the methods of key for

the field identification of the various collected spices of freshwater *Macrobrachium* (Joychandran & Joseph - 1990).

(i) Carpus distinctly longer or shorter than merus.

(ii) Rostrum may be short, moderately long, very long and distinctly curyed upwards. Rostrum is uniformly toothed on upper margin with or without an elevated based crest. Rostrum with various rostrum with rostral formula was shown by the collected species of *Macrobrachium.*

(iii) Carapace smooth, with teeth arrangement dorsolaterally. Upper margine of rostrum with sub-distal and post orbital teeth.

(iv) Sensory antenna and antennual, slender 2nd chelate legs is strongly bult, Jriangular, cylindrical carpus, highly flattened to swollen palm, figer shorter than palm. Non chelate legs unidentical progressively increasing in length.

SYSTEMATIC POSITION

Kingdom - Animalia

Subkingdom - Eumetazoa

Phylum – Arthropoda

Class – Crustacea

Sub-class – Malacostracha

Division – Eucaridia

Order – Decapoda

Sub-order - Micrura

Family - Palaemonidae

Genus - *Macrobrachium* (Bate, 1868)

3.3: Ecology of *Macrobachium*

3.3.1: Physico-chemical Parameter of Water:

3.3.1.1: Collection and Preservation:

The study was conducted for the consecutive three years (January 2004 to December 2006). Collection of samples were made thrice, early, middle and late period of every season (Borthakur, 1986). Though all the parameters were repeated in three years but their seasonal mean for various parameters have been recorded with minimum and maximum values (Table 7.1 to 8.3)

Water samples from different habitat of prawns (River, beel, pond, paddy field and swamp) were collected in one litre sized pyrex bottles during 8 am to 10 am. Samples were brought to the laboratory within 2 to 3 hours and preserved at 4^0C in freeze prior to further analysis.

The investigation of parameters were done following the procedures outlined by Golterman et al. (1978), Triverdy and Goel (1986), Santhanam et al. (1989) and APHA. 1989.

3.3.1.2: Physical Characteristics of Water:

1. **Temperature:** Temperature was measured by reversible sensitive mercury thermometer at the time of sample collection. The atmospheric temperature was determined by exposing the thermometer in sunlight upto five minutes and the water temperature was be dipping the thermometer in water upto 0.5 meter depth and the unit expressed as degree centigrade (^{0}C)

2. **Transparency:** Transparency is another factor that affects growth and development of prawn species and indicates the amount of suspended particles and biotic community in water, which interferes with the passage of light into the water, hence it becomes limiting factor in the productivity of the pond. It is also an indicator of eutrophication (Jhingran, 1985) as the transparency of a water body is affected by the abundance of the biotic community particularly if there is any algal bloom.

However, 30 cm or less transparency due to suspended particles, eroded soil, silt etc. (Benerjee, 1967) may prevent growth of plankton in water while more than 70 cm indicates less productivity of water. Therefore, the low productivity of the water body affects the productivity of fish and prawn as the prawns are deprived of their natural food in such water body.

Transparency is the measurement of the light penetration capacity of a water body. Light penetration in the water body was determined by immerging the Secchi disc and observed visually until just disappear and reappears. The Secchi disc is 20 cm diameter alternatively painted black and white circular metal disc. Tied with a centimeter marked string. The measuring unit expressed as centimeter (cm)

Calculation:

$$\text{Transparency (cm)} = \frac{A+B}{2}$$

Where A = depth of disappearance

B = depth of reappearance.

Conductivity:

Conductivity of a solution is a measure on its capacity to convey an electric current. The systronic model – 304 digital conductivity meter was used to determine the conductivity of the solution. The instrument was calibrated in 0.01 M KCl according to the company instruction. The unit is expressed □mho/cm at 25^0C.

Calculation:

Conductivity (□mho) = observed conductance X cell constant X temperature factor at 25^0C

3.3.1.3: Chemical Characteristics of Water:

1. **P^H:** The hydrogen ions concentration of water i.e. pH is an important factor, which has different effects on the growth and development of fish and other aquatic organisms especially the fish food organisms. The pH of any water body, in general, undergoes diurnal change and become alkaline during the mid-day whereas the acidity increases at its peak at the daybreak.

   A change in the water surface temperature and water pH was related to change in volume of ovary, morphology and ganadosomatic index.

P^H also measures the intensity of acidity and alkalinity. The pH was measured at sampling by using digital stick meter. The standard buffer solution was used to adjust the instrument at pH 7.0

2. **Dissolved Oxygen (DO):** It is of paramount importance to all living organisms and it is considered to be the lone factor which reveals the nature of the whole aquatic system at a glance, even without the assessment of other Physico – Chemical biological conditions. The importance of dissolved oxygen concentration in prawn culture is well known. In a water body it is important to monitor in dissolved oxygen concentration by supplemental aeration for prawn culture if it becomes difficult naturally.

Dissolved oxygen was determined by Winkler's modified iodometric method. Manganous Sulphate react with alkali (KOH or NaOH) to form white precipitate of manganous hydroxide, in presence of oxygen it is oxidized to brown coloured compound. Manganese ions are reduced by iodide ions and converted into iodide equivalent to the oxygen concentration in the sample in strong acid medium. Using starch as indicator iodine can be titrated against sodium thiosulphate. The measuring unit is expressed as mg/L.

Calculation:

When whole content is titrated

DO_2 mg/L = ml X N of titrant X 8 X 1000 / V_1 – V

When a part of the content is titrated

$$DO_2 \text{ mg/L} = \text{ml X N of titrant X 8 X } \frac{1000}{V_2}\left(V_1 - \frac{V}{V_1}\right)$$

Where, V_1 = Volume of the sample bottle after placing stopper.

V_2 = Volume of the part of content titrated.

V = Volume of $MnSO_4$ and Kl

3. **Free Carbon Dioxide (FCO_2):** The concentration of free Carbon dioxide content of any water varies depending on various factors. There are different sources from where the free CO_2 can get into a natural water body. These sources may be the atmosphere through the respiration of aquatic living beings, microbial action on decomposition of organic matter, chemical composition of the soil and rocks or combination of Calcium and magnesium with other substances in the water body itself. In a pond, the carbon dioxide mainly increases by the respiration of aquatic organisms or from calcium – magnesium combination. In general, the amount of free CO_2 is negatively correlated with the presence of phytoplankton as it releases carbon dioxide at night. The presence of high amount of carbon dioxide is also related to low temperature on a cloudy day.

The free carbon dioxide of water plays an important role in the life of any aquatic organism especially in prawns. Free CO_2 in a water body should be minimum () as it has a toxic affect on living beings

(Jhingaram 1985). The action of carbon dioxide upon prawn is similar to that of effect of it upon other animals as it consists of the reduction of the dissolved oxygen absorption capacity of blood.

F. CO_2 reacts with sodium carbonated or sodium hydroxide to form sodium bicarbonate. The development of pink colour of phenolphthalein indicator indicated the completion of the reaction at near to the pH 8.3. Measuring unit for FCO_2 is used as mg/L

Calculation:

FCO_2 mg/L = mL X N of titrant X 1000 X 44/V

Where N = normality of NaOH

V = Volume of sample.

4. **Total Alkalinity (TA as $CaCO_3$):** The principle based on hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes reacts with addition of standard acid, it depends on the end point of pH used. The amount of CO_2 + $OH.HCO_3$ is equivalence pH 4.2 – 5.4. the total alkalinity is measured as mg/L

Calculation:

Phenolphlthalein alkalinity (PA) as $CaCO_3$ mg/L = A X N of HCl X 1000 X 50/V

Total alkalinity (TA) as $CaCO_3$ mg/L = B X N of HCl X 1000 X 50/V

Where

A = ml. of HCl used with only Phenolphlthalein

B = ml. of total HCl used with Phenolphlthalein and methyl.

N = Normality of HCl used.

V = Volume of sample used.

5. **Hardness (as $CaCO_3$):** The calcium and magnesium ions of the sample are titrated with ethylene diamine tetra acetic acid disodium salt (EDTA) to form a stable CaEDTA and MgEDTA, calcium and magnesium form a complex of wine red colour with Erichrome black T at pH of 10.00 ± 0.1. Due to strong affinity of EDTA towards Ca^{++} and Mg^{++}, more addition of EDTA formed new complex of blue colour by breaking former complex. The measuring unit of hardness is expressed as mg/L

   Hardness mg/L (as $CaCO_3$) = ml of EDTA used X ml of sample.

6. **Chloride:** Silver nitrate reacts with chloride to form very slightly soluble white precipitate of AgCl. At the end of the point when all the chlorides get precipitated, free silver ions react with chromate to form silver chromate of reddish brown colour. The unit used for the measuring of chloride is mg/L.

   Calculation:

   Chloride mg/L = (ml X N) of $AgNO_3$ X 1000 X 35.5 / ml sample.

7. **Nitrate:** Nitrate in alkaline ammonium chloride solution quantitatively reduced to nitrate on treating the sample in a cadmium column. Then the resulting nitrate is determined by forming the pink azo dye. The

ammonium chloride serves both as complexant and buffer. The unit for the measurement is expressed as mg/L.

Calculation:

NO_3^- mg/L = $F/E_1 - EB_1$

The concentration of NO_2^-N in sample = $F \times E_1 - (E_0 + EB_1)$

Where,

EB_1 = absorbance of diluted water + reagent

E_o = absorbance of sample without NEDD reagent

E_1 = Absorbance of standard or sample with reagent.

F = Standard concentration (□ $NO_2 - N/L$)

8. **Phosphate (PO_4^{--}):** The filtered water sample is allowed to react first with a mixed reagent of molybdate, ascorbic acid and tetravalent antimony. The molybdate acids formed are converted by the reducing agents to a blue coloured complex. The unit termed for measurement of PO_4^- is mg/L.

Calculation:

PO_4^- mg/L = $F/E_1 - EB_1$

□g $PO_4^-/L = F (E_1 - E_o + EB_1)$

Where

E_o = absorbance of sample without reductant

E_1 = absorbance of sample or standard with reductant.

EB_1 = absorbance of diluted water and reagent

F = a unit centinction factor.

Spectrophotometer procedure was followed to investigate the PO_4^- level outlined by Santhanam at el. (1989).

3.3.2: Physico – Chemical Analysis of Soil:

3.3.2.1: Collection and Preservation of Samples:

The soil of beel, paddy field, swamp, pond and river were collected with the help of bamboo stick [5cm (diameter) x 200cm (length)]. Three replicants of samples were lifted from each sampling station, well mixed and 100 – 200 gm samples were packed in polythene packet and brought to the laboratory for further analysis. (Table....)

The homogeneous sample solution was made by mixing fresh soil and distilled water at 1:5 ratio respectively and the pH was determined by using digital stick pH meter at sampling station. The samples for organic carbon, organic matter, NO_3, SO_4^-, exchangeable calcium and exchangeable magnesium were brought to the laboratory. Samples dried for some experimental procedures were followed after Trivedy and Goel (1986), Santhanam et al (1989)

Parameters:

1. **Organic Carbon:** Potasium dichromate reacts with sample carbon and phosphoric acid and gives precipitate, in addition of excess sulphuric acid it gets dissolved. Diphemylamine is used as an indicator, which indicates

change of colour in the reaction with ferrous ammonium sulphate. The unit for the measurement of organic carbon is express as %

Calculation:

Carbon is simple (%) = 3.951/g X (1 – T/S)

Where g = Volume of sample (ml)

T = Volume of $Fe(NH_4)_2SO_4$ solution used for titration

S = Volume of $Fe(NH_4)_2SO_4$, solution used for blank titration.

% organic matter = % organic carbon X 1.724.

2. **Nitrate (NO_3^-):** The nitrate is quantitatively reduced to nitrate in alkaline ammonium chloride solution by treating the sample in a cadmium colum. The resulting nitrate is then determined by following the pink azo dye. Nitrate was measured in spectrophotometer (spectronic 20D Roy and Milton Model) and unit expressed as mg/100 gm of soil.

Calculation:

$NO_2^- = F/E_1 - E_{B1}$

The concentration of NO2 – N in sample = F X $E_1 - (E_o + E_{B1})$

Where

E_{B1} – absorbance of distilled water and reagent

E_o = Absorbance of sample without NEDD reagent

(measured on visible brownish colour)

E_1 = Absorbance of standard or sample with reagent.

F = Standard concentration (mg NO_2 – N/L).

NO_3^- = FXV/1000 X W

Where,

F = NO_3^- determined in filtrate (mg/L)

V = Total volume of filtrate (ml)

W = Weight of dried sediment.

If the concentration of NO_3^- N + NO_2 – N = X and concentration of NO_2 – N = Y, then NO_3 – N = X – Y.

3. **Sulphate:** Sulphate ion is precipitated in the form of barium sulphate by adding barium chloride in hydrochloric acid medium. The accumulation of sulphate is determined from barium sulphate by comparing with standard curve (Trivedy and Goel, 1986) SO_4^- content is measured in spectrophotometer (spectronic 20D of Roy and Milton Model) and unit expressed as mg/100gm of soil.

Exchangeable Calcium (E Ca^{++}) and Exchangeable Magnesium (E Mg^{++}):

Cation present in the exchangeable complex of the soil can be removed by leaching in the soil with ammonium acetate solution. Different exchangeable cations are then estimated separately in this ammonium acetate leachate. The unit for the measurement of ECa^{++} and EMg^{++} are expressed as mg/100 gm of soil.

Calculation:

Ca^{++} mg/100gm = A X 400.8 X V/v X 10 X S

Mg^{++} mg/100gm = (B – A) X 400.8 X V/v X 1.645 X 120 X S

Where,

A = Volume of EDTA used for Ca^{++} determination (ml)

B = Volume of EDTA used for Ca^{++} + Mg^{++} determination (ml)

V = Total volume of soil extract prepared (in the case of 500 ml)

V = Volume of soil extract titrated,

3.4: Biochemical (Nutritional) Qualities Estimation

Biochemical (Nutritional) qualities of different *Macrobrachium* species were carried out along with some elements during the study period (January to December). Within the three years (ten) experiments for each parameter were done and their minimum and maximum values are recoded and presented in a tabular form (Table-6)

Biochemical estimations were carried out from edible muscle parts of each *Macrobrachium* species. But in case of study on carotenoid the study is done both in muscles and carapace and carotenoids have some impact on the body colouration of the species.

3.4.1. - Determination of carotenoid:

To estimate the quantitative analysis of carotenoid pigment of these four types of *Macrobrachium* species, the extract is studied with help of **Spectrophotometer (Model SPD MIOAVP)** and the optical density (OD)

value was obtained. The study was carried out both in muscles and carapace and the result is expressed in μg. The extract was analyzed by **HPLC** and different fractions were identified on the peak showing the chromatogram and their corresponding UV visible spectra.

Calculation

$$\frac{\text{OD x Total volume}}{E^{\varnothing} \text{ x } 100} \times 10^6 \ \mu g$$

$E^{\varnothing}$ = molar extrinction co-efficient.

3.4.2. - Determination of Ash:

Ash content was determined after ingintion of the sample at 550-600^{0}c in an oven for about four hours, as per method described by Ravindranath (1981) and Silas (1982)

Calculation :

$$\text{Ash Content (\%)} = \frac{weiofash}{wightofdrysample} \times 100$$

3.4.3. - Estimation of Dry Matter:

Dry matter was estimated after grinding about 100gm of the samples so that it passes through AIS sieve 100 (aperture 1000mm). Now the prepared sample is transferred to a well –stoppered glass bottle

Calculation :

$$\text{Moisture percent by weight} = \frac{w_1 - w_2}{w_1 - w} \times 100$$

Where

W_1 = Weight in gm of the dish with materials before drying

W_2 = Weight in gm of the dish with the pried materials.

and W= Weight in gm of the empty dish % of DM = 100 (% of Moisture)

3.4.4. - Determination of crude Protein

(Micro-Kjeldahl Method)

Total protein values were obtained by following Micro-Kjeldahl Method described by Ravindranath (1981) and Silas (1982). Organic nitrogen when digested with concentrated H_2So_4 in the presence of a calalyst – (Selenium oxide or potassium sulphate and copper sulphate) is converted into ammonium sulphate, Ammonia liberated by making the solution alkaline is distilled into a known volume of standard acid, which is then back titrated. The protein content is obtained by multiplying the nitrogen value with 6.25

Calculation :

$$\% \text{ of Nitrogen} = \frac{\text{Titre Value x } 0.14008 \text{ x } 100}{\text{Amount of Sample}}$$

Protein content (%) = % of Nitrogen x 6.25

3.4.5. - Determination of Crude Fibre

Crude fibre content was estimated using Anthone method described by Rabindranath (1981)

$$\text{Crude Fibre (\%)} \frac{\text{O.D. of the unknown}}{\text{O.D. of the standard}} \times \text{Conc.of standard} \times \text{dilution factor} \times 100$$

3.4.6. - Crude fat :

Crude fat content of various *Macrobroachium* were analysed following Blighat and Dyer method described by Silas (1982). The reagent used for this experiment was chaloroform extract so obtained is corresponding to total fat (%) of fresh matter.

3.4.7. - Determination of calcium :

The amount of calcium can be estimated when calcium containing solution is treated with ammonium oxalate, all the calcium present is prescipitated as

calcium oxalate. The precipitate when treated with sulphuric acid, dissolves, forming calcium sulphate liberating free oxatic acid which is quantitatively estimated by titration against standard $^{N}/_{10}$ potassium permanganate solution to arrive at the calcium content present in the given solution.

Calculation :

1ml of $^{N}/_{10}$ KM_n0_4 = 0.002g calcium

$$\% \text{ of Ca} = \frac{1\text{ml of } {}^{N}/_{10}\, KMn0_4 = 0.002 \times \text{volume of sol. Ash made}}{\text{volume of aliquot taken x weight of sample taken for ashing}} \times 100$$

3.4.8. - Estimation of Phosphorus:

Phosphorus is precipitated as yellow precipitate of ammonium phosphomolybdate by adding ammonium molybdate solution and concentrated $HN0_3$. The precipitate is washed till free from acid and dissolved in a measured volume of $^{N}/_{10}$ NaOH. The excess alkali is back titrated against n/10 HCL to arrive at the exact quantity of N/10 NaOH required.

Calculation :

Volume of N/10 NaOH used up = (ml of N/10 NaOH taken -ml of N/10 HCL)

1ml of N/10 NaOH= 0.000135 g Phosphorus.

$$\% \text{ of Phosphorus} = \frac{\text{Ml of N/10 NaOH used x 0.000135 x vol. of soluble ash made}}{\text{Volume of aliquot taken x weight of sample taken for ashing}} \times 100$$