

Consolidated Progress Report [till March 2016]

Section-A: Project Details

A1. Project Title: Centre of Excellence in Fisheries and Aquaculture Biotechnology

Under this FAB-CoE following are the lead project and two subprojects:

Lead-Project:

Inventorization and cataloging of fish diversity of north-eastern region based on classical and molecular tools.

Sub-Projects:

- 1. Development of breeding protocol and intensified culture of minor carps for aquaculture production enhancement in NE region through diversified species culture systems**
- 2. Standardization of culture and captive breeding protocol of selected indigenous ornamental fish species of NE region of India and attribute enhancement: (*Danio rerio*, *Puntius spp.* *Botia dario*)**

A2. DBT Sanction Order No.: DBT-NER/LIVE/05/2011 Date: 25/09/2012

A3. Name of Team Leader: Dr. Pramod Kumar Pandey, Dean, College of Fisheries, CAU(I)
Consultant: Prof. J. R. Dhanze, Former Dean College of Fisheries, CAU(I)

Name of Investigators:

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Co-ordinator, Mizoram
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A6. Total Cost: 1073.302 Lakhs

A7. Duration: Five years

A8. Approved Objectives of the Project:

Under these focal projects the FAB-CoE, the following are composite objectives:

1. The main focus of CoE will be on improving the yield of fish production system in the entire NER and raising the livelihood of communities involved in fish farming
2. Inventorisation of freshwater fish diversity of NER of India and characterization by molecular tools (DNA Bar coding)
3. Development of protocol for breeding, seed production and grow-out farming of economically-important endemic species of food and ornamental value for diversified farming
4. Focus the research on feed development, with particular reference to larval feed and their management and use of locally available ingredients
5. Fish-health including trans-boundary disease and parasites
6. Post-harvest processing including fermentation and risks analysis on the cross-border movement of processed products
7. To pursue fish resource management education and capacity building of stakeholders and fish farming communities
8. To develop linkages with state plan and programmes
9. To develop mechanism of transfer and dissemination of technologies.

Executive summary

Title of the project: Centre of Excellence on Fisheries and Aquaculture Biotechnology (FAB-CoE) (Funding agency – DBT, Total budget Rs. 1073.302 lakhs)

Name of the PI/Scientist Dr. Pramod Kumar Pandey, Dean, College of Fisheries, Lembucherra

Lead project: Inventorization and cataloging of fish diversity of north-eastern region based on classical and molecular tools

Sub-Projects:

1. Development of breeding protocol and intensified culture of minor carps for aquaculture production enhancement in NE region through diversified species culture systems
2. Standardization of culture and captive breeding protocol of selected indigenous ornamental fish species of NE region of India and attribute enhancement: (*Trichogaster* spp., *Esomus danricus*, *Botia dario*)

Objectives of the Project:

- To enhance the yield of the fish production system in the entire NER and thereby improving the livelihood of communities involved in fish farming.
- Inventorization of fish diversity of NE region and characterisation by molecular tools (DNA bar coding).
- To identify the reasons for low productivity of the fish production in NE, component wise, species wise and eco-regime wise and propose solutions and mitigation strategy.
- Development of protocol for breeding, seed production and grow-out farming of economically-important endemic fish species of food and ornamental value for diversified farming.
- To focus the research on feed development, with particular reference to larval feed, management, and use of locally available ingredients.
- Capacity building through development of effective linkage with State plan and programme and dissemination of proven technologies to the stake holders.
- Study fish health including trans-boundary disease and parasites.

Summary of the Progress made

1. Inventorization and Cataloging of Fish Diversity Based on Classical and Molecular Tools

1.1. Species Inventorization

- In Assam 10 habitat systems were surveyed and more than 983 fish samples were collected under 9 Order, 26 Family, 63 Genus and 138 Species.

- In Arunachal so far 1286 fish specimens belonging to 7 Order, 18 Family, 56 Genus and 123 species were collected.
- In Manipur 9 habitat systems were surveyed and more than 1225 Fish samples collected belonging to 6 Order, 20 Family, 64 Genus and 103 Species.
- In Meghalaya 30 habitat systems were surveyed and more than 706 Fish samples were collected belonging to 9 Order, 25 Family, 59 Genus and 102 Species.
- In Mizoram 18 habitat systems were surveyed and more than 906 Fish samples collected belonging to 6 Order, 16 Family, 36 Genus and 74 Species.
- In Nagaland 2 habitat systems were surveyed and more than 230 Fish samples collected belonging to 4 Order, 9 Family, 15 Genus and 21 Species.
- In Sikkim so far 3 Order, 4 Family, 12 Genus and 22 Species were collected.
- In Tripura 12 habitat systems were surveyed and more than 5753 Fish samples collected belonging to 11 Order, 31 Family, 57 Genus and 97 Species.
- A total of more than 86 aquatic ecosystems belonging to eight northeastern states have been extensively surveyed
- A total of 269 fish species belonging to 13 order, 34 families and 78 genus have been collected, identified and their topotype catalogued in the museum
- During the year 2015- 2016 the Morphometric and Meristic data comprising of 59 characters have been mensurated from 1650 specimens belonging to 77 species under 21 families for further statistically analysis to confirm the specific identity of each species.

1.2. Bar Coding of Fish Fauna of NE Region

- A total of 158 species have been bar-coded from all over the North Eastern states.
- DNA barcode of 96 fish species (51 from Mizoram, 40 from Meghalaya, 21 from Manipur and 10 from Nagaland) have been generated and COI sequences were submitted in NCBI GenBank Database. DNA barcode of nine species namely, *Pillaia indica* collected from Ganol/Kalu river, Meghalaya, *Channa ornatipinnis*, *Parambassis vollmeri* and *Garra naganensis* collected from Kaladan and Tlwang River, Mizoram, *Akysis manipurensis*, *Lepidocephlichthys berdmorei* and *Mystus cineraceus* from Khujai lake, Chakpi and Lokchao River in Manipur and *Schistura nagaensis* & *Paracanthocobitis adelaideae* collected from Chate and Tizu river of Nagaland, were generated for the first time.

2. Fish-Health including Trans-boundary Disease and Parasites

- In 2014-15, A tape worm (*Ligula* sp.) in *Schizothorax richardsonii* in river ecosystem was noticed at Rangpo, Sikkim
- In Tripura,
 - EUS in IMCs in three fish farm at Dhalai, Sepahijila districts

- *Aeromonas hydrophila* infection in IMCs, common carp and silver barb from five farms located in districts of West Tripura, Gomati and Sepahijala observed.
- Myxobolus and Dactylogyrus infection in IMCs from five farms in districts of Gomati, South Tripura and West Tripura
- *Saprolegnia parasitica* infestation in IMCs, Silver carp, Common carp, Silver barb, pabda, reba in farms at College of fisheries and Gomati district.
- A specimen of *Heteropneustes fossilis* from natural stock collected from Assam was diagnosed to be suffering from scoliosis.
- *Systemus sarana* collected from lower stream of Teesta river in Sikkim was infected with Epizootic ulcerative syndrome (EUS).
- Fish diseases were also screened from different culture systems viz., parasitic diseases like *Dactylogyrus* sp., *Myxobolus* sp., *Ergasilus* sp., *Lernaea* sp., bacterial diseases like Motile *Aeromonas* Septicemia (MAS), fungal infection like *Saprolegnia parasitica* (Cotton wool Disease) & *Aphanomyces invadans* infection (EUS).
- In Dec. 2016, Eye fluke in the chocolate mahseer and Gill haemorrhages in common carp at Wakha district of Nagaland were recorded.

3. Sub-project 1A: Development of breeding protocol and optimization of stocking density during seed nursing and rearing of Minor carps

3.1. Collection of prioritized species:

- Live juvenile and adult specimens of *Cirrhinus reba* from natural habitat of Tripura and Assam.
- Live fingerlings of *Osteobrama belangeri* were collected from Manipur.
- Rearing for maturity in earthen ponds at College of Fisheries, Lembucherra.

3.2. Seed Production of *Cirrhinus reba*

- Induced with ovatide @0.4 (Male) – 0.5 (Female) ml / kg body wt.
- Average fertilization rate of 86% and average hatching rate of 83%.
- Average survival from spawn to fry was 13.6% (2014-15) at stocking density of 3-3.5 million ha⁻¹ but 55.8±0.81% during 2015-16.
- Average survival of rearing of fry to fingerling was 26 % (2014-15) and 88.3±2.68% in the year 2015-16 @ 1.0 lakh/ha for 45 days.

3.3. Seed Production of *Osetobrama belangeri*

- Induced with ovatide @0.4(Male) – 0.5 (Female) ml / kg body wt.
- Average fertilization rate of 90% and hatching rate of 86%.
- Survival from spawn to fry @70.67±13.00 was achieved with stocking density of 3 mill/ha of spawns.

- Survival during rearing of fry to fingerling was $87.28 \pm 4.88\%$ was achieved when fry were stocked @ 1.0 lakh/ha for 45 days.

3.4. Optimization of larval and fry stocking density on growth performance of butter catfish, *Ompok bimaculatus* (Bloch, 1794)

- The stocking density of 10 larvae l^{-1} was observed to be appropriate for larval rearing of *Ompok bimaculatus* with 4 times feeding frequencies.
- The stocking density @ 100 fry m^{-3} is found to be optimum and quite profitable for fingerling production in cages installed in ponds.

4. Sub-project 1B: Fish Feed Development using Local Ingredients

4.1. Screening of potential local non-conventional ingredients

- Proximate composition of local unconventional potential feed ingredients including jackfruit (whole, flesh and seed), papaya (peel, raw, ripe), taro corms, *Wolffia arrhiza*, and tapioca were formulated.

4.2. Evaluation of *Wolffia arrhiza* meal as a local replacement of dietary mustard oil cake and corn starch in supplementary carp feed

- *Wolffia arrhiza* was grown at College of Fisheries and utilized to replace mustard oil cake and corn starch in supplementary carp feed and evaluated in polyculture of Indian Major Carps (catla: rohu: mrigal:: 3:4:3 @ 15,000 ha^{-1}).
- Replacement levels of mustard oil cake and corn starch with wolffia meal were 25%, 50% & 100% on crude protein and starch basis, respectively.
- No significant difference in net fish yield/net gain in overall fish biomass, feed utilization and proximate composition of fishes were evident.
- Remarkable reduction of about 15- 30% in feed cost of production was achieved (partial budgeting).
- Complete replacement of both mustard oil cake and corn starch is possible in carp feed without any reduction in fish yield

4.3. Evaluation of unprocessed and processed taro corms as local replacement of corn starch in carp feeds

- The unprocessed (simple dried) and processed (boiled, extruded and soaked) corms of a wild variety of taro were evaluated as local starch source to replace dietary corn starch for Amur common carp (*Cyprinus carpio*).
- Compared to unprocessed corms, the total oxalate contents (mg/100g DM) was reduced by 20% (extrusion)-50% (soaking).
- Unprocessed taro corms lead to remarkably inferior growth, feed utilization and health status.
- Extruded and soaked taro corms led to significantly higher growth than that with control diet.

4.4. Evaluation of *Wolffia arrhiza* meal as a local replacement of dietary fish meal and wheat flour in supplementary feed for pengba (*Osteobrama belangeri*)

- Farm grown *Wolffia arrhiza* grown at farm was utilized to replace fish meal (at inclusion level of 20%) and wheat meal (at 43%), simultaneously.
- Replacement levels of 0% (control), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) on crude protein and starch basis in a half-year experiment were employed.
- 100% replacement of both fish meal and wheat starch with wolffia meal led to significantly higher growth performance than that with control.
- The apparent feed utilization parameters showed markedly improved values for treatments wherein 50-100% replacement levels were employed.
- Higher gumminess, chewiness, lower adhesiveness, and higher whiteness index for flesh were obtained with 50-100% replacement levels of fish meal and wheat meal.
- The highest sensory score was obtained for pengba grown at 50% replacement of fish meal and wheat meal with wolffia meal.
- The feed cost of fish production was reduced upto 35% with 100% replacement levels.

4.5. Evaluation of optimum dietary protein requirement of *Cirrhinus reba*

- The dietary requirements of *Cirrhinus reba* was evaluated using semi-purified diet under wet laboratory conditions.
- The employed dietary crude protein levels were 20%, 25%, 30%, 35%, and 40%.
- Both mean weight gain and feed utilization was the highest with 30% crude protein diet.

4.6. Evaluation of fresh *Wolffia arrhiza* as live feed for fry rearing of *Osteobrama belangeri*

- Fresh *Wolffia arrhiza* was evaluated as a sole live food for 14 days old pengba fry against semi-purified feeds of crude protein contents ranging 20-40%. Under laboratory conditions pengba fry exhibited 8-10 fold higher mean weight and 2 fold higher SGR compared to those with artificial diets, respectively.

5. Sub-project 2: Standardization of culture and captive breeding of selected indigenous ornamental fish species of NE Region of India and attribute enhancement

5.1. Screening of different feed ingredients for carotenoid content

- Screening of different feed ingredient namely apple, beetroot, brinjal, marigold flower yellow and orange, pomegranate, pumpkin, spirulina, tomato and wolffia were undertaken for carotenoid contents.
- Spirulina and marigold orange show a significantly higher content of carotenoid in the extraction using Diethyl ether solvent,
- Spirulina and wolffia show a significantly higher content of carotenoid in DMSO and methanol solvent extraction

5.2. Effect of dietary *Wolffia arrhiza* and *Spirulina platensis* on the growth performance and pigmentation of *Botia dario* and *Trichogaster fasciata*

- The final carotenoids content in skin of male and female *Botia dario* showed an increasing trend with increasing dietary wolffia percentage level.
- *Wolffia* is more suitable for colour enhancement of *Trichogaster fasciata* as the skin colour of the fishes are gradually enhanced with the increasing level of wolffia in the feed incorporated in the diet irrespective of the dosage of spirulina in the feed. In addition, the skin colour of female fishes was greatly enhanced as compared to control diet.

5.3. Breeding of Indian Flying Barb *Esomus danricus* (Hamilton, 1822) in Aquarium

- *Esomus danricus* were collected from the nearby water bodies and acclimatized in captive conditions.
- Fully matured three each of male and female breeders were released in another aquarium of 150 l capacity provided with pebbles at the bottom for protection of eggs and hiding spaces for newly hatched larvae.
- Successful breeding trial on *Esomus danricus* indicated that the fecundity was found about 145 numbers of eggs per 0.7 ± 0.21 g.
- Hatching percentage was about 53% and larval survival percentage found to be lower i.e. 31 %.

5.4. Induced Breeding of Asian Striped Dwarf Catfish, *Mystus vittatus* (Bloch, 1794) in Captivity

- *Mystus vittatus* were collected from their natural environment reared at the college for attaining maturity.
- Matured male and females were injected with ovotide and released in aquarium half of which was covered with thermocol sheets and few aquatic plants like hydrilla were put inside the tank.
- Spawning was observed after 12 hours of injection with adhesive eggs.
- Newly born hatchlings were fed with infusoria, sieved plankton and chopped tubifex once yolk sac was exhausted in 3 days.

6. Capacity building programmes

- Two days Awareness cum Training Programme on “**Fisheries Resource Management and Aquaculture Production Enhancement in Mizoram**” was conducted on 23rd to 24th November, 2015 organised by College of Fisheries, Central Agricultural University, Imphal in collaboration with Department of Fisheries, Govt. of Mizoram and College of Veterinary Sciences and Animal Husbandry (CAU), Aizawl, Mizoram. A total of 35 fish farmers and 5 extension functionaries from Department of Fisheries, Government of Mizoram participated in the programme.

- Two days workshop on **“Fisheries Resource Management and Aquaculture Production Enhancement in Assam”** jointly organized by College of Fisheries, Central Agricultural University, Imphal & Directorate of Fisheries, Assam was conducted on 21-22 August, 2015 at the training hall of North Eastern Regional Centre of CIFRI, Housefed Complex, Guwahati. 61 progressive farmers in addition to fisheries officials attended the training.
- Two days awareness cum training programme on **“ Fisheries resource Management and Aquaculture Production enhancement in Meghalaya”** jointly organized by College of Fisheries, Central Agricultural University, Imphal & Directorate of Fisheries, Meghalaya was conducted at College of Post Graduate Studies, Meghalaya (14-15th July 2015). 55 progressive farmers and officials from the 11 districts attended the training.
- Two days workshop on **“Fisheries Resource Management & Aquaculture production Enhancement in Sikkim”** jointly organized by College of Fisheries, Central Agricultural University, Imphal & Directorate of Fisheries, Sikkim was held at Dept. of Fisheries, Sikkim on 11th-12th June 2015. A total of 51 farmers attended the workshop.
- Awareness cum training on **“Fisheries Resource Management & Enhancement of Aquaculture production in Tripura”** in College of Fisheries, Lembucherra (CAU-I) in collaboration with Dept. of Fisheries Tripura on 20th to 21st May 2015 which was attended by 41 progressive farmers.
- Two days awareness cum training Programme on **“Fisheries Resource Management and Aquaculture Production Enhancement in Manipur”** was conducted on 17-18 May 2015 in collaboration with Dept. of Fisheries, Govt. of Manipur in Training hall of the Department of Fisheries, Government of Manipur, Lamphelpat, Manipur. Around 70 farmers attended the workshop.
- One day workshop on **“Fish biodiversity in north-eastern region with special reference to Tripura”** on 9th February, 2015. A total of 20 line department officials and field staffs participated. The participants were sensitized about needs and measures to conserve the biodiversity.
- Five-days training programme on **“Collection, preservation, cataloguing, preliminary identification and application of molecular tools for confirmation of systematic identity”** during 27-31 March, 2014. A total of 21 farmers and field level staffs participated. Hands on training on these techniques in the field of fish inventorization were provided.
- Four days training on **“Fish germplasm collection preservation cataloguing, preliminary identification and application of molecular tools for confirmation of systematic identity”** during 4-7, November, 2013. A total of 18 field level workers were imparted hands on training on techniques.

- Five-days training programme on **“Fish germplasm collection, preservation, cataloguing, preliminary identification and application of molecular tools for confirmation of systematic identity”** during 08-12 March, 2013. A total of 23 farmers and field level staffs participated. Hands on training on these techniques in the field of fish inventorization were provided

7. Inventorization of Indigenous Technical Knowledge (ITK) in fisheries in North East India

A total of 35 ITKs in fisheries and allied field have also been recorded from the eight states of north east.

Sampling sites

Map of the entire riverine system of the north-eastern part of India was drawn. Study areas included different River systems and its various primary, secondary, tertiary tributaries and wetlands in the eight states of the region. Maps of the study areas, showing locations surveyed and sampled during the study period, are given in Figure 1-9. Surveys and sampling were conducted giving due representations to pre-monsoon, monsoon and post-monsoon seasons. Habitat diversity was given prime importance in the selection of locations within the river system.

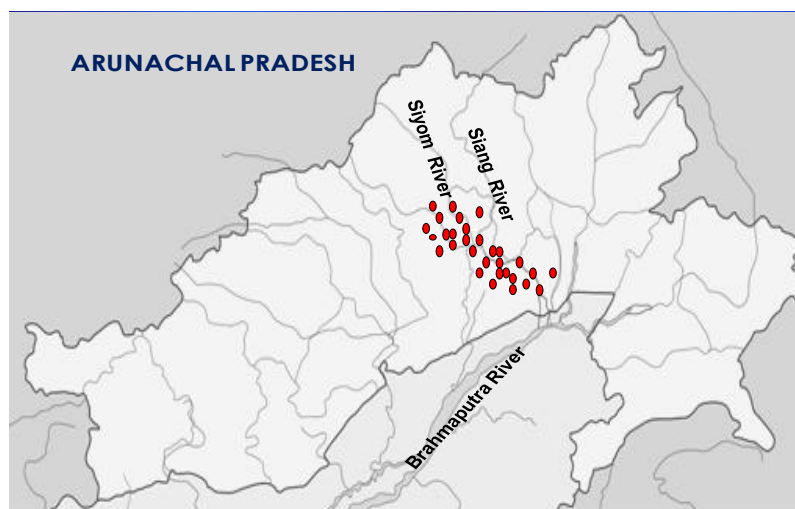


Fig. 2: Study locations of Arunachal Pradesh

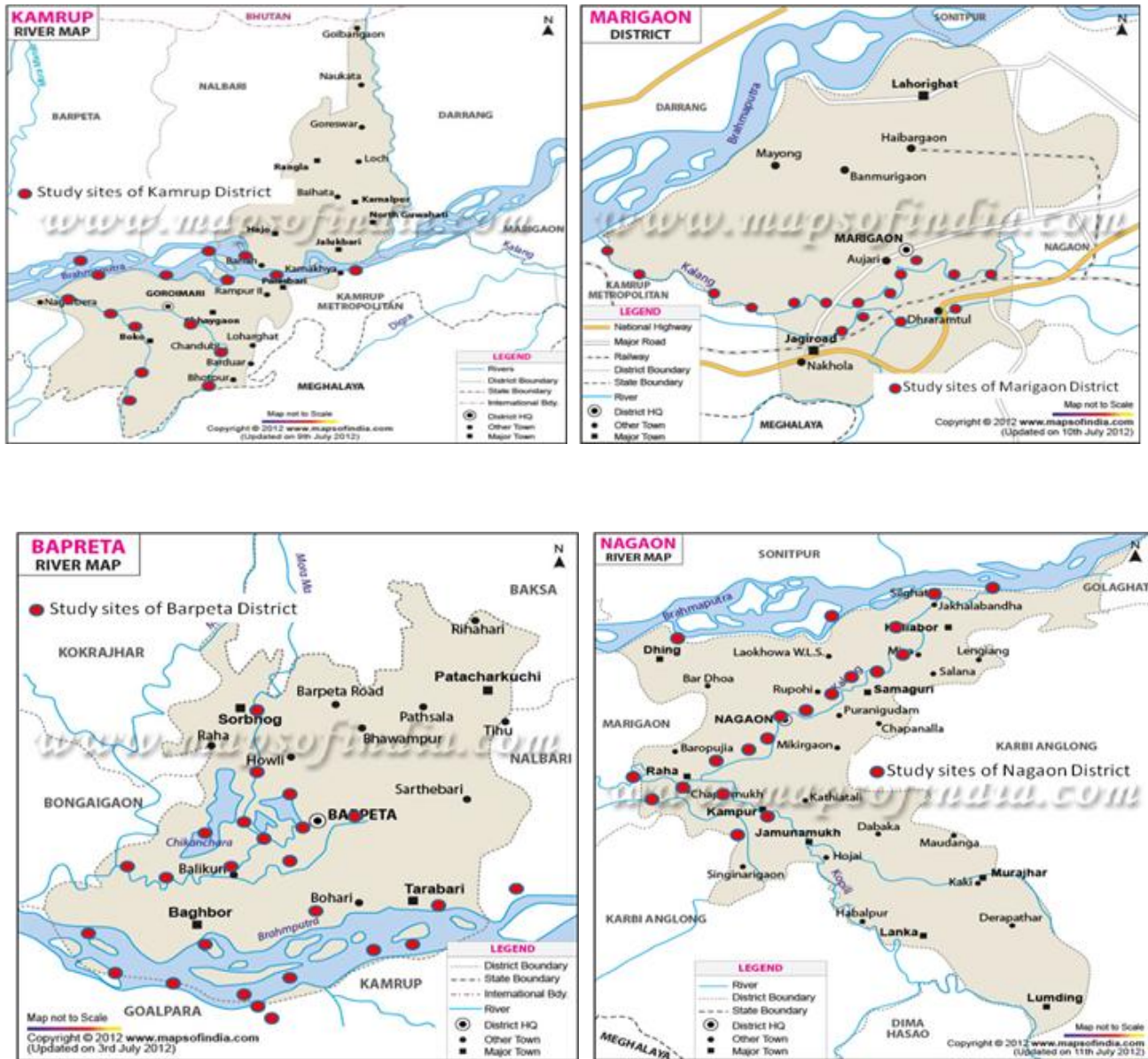


Fig. 3: Study locations of Assam

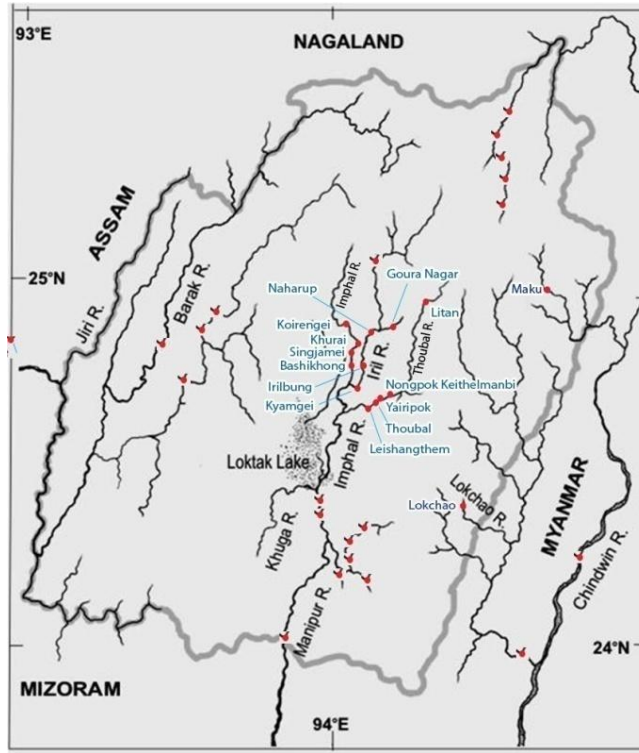


Fig. 4: Map of study sites in Manipur

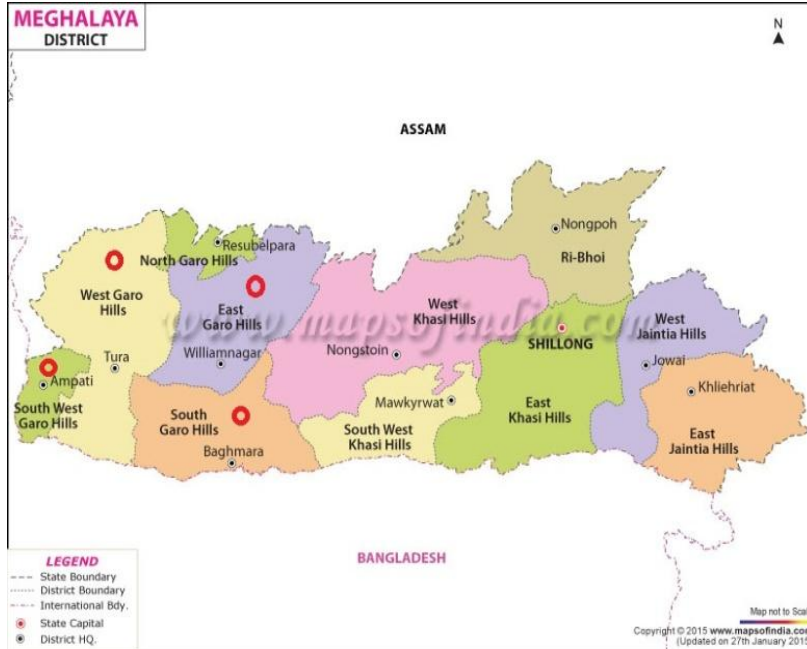


Fig. 5: Study locations of Meghalaya



Fig. 6: Study locations of Mizoram

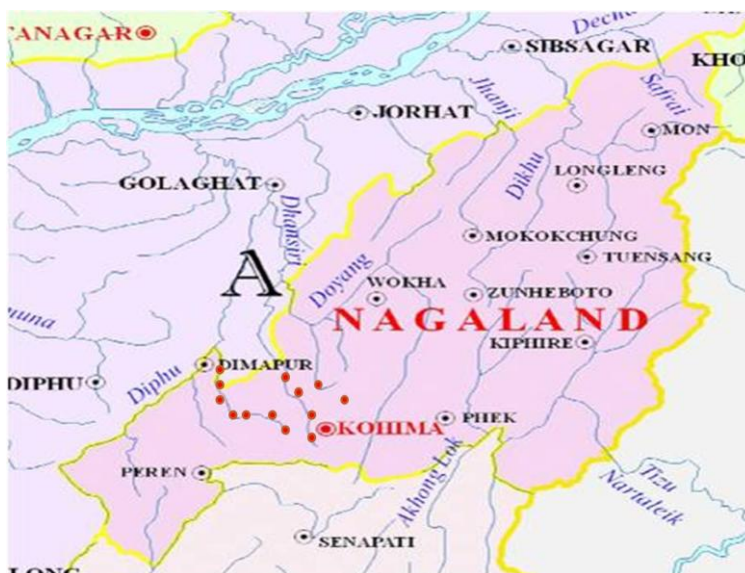


Fig. 7: Study locations of Nagaland

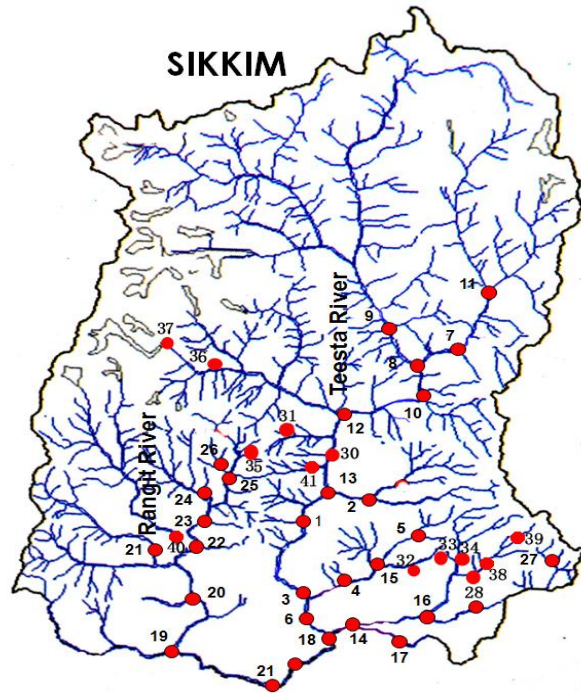


Fig. 8: Study locations of Sikkim

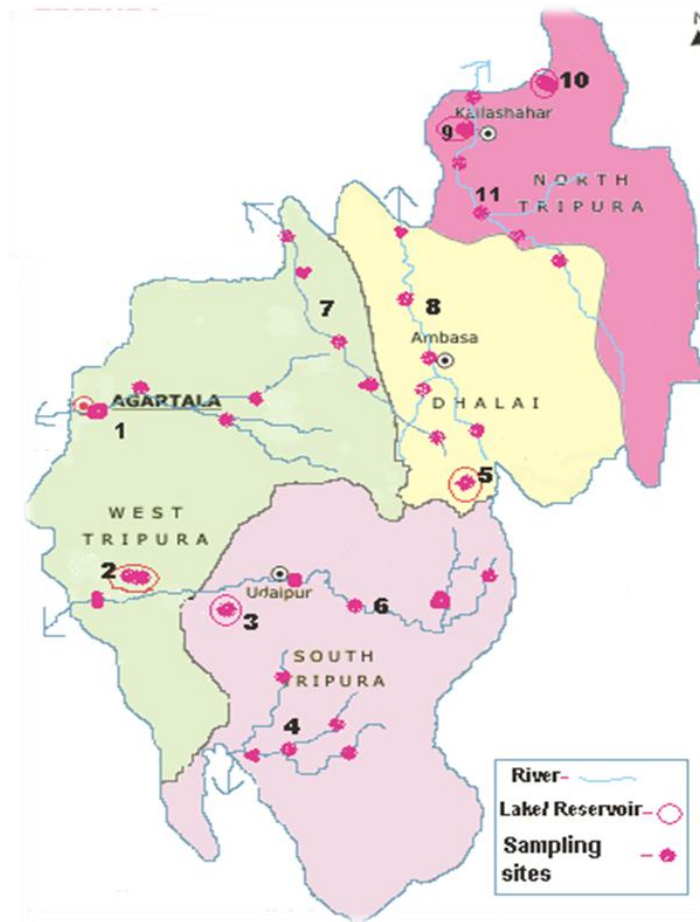


Fig. 9: Study locations of Tripura