CHAPTER 2
MATERIALS AND METHODS

For the taxonomic study, a large number of specimens covering both the sexes are collected from different lotic and lentic water bodies of the North Eastern Region. In Assam the collecting centers are Garjan beel, Hajo, 7.4-13.1 cm (11 exs); Deepar beel, Guwahati, 6.2 – 15.5 cm (14 exs); Haribhanga beel, Nowgoan, 9.9 – 14.8 cm (35 exs); Zahazduba beel, Sonitpur, 11.6 – 13.0 cm (8 exs); Khaloi beel, Sonitpur, 6.5 – 12.2 (10 exs). In Arunachal Pradesh the collecting sites are Sijusa, East Kameng 7.3 – 8.4 cm (3 exs); Itanagar Fish Market 7.9 – 9.8 cm (7 exs); Meghalaya, Garo hills 8.2 – 11.9 cm (3 exs); Manipur: Imphal fish market, 7.2 – 9.6 cm (10 exs) and Tripura: main Agartala market, 8.0 – 11.2 cm (12 exs). The colouration of the test specimens is recorded in fresh conditions.

The fish samples are preserved in 8 to 10% formaldehyde in the field. Detailed taxonomic studies are made in the Industrial Fishery Laboratory of Gauhati University as well as Laboratory of Zoology, Hojai College, Hojai.

Measurements of various body proportions are taken with utmost care. All are straight point to point measurements taken with dial-reading calipers and with fine pointed dividers and recorded to the nearest one-tenth of a millimeter, notably standard length, head breadth, head depth, gape of mouth, snout length, inter nasal distance, eye diameter, inter-orbital distance, pre-orbital distance, post-orbital distance, body depth, body width, dorsal height, dorsal length, pre-dorsal distance, post dorsal distance, prepectoral distance, pre-pelvic distance, pre-anal distance, distance between origin of pectoral fin and origin of pelvic fin and distance between origin of pelvic fin and origin of anal fin, length of caudal fin, length of caudal peduncle, least height of caudal peduncle and length of key scale of both male and female of each species are incorporated in the present communication.

All the relative data are given in ranges with mean in parenthesis under morphometrics, while only 10 specimens of both male and female have been presented under meristic measurements. Besides, the zoogeography of each species in India and elsewhere is also appended to culminate the taxonomic account. And for the physico-chemical analysis of the habitat standards methods are followed.

The different aspect of ethological perspectives of *Nandus nandus* (Ham) are resting behaviour, locomotive deportment, ingestive conation, agonistic mean and procreatic demeanor and the laboratory observations are conducted after Harris (1936), Bainbridge (1958), Brawn (1961), O’Brien (1990), Hart (1993), Riehl and Baensch (1996) and Marshall (1999).

The speed attained by a fish on water is equal to the thrust less the drag or resistance of water. Bainbridge (1958) was able to equate the speed of swimming \((v)\) to the length of the fish \((L)\) and frequency \((f)\) of the tail movements.
The equation is: \( V = \frac{1}{4}L(3f - 4) \), where, \( V \), being expressed in cm/sec; \( L \), being expressed in cm; \( f \), being expressed in frequency of tail beat/sec. The locomotive velocity and the speed, both cruising and top level are two different features, which are critically investigated, in the present species. Further, in the locomotive velocity, forward movements in horizontal and oblique direction, which are found pertinent in the test species, are only documented.

The feeding behaviour of the test species is also precisely investigated in the aquaria. Responses and reactions of the test specimens towards their food elements introduced into the eco-system are visually analyzed. The modus operandi of food capture, period of quiescence, change of equilibrium, operculum and fin movement, surfacing behaviour, postures of feeding, actual mode of movement and other related changes of the fish are precisely documented.

In the present investigation, aggressive behaviour of the test species towards a newly introduced fish as well as among the occupants of the aquaria is visually observed and documented through schematic figures.

The procreatic demeanor of *Nandus nandus* is visually observed and deduced schematically. The test specimens are observed to become restless 2 hrs. after inducing the brooders with hormone. Actual pre-spawning mating behaviour is displayed 6 hrs. after inducing the fishes.

On Bionomics profile, the gut content of *Nandus nandus* is analyzed after Hynes (1950), and Lagler (1952, 1962). On an average of c 50 fishes per season is analysed for victual spectra. The gut of each fish is cut open lengthwise on the ventral side by means of a pair of scissors and the entire gut is carefully removed from end to end. The entire alimentary canal is separated and spread on a board and the length of the alimentary canal is recorded with a graduated scale.
The entire digestive tube is cut open lengthwise and the contents are emptied into petri dishes for analysis and the different food items are separated. The large food items are isolated and identified and the smaller food constituents are identified with the aid of microscope. All the food items are ascertained up to the genus or the family level, depending upon the completeness of the organism and the extent of digestion. If digestion has progressed to an advanced state making identification difficult, it is treated as digested matter. The food items are identified according to authoritative sources. The number of empty and non-empty guts is also observed.

The relative length of the gut (RLG) exhibits the precise relation between the gut dimension to the actual body length. The RLG is analysed after Jacobshagen (1913) using the formula, \( \text{RLG} = \frac{\text{GL}}{\text{TL}} \), where GL – gut length and TL – total length of the fish in cm.

The hepato-somatic index (HSI), which is an estimation of the feeding intensity of the fish, is calculated by the formula, \( \text{HSI} = \frac{w \times 100}{W} \), where \( w \) and \( W \) are the weight of the gut content and the fish respectively.

In order to give a summary picture of the frequency of occurrence in conjugation with the bulk of the various food items consumed, an index taking two variable factors into consideration seems desirable. Such an index is given by Natarajan and Jhingran (1961) designated as the index of preponderance which is deduced by,

\[
\text{PI} = \frac{v_i O_i \times 100}{\sum v_i O_i},
\]

where \( v_i \) and \( O_i \) are the volume and occurrence indices of food items as indicated by their percentage.

The gills of the test species are dissected out and studied under the microscope to find out gill raker condition and correlated with food habit after Nikolsky (1963).
In Reproductive biology, fish specimens are collected from different regions of North East India for ascertaining the sexual dimorphism. 50 healthy specimens of *Nandus nandus* (Ham) is kept in batteries of glass aquaria separately. In vitro sexual dimorphism is ascertained after Furkayama and Hiroya (1982), Gota (1984), Dey and Roy (1991) and Sarmah and Dey (2004).

Male and female of each species are identified through various morphological characteristic i.e. body shape, mouth, origin of dorsal fin, dorsal fin spine with the conformation from anatomical studies after examining each specimen independently. Measuring board, weighing balance, magnifying lens, dissecting tools, graduated scale and soft cushion platform are some of the simple requisites found useful for the present study. The sexual dimorphism characteristics are recorded in both breeding and non-breeding seasons. Sex ratio has been ascertained from natural stock through random sampling.

The size at first maturity of *Nandus nandus* is assessed after Wood (1930) by considering different parameters like gonado-somatic index and fecundity.

For testes, milt and spermatozoa trait, the males are reared in separate aquaria. If milt oozes out on slight pressure over the belly by index finger is considered as fully matured. A few male specimens are dissected for anatomical structure of testis, mainly shape and colour. For collection of milt, the previously maintained matured fish are netted out and the total length is recorded as soon as possible. After that the fish is held between the fingers with belly facing upward. By pressing the index finger gently on the side of the belly towards the vent the light pressure is sufficient to cause oozing of milt and the colour and type of milt is recorded. The milt is collected by Pasteur micropipettes. Since the volume of the milt is found in less amount, therefore 0.01 ml of milt is collected and diluted upto 0.1 ml with Ringer’s solution of Mukherjee and Bhattacharya (1949 a and 1949 b) and normal saline (0.9%) solution. Since, there is no
difference between these dilutents, normal saline is preferable due to its simplicity and preparing smears on the slide. The smeared slides are now stained with Giemsa. The Giemsa staining method for cold blooded vertebrate (Piennar, 1962) is the most effective stain for present investigation. The micrograph are taken under a C–Z NFPK stereoscopic research binocular microscope.

For estimating ova and maturity stage of gonad several criteria including size, amount and distribution of various cell inclusions specially yolk granules have been used for designating the stage of oogenesis in fishes (Nagahama, 1983 and Guraya, 1986).

The maturity stage in female is assessed by critical examination of ovaries. The live specimens are dissected on the spot at monthly intervals and gonads are taken out as soon as possible and transferred to physiological saline solution. The colour of the ovary is recorded and the gonads are immediately fixed with 8% formalin. Morphological stages of ovary are assessed on the basis of colour, size, weight and maturity of ova. The ovaries are classified into I to VII stages adopted by ICES (Wood, 1930).

For estimating the fecundity of the test fish, the ovaries of stage IV & V are taken into consideration. The ovaries are preserved in Simpson’s (1951) modification of Gilson fluid. From an ovary of known weight, three small portion are cut & weighed separately in mono pan electric balance to the nearest milligram. Each portion of ova are teased out of the follicle and counts are made of all ova comprising the mature group under a zoom citoval dissecting microscope. From the total number of the ova of three portion, the average number of ova per milligram is computed. Based on this method the total number of ova (fecundity) in the studied fishes is estimated after Lagler (1952). The fecundity data are then analyzed in relation to variables like, length and weight of fish & weight of ovaries by applying regression equation. Once such equation is established fecundity can be estimated by using any of the above-mentioned variable (Bal &Rao, 1984).
Fecundity (F) as the dependent variables is regressed with total length (TL), total weight (TW) and ovary weight (OW) as independent variables to estimate Karl Pearseon co-efficient of co-relation with standard error. The result is tested through t-test for its significance. The gonado-somatic index (GSI) of matured female fish is studied after Le Cren (1951), Marichamy (1971) and Wotton (1973).

Females in the size range of 13.00 – 14.00 cm are collected randomly each month and preserved in 8 – 10% formaldehyde. The weight of the females is precisely taken in a single pan balance to the nearest 5 mg. The gonad is dissected out and weighed in a mono pan electric balance to the nearest milligram and the values are computed using the formula, GSI = \( \frac{\text{Total ovary weight}}{\text{Total weight of the body}} \times 100 \). Plausible spawning ground of *Nandus nandus* (Ham) is estimated by making frequent visits to the field and ascertaining by the presence of eggs/fries. The physico-chemical parameter of the spawning ground is estimated after APHA (1985).


Feeds are the major components for the maintenance of brooders. Live fishes (minnows) and shrimps are collected from wetland of Jalukbari and stocked in separate aquaria for each application.
Administration of synthetic hormone has been attempted successfully to induce breeding in the test species. Dechlorinated tap water treated with 5% methylene blue solution processed for 2 – 3 days is needed before brooders are released for rearing. Brooders glass tank of the size 60x45x40 cm and 60x45x40 cm are used to rear separately the male and female stock. Corner filters are used with artificial oxygenation for 24 hours. The faecal matter and uneaten food particles of the tank are siphoned out everyday and the water is changed partially every alternate days. For embryonic and larval development, fertilized egg samples are taken every 10 – 15 mins. in the first 1 hour to determine the cleavage and then at 1 hour interval. Microphotographs of the different stages of development of each species are taken as far as practicable.

Free embryo is reared in the aquaria with a fix temperature and one third of the water is changed daily. 56 hours after hatching, live food (mainly infusoria) and nanoplanptons are added into a cloth hapa fitted in a tank where the hatchlings are shifted. Sampling of hatchling is done daily and are examined under microscope to document the developmental stages. Length of the hatchling is measured with micrometer and photographs are taken. The critical progressive developmental stages of the larva are recorded in aquaria and under microscope to define phase after Blaxter(1969), Balon (1975 a, 1975 b) Dujakovic et al, (1995), Chakrabarti (1998) and Unal et al,(2000).

In the present investigation, laboratory rearing of fry are done through rearing tank set-up, maintenance of abiotic condition of water, stocking density of fry, food and feeding of fry and rearing duration.

Nayak *et al.*, and Sarmah (2002, 2003) are also taken into consideration, while developing the technology in the fry rearing of the test ornamental fish species.