CHAPTER-2

MATERIALS AND METHODS

For the taxonomic study, a large number of specimens of *Danio dangila* and *Puntius chola* of both the sexes were collected from different lotic and lentic water bodies of Nagaland. The lotic water bodies include the Rivers: Milak, Tsurang, Tesuru, Dhansiri, Dzüza and Kehoru for *Danio dangila* where as Lentic water bodies are Rice fields of Southern Angami Villages of Kohima, District. The collection sites of *Puntius chola* are Rivers: Doyang, Milak, Tsurang, Intangki and lentic water bodies include Rice fields of Changki valley of Mokokchung District. The colouration of the test specimens were recorded in fresh conditions on the collection site.

For the taxonomic study, the fish samples were preserved in 8 to 10% formaldehyde in the field. Detailed taxonomic studies were carried out in Lab cum Awareness Centre, Half Nagarjan, Department of Fisheries, Dimapur, and as well as in the Department of Zoology, Nagaland University, Lumami.

The fish specimens were taxonomically identified and confirmed after Menon (1954), Dutta and Srivastava (1988), Talwar and Jhingran (1991), Jayaram (1994), Nath and Dey (2000 a, 2000 b) and Vishwanath (2002).

Measurements of various body proportions were taken with utmost care. All measurements were taken with dial-reading calipers and fine pointed dividers and were recorded to the nearest one-tenth of a millimeter. All the morphometric measurements like, 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, pre-pectoral distance, pre-pelvic 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 depth of caudal peduncle, highest depth of caudal peduncle and length of key scale of both male and female of each species were incorporated in the present communication.

All the relative data were given in ranges with mean in parenthesis under morphometrics, while only 10 specimens of both males and females of *Danio dangila* and *Puntius chola* have been presented under meristic measurements. Besides, the zoo-geography of each species in India and elsewhere were also appended to culminate the taxonomic account.

The different aspect of ethological perspectives studied under the two test species *Danio dangila* and *Puntius chola* were ingestive conation, and procreatic demeanor. These ethological investigations were undertaken in different sets of aquarium after the methodology used by different workers (Bainbridge, 1958; Hart, 1993; Riehl and Baensch, 1996; Marshall, 2000 and Sarmah, 2002).

On Bionomics profile, the gut content of the two test species were analyzed after Hynes (1950), and Lagler (1952, 1956). On an average of 20 fishes of each species both male and female per season were analysed for victual spectra, relative gut length, hepato-somatic index and index of preponderance. The guts of each fish were cut open lengthwise on the ventral side by means of a pair of scissors and the entire guts were carefully removed from end to end. The entire alimentary canal was separated and spread on a board and the length of the alimentary canal was recorded with a graduated scale.

The contents of the guts were emptied into petri-dishes for analysis. The different food items were separated and the large food particles were isolated and identified whereas the smaller food constituents were identified with the aid of microscope. All the food items were ascertained, depending upon the completeness of the organism and the extent of digestion. If digestion has progressed to an advanced state making identification of the food particle difficult, it was treated as digested waste. The numbers of empty and no-empty guts were also observed.

The relative length of the gut (RLG) exhibits the precise relation between the gut dimensions to the actual body length. The RLG were 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, was calculated by the formula, $\text{HSI} = \frac{\text{w} \times 100}{\text{W}}$, where w and W are the weight of the gut content and the fish respectively.

In order to give a complete picture on the frequency of occurrence in conjugation with the bulk of the various food items consumed, an index taking two variable factors into consideration was taken. Such an index was given by Natarajan and Jhingran (1961) and designated as the index of preponderance which was deduced by, $PI = \frac{V_i O_i \times 100}{\sum V_i O_i}$, where '$v_i$' *and* '$o_i$' are the volume and occurrence indices of food items as indicated by their percentage. The characteristics of gill rakers such as, number of gill raker/gill arch, size of gill raker, length of gill lamella, and length of gill arch were studied after Nikolsky (1963).

To study the length-weight relationship, live fresh specimens of *Danio dangila* and *Puntius chola* were collected from both lotic and lentic water bodies which were described elsewhere. Fifty specimens of each *Danio dangila* whose length varies from 4.8 cm to 8.0 cm and *Puntius chola* whose length varies from 5.8 cm to 8.7 cm were preserved in 8% formalin solution. They were subsequently dried for 30 seconds to 1 minute in a blotting paper, and then measured by a meter scale and weighed on an electric balance. Biostatistical tables were used throughout the study for the calculation of various factors necessary for the expression of length-weight relationship. Length was used as type and weight as the array. The equation adopted was that of the general parabola: $W=cL^n$. This equation when expressed in logarithmic form becomes log W= log c + n log L, which

when graphically represented assume a linear form. The value of **c** and **n** were determined empirically by the following formulae,

$$\text{Log } c = \frac{\Sigma \log W.\ \Sigma (\log l)^2 - \Sigma \log L.\ \Sigma (\log L.\ \log W)}{N.\ \Sigma (\log l)^2 - (\Sigma \log l)^2}$$

And $$n = \frac{\Sigma \log W - N.\log c}{\Sigma \log L}$$

The coefficient of condition is estimated by using LeCren's relative condition factor (Kn) as Fulton's condition factor (K) gave erroneous conclusion in the heterogeneous sized fishes. LeCren's relative condition factor (Kn) was estimated with the equation, Kn=observed weight/expected weight after the equation $W=cL^n$. Where, all the weights were taken in grams.

The main objective was to derive mathematical formulae, correlating the two variables length and weight in a very general manner, for calculating one from the other within a range of error. In view of this, a reasonable size range of both the wild caught species were included in the study. The sex factor was not reckoned as also the gonad condition and the gut contents. The material was, therefore, heterogeneous. The general fact which has been elucidated by Clark, (1928), Walford, (1932) and Schultz, (1933) for other species has also been taken into consideration in the present study.

To study the reproductive biology, fish specimens were collected from different drainage system of Nagaland for ascertaining the sexual dimorphism. 20 healthy specimens each of *Danio dangila* and *Puntius chola* were kept in batteries of glass aquaria separately. In- vitro sexual dimorphism was ascertained after Furkayama and Hiroya, (1982), Goto,

(1984), Dey and Roy, (1991), Kurian and Inasu, (1997) and Sarmah and Dey, (2003).

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

The size at first maturity of *Danio ganglia* and *Puntius chola* were assessed after Wood (1930) by considering different parameters like gonado-somatic index and fecundity. For testes and milt, the males were reared in separate aquaria. If milt oozes out on slight pressure over the belly by index finger, it was considered as fully matured.

For estimating ova and maturity stage of ovary, several criteria including size, amount and distribution of various cell inclusions, specially, yolk granules were used for designating the stage of oogenesis in fishes. The maturity stages in females were assessed by critical examination of ovaries as described by Nagahama, (1983) and Guraya, (1986).

The live specimens were dissected on the spot at monthly intervals and gonads were taken out as soon as possible and transferred to physiological saline solution (0.3%). The colours of the ovaries were recorded and were immediately fixed with 8% formalin solution. Morphological stages of ovary were assessed on the basis of colour, size, weight and maturity of ova. The ovaries were classified into I to VII stages.

For estimating the fecundity of the test fishes, the ovaries of stage IV & V were taken into consideration. The ovaries were preserved in Simpson's (1951) modification of Gilson fluid. From an ovary of known weight, three small portions were cut & weighed separately in mono pan

electric balance to the nearest milligram. Each portion of ova was teased out of the follicle and ova counts were made under microscope. From the total number of the ova of three portions, the average number of ova per milligram was computed. Based on this method the total number of ova (fecundity) in the study fishes was estimated after Lagler (1952). The fecundity data were then analysed in relation to variables like, length and weight of fish and weight of ovaries by applying regression equation. Once such equation is established, fecundity can be estimated by using any of the above mentioned variables as reported by Bal and Rao, (1984).

For estimation of Karl Pearsonean co-efficient of co-relation with standard error. Fecundity (F) was taken as the dependent variable and total length (TL), total weight (TW) and ovary weight (OW) as independent variables. The result was tested through t-test for its significance. The gonado-somatic indexes (GSI) of matured female fishes were studied after Le Cren, (1951) and Wotton, (1973). Females in the size range of 5.00 – 6.8 cm *Danio dangila* and 5.8 – 7.8 cm *Puntius chola* were collected randomly each month and preserved in 8 – 10% formaldehyde. The weights of the females were precisely taken in a mono pan electric balance to the nearest 3 mg. The overies were dissected out and weighed in a mono pan electric balance to the nearest milligram and the values were computed using the formula, $\text{GSI} = \frac{\text{Total ovary weight}}{\text{Total weight of the body}} \times 100$. Plausible spawning ground of *Danio dangila* and *Puntius chola* were estimated by making frequent visits to the field to ascertain the presence of eggs/fries. The physico-chemical parameter of the spawning ground is estimated after APHA (1998).

The ornamental fishes can be categorized into two broad categories based on their spawning habit such as (i) oviparous fishes- fishes that lay egg (ii) viviparous fishes- live bearers (Devraj 1989). The majority of the freshwater ornamental fishes are egg layers. Oviparous fishes release adhesive / non-adhesive and semi-adhesive eggs.

On Laboratory propagation for ex-situ breeding, live specimens of both the test species were collected from the wild and acclimatized as warranted in aquariums after methods of *Dey et.al,* (2002). Acclimatized fishes were then reared in cement cisterns and fibre reinforced plastic tubs with optimum quality control measures. The process involves selection of brood stock, breeding set-up and breeding technique. For the selection of brood stock authorative methods of Sunny, (2002), Sarmah and Dey, (2004) and Swain, (2005) were followed. Maintenance of brooders, stocking density and breeding technique were made after Huet, (1986), Nandesha *et.al,* (1991) and Parazo *et.al,* (1998). The physico-chemical variables such as pH, air and water temperature, total alkalinity, dissolved oxygen, of the brooders tanks were estimated after APHA (1998).

Under-ground water treated with 5% methylene blue solution was needed before brooders were released for rearing. Cemented cisterns and FRP tubs of the size 240×120×60 cm were used to rear separately the male and female stocks. Filters and aerators were used for oxygenation for 24 hours. The faecal matter and uneaten food particles of the tank were siphoned out every day and the water was changed partially every alternate day. The captive breeding techonology of the two test species were standardized by trial and error experiment and details were given elsewhere in chapter 3. Administration of synthetic hormone (ovaprim) was attempted to breed the two test species *Danio dangila* and *Puntius chola*.

For embryonic and larval development, fertilized egg samples were taken every 10 – 15 mins in the first 2 hours to determine the first cleavage and then at 1 hour interval till hatching. Microphotographs of the different stages of development of the two test species were taken as far as practicable. Line diagrams were drawn to depict the different stages of larval development. Several criteria including size, amount and distribution of various cell inclusions specially yolk granules were used for designating the stages of oogenesis in fishes Nagahama, (1983) and Guraya, (1986).

Laboratory rearing of fries of *Danio dangila* and *Puntius chola* were done through different rearing tank setup, maintenance of abiotic condition of water, stocking density of fry, food and feeding of fry and rearing duration. The embryos were reared in the aquaria where the temperature was constantly monitored and one third of the water changed daily. 3 to 5 days after hatching, live food (mainly infusoria) were added into the aquaria. Samplings of hatchlings were done daily and were examined under microscope to document the developmental stages. Hatchling lengths were measured with micrometer and photographs were taken. The progressive developmental stages of the larva were observed under microscope to define phase after Blaxter (1969), Balon (1975 a), Dujakovic *et.al*, (1995), Chakrabarti (1998) and Unal *et.al*, (2000).

Approaches followed by Charles (1975), Dawes (1984), Goldstein (1987), Kelly (1987), Lazarus (1987), Kiran and Paulraj (1988), Tomey (1988 b), Baskar (1993), Krishnakumar (1997), Sarmah and Dey (2000), and Swain (2008) were also taken into consideration, while developing the technology in rearing of fries of the two test ornamental fish species.

Fish disease, its type, trait and prophylactic measures were evaluated after Foster and Woodbery (1936), Gopalakrishnan (1963, 1964 and 1968), Snieszko (1974), Richard (1977a, 1977b, and 1977c), Schaperclaus (1986), Varghese (1988), Sood (1988), Jhingran (1991), and Biswas (2002). Fishes were constantly monitored to detect any abnormal behaviour. A quarantine / hospital tank was always maintained to accommodate diseased fishes. Specific treatment and control measures were formulated and administered to the diseased fishes.