STUDIES ON THE BIONOMICS AND BREEDING BIOLOGY OF *DANIO DANGILA* (HAMILTON, 1822) AND *PUNTIUS CHOLA* (HAMILTON, 1822), TWO RHEOPHILIC ORNAMENTAL FISHES OF NAGALAND.

THESIS
SUBMITTED TO THE NAGALAND UNIVERSITY IN FULFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

By

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Regd No. 327/2007

DEPARTMENT OF ZOOLOGY,

NAGALAND UNIVERSITY

LUMAMI-798627

2012

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Certificate

This is to certify that, the thesis entitled "Studies on the Bionomics and Breeding Biology of Danio dangila (Hamilton, 1822) and Puntius chola (Hamilton, 1822), Two Rheophilic Ornamental Fishes of Nagaland" incorporates the results of the original findings carried out by Mr. Vimezo Kire Angami under my guidance and supervision. He is a registered research scholar (Regd. No- 327/2007) of the Department and has fulfilled all the requirements of Ph.D regulations of Nagaland University for the submission of his thesis.

The work is original and neither the thesis nor any part of it has been submitted elsewhere for the award of any degree or distinctions.

The thesis is therefore, forwarded for adjudication and consideration for the award of degree of Doctor of Philosophy in Zoology under Nagaland University.

Dated: October, 2012
Place: Lumami

Head
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Prof. Sharif U. Ahmed
Supervisor

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DECLARATION

I, hereby declare that, the Thesis entitled **"Studies on the bionomics and breeding biology of *Danio dangila* (Hamilton, 1822) and *Puntius chola* (Hamilton, 1822), two rheophilic ornamental fishes of Nagaland"**. Submitted by me is entirely the research work of my own. The thesis or part thereof has not been submitted elsewhere for any research degree or distinction.

Dated, Lumami the October, 2012

(VIMEZO KIRE ANGAMI)

Ph.D Regd. No. 327/2007

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CHAPTER-1

INTRODUCTION

The North Eastern Region (NER) is endowed with vast and varied aquatic resources. It is blessed with 56 notable rivers/tributaries and several small rivulets/hill-streams. The rivers Brahamaputra and Barak form the principal drainage of NE India with its numerous tributaries flowing through the different states along with the myriads of rivulets and lentic water bodies (Sarkar and Ponniah, 2000). The region with its diversified lotic and lentic water bodies are considered as the global hotspot for fish bio-diversity (Kottelat and Whitten, 1996). It harbours valuable fish garmplasm resources. Out of the total 806 fish species found in India (Talwar and Jhingran, 1991), the NER is represented by 266 fish species, belonging 114 genera under 38 families and 10 orders (Sen, 2000). In India, 288 species of exotic ornamental fishes exist of which 261 species are egg layers and 27 species are live bearers. The egg laying exotic ornamental fish species belong to 10 orders, 26 families, 123 genera and 1 sub genus, while the livebearer species are represented by 12 genera and 3 families of the order Cyprinodontiformes (Tiwari *et. al*, 2005). The NER is represented by a total of 196 species of potential ornamental fishes (Dey, 2001).

Like most of the montane state of NE region, Nagaland with an area of 16,579 sq.kms lies between 25^0 6’ and 27^0 4’ N latitudes and between 93^0 20’ and 95^0 15’ E longitudes abounds a good many rheophilic rivers and torrential hill streams besides lentic water bodies. This provides a lucrative abode of her ichthyo-diversities including ornamental fishes. A total of 149 species of Ichthyofauna belonging to 64 genera under 22 families and 6 orders are recorded from lotic, lentic and mixed habitat of the state. Out of these as many as 118 species of fishes having good export potential are identified as ornamental fishes (Ao *et.al*, 2008).

Ornamental fishes may be defined as fishes, which are reared as pets and not for consumption (Anon 2001). Ornamental fishes in general are smaller in size, attractively coloured with majestic movement exposure in the aquarium. However, non-colourful fish will also receive ornamental status if, they exhibit peculiar body morphology, strange locomotive deportment and rare occurrences (Dey, 1984). The present day concept of ornamental fishes varies from man to man and from nation to nation. An unattractive fish for one person may be very attractive for another and a common fish in a country may be an ornamental fish in another country.

Keeping colorful fishes as pets in aquaria or garden pools is an age-old hobby. It originated in China with keeping of gold fish in glass bowl several hundred years ago (Kelly, 1987). It was during 17th century that gold fish was introduced to several countries and became popular in England and Scotland. The first public display aquarium was opened at Regent Park in England in 1853 (Swain *et.al*, 2003). Presently there are over 500 display aquaria functioning worldwide. However, the market for public aquaria of ornamental fish is less than 1% as 99% of the market is still confined to hobbyist (Bhattacharjya and Choudhury, 2004).

In India, the hobby of keeping ornamental fishes as pets is of recent origin with the opening of the Taraporevala aquarium in Mumbai in 1951. In Northeastern region, aquarium keeping as a hobby got a boost with the setting up of first aquarium shop in Guwahati around 1977 (Bhattacharjya and Choudhury, 2004). During 2002 the Department of Fisheries, Govt. of Nagaland, has set up an aquarium display unit at Dimapur.

In recent years, there has been an insatiable demand for newer unique or bizarre shaped fishes by overseas hobbyists, which may not be beautiful in the conventional sense. Presently ornamental fish keeping has emerged as the second most popular hobby next to photography (Chapman, 1997). What started as a hobby has now expanded in to a booming international trade valued at US $150 billion (Bartly 2000). India’s present overall trade in ornamental fish has crossed Rs 150 million. Export of ornamental fishes from India accounted for Rs. 226.00 lakh during the year 2000 (Palanisamy, 2003). The ornamental fish trade although is growing almost continuously, however, India's contribution to the global trade is insignificant (0.007%). Therefore it may be possible for India to capture at least 10% of the market by utilizing its vast indigenous stock of fish species and unemployed trained manpower (Vijayakumar, 2001).

In addition to the export market, the domestic demand for ornamental fishes has been estimated to be of Rs. 10.00 crore per year. The demand is increasing at the rate of 20% per year (Vijayakumar, 2001) thereby, offering enough scope for development of ornamental fish breeding and rearing on a commercial scale. Kolkata, Mumbai and Chennai have emerged as the pioneer breeding centers of India.

The U.S.A. is the largest market for ornamental fish import followed by Japan, U.K and Germany. Singapore, the world's largest exporter of ornamental fish with 30% of global supply, has already produced numerous lucrative new varieties through selective breeding. Out of the total export of ornamental fishes, freshwater fish accounts for around 90%, with almost 90% bred in captivity. The global ornamental fish trade increased from US$ 4.5 billion (1995) to US$ 7 billion today. Though India's share (US$ 0.25 million in 1997) in global trade is very less, it has been noticed that Indian ornamental fishes are of great demand in international market (Swain and Chakrabarty, 2008).

The export of indigenous ornamental fishes from the country are mainly confined to freshwater varieties and is limited to the fishes from the Northeastern states (85%) and a few bred varities of exotic species (Swain et.al, 2003). The status and export potentialities of indigenous ornamental fishes of India have been highlighted by many workers (Anon, 1982; Nopany, 1987; Shenoy, 1987; Elampasithy, 1989, 1995; Banerji, 2001; Belsesware and Naik, 2001; Ghosh *et.al*, 2002; Nair, 2001; Swain *et.al*, 2003; Bhattacharjya *et.al*, 2000; Bhattacharjya and Choudhury, 2004; Swain and Chakrabarty, 2008 and Ao *et.al*, 2008).

The paragon of pre-investment feasibility study extensively made by Dey *et.al*, (2002) on the prospect of ornamental aquaculture in NER has revealed that, these Ornamental Fish Species (OFS) are all traded on wild caught and none venture for their culture and breeding. Therefore, the population of these valuable ichthyo-species is gradually declining due to over exploitations from their natural stock. Hence, there is enough scope and potentialities for the OFS of NER to venture into the Ornamental Fish Farming and Trade in International market.

Review of literatures

A beginner, hobbyist or entrepreneur would naturally like to know more about this beautiful ornamental fish, about its life, feeding behavior, compatibility, courtship and above all the breeding behaviour and its techniques. Notable contributions have been made by Mills (1990); Sands (1986); Kelly (1987), Nelson (1994); Riel and Baensch (1996), Vinci (1998) towards cataloging and recording the worldwide distribution of tropical freshwater ornamental fishes.

Several workers (Dey, 1973; 1975; 1982 and 1995; Sen, 2000; Nath and Dey, 1982; 2000 a, b; Das, 1989; Dey *et.al*, 2002; Bhattacharjya *et.al*, 2004 and Mahapatra, 2004) have also made valuable contribution on the fish germplasm of North East India, which have thrown light on the ornamental fish species of the region.

Fishes have remarkably wide range of biological adaptation to diverse habit. Fundamental work on fish behaviour has been a rapidly moving field. The behaviour of fishes is unique. A newly fertilized egg does not behave but an adult fish responds to its environment with repertoire of complex, adaptive behaviour pattern.

The ethological perspectives of the fishes such as ingestive and procreatic behaviour have drawn the attention of many scientific workers (Gray, 1953; Harris, 1960; Benkema, 1964; Boer, 1980; Halliday, 1983; Lauder, 1983; Wainwright and Lander. 1986; Gladstone, 1987; Houde, 1987; Bisazza *et.al*, 1989; Bells *et.al*, 1990; Mc Adam *et.al*, 1999 and Spears 2000)

Studies on the food and feeding behaviour helps in understanding and identifying the feeding ecology of the species. The feeding behaviour of fish is a species characteristic. Nikolsky (1963), categorized fishes according to their extent of variation and types of food consumed by them such as, a) Euryphagic: feeding on varieties of food. b) Stenophagic: feeding on few selected types of food and, c): Monophagic: feeding on single type of food. The food and feeding habits of cultured fishes have been studied by several workers (Fagade and Olaniyan, 1973; Ajah, *et.al*, 2006; Soyinka and Olufemi, 2008). However, report on ornamental fish culture and ingestive behaviour of the species in confined water is very fragmentary and scanty.

The mathematical relationship between length and weight of fish is an important parameter in the fishery biology (Sinha, 1981). The relationship is of significant importance in studying the growth, gonadal development, general well-being of the fish population and management of the species and their fisheries (Le Cren 1951, Shafi and Quddus 1974). The growth in weight of fish, in general, is directly proportional to the cube of its length, but sometimes values of the relationship may deviate from the cube law, either due to environmental factors or condition of fish (Le Cren, 1951; Solanki *et.al*, 2004). Pervin and Mortuza (2008) cited that, the length-weight relationship is very important for proper exploitation and management of fish species population. The economic value of fish depends upon its length weight relationship.

Ever since Harbert Spencer first enunciated the cube law in 1871, numerous workers have carried out its application to fish measurements. During earlier investigations on the applicability of the cube law to fish measurement, beginning with Hensen (1899) the constant **c** was found to fluctuate and Heincke (1908) proposed the use of this factor as an index of the well being of the fish. This factor has been variously termed as coefficient of condition, length-weight factor etc. Crozier and Hect (1914) and Hect (1916) found the cube law applicable to the fishes they investigated, but these instances appear to be exceptions rather than the rule. Fulton’s findings showed the inadequacy of the cube law in describing the length-weight relationship of fishes. In recent years a much more satisfactory way of describing the length-weight relationship of fishes has been developed through the use of more general equation : $W=cL^n$, where W= weight in grams, L=length in centimeter and **c** and **n** are two exponents. The value of **c** and the exponent **n** are determinable empirically. Such a relationship has been worked by a host of workers on different fishes, who, among others include, Walford (1932), Hile (1936), Hile and Jobes (1941), Jhingran (1952), Das and Mitra (1958), Sarma *et.al*, (1979), Baragi and James (1980), Dey (1987), Subba and Ghosh (2000), Pawar and Mane (2006) and Pervin and Mortuza (2008). Most of the above stated authors, determining the length-weight relationship of the fish they investigated, have also determined the condition factor of the fish. A great confusion appears to have arisen in describing the condition of a fish and the expression of the length-weight relationship. Hile (1936) has thrown light by elaborating upon the theme coefficient based on empirical exponents fail to reflect differences in form or relative heaviness independent of general length-weight relationship and comparable as measures of relative heaviness between fish of any length. In India, Lacey and Cretin (1905) and Treven (1952) worked on length-weight relationship of *Tor putitora* (Hamilton) for which they advanced some formulae. The formula mentioned by Lecey and Cretin is 1 ¼ length of the fish multiplied by the square of the girth in inches and divided by 1000 gives the weight of the fish in pounds. However, this approach appeared insignificant in its applicability by different workers.

The reproductive biology of freshwater ornamental fishes is a discipline of increasing importance. A perusal of literature reveals that, a great deal of scattered information is available on reproductive biology of different freshwater ornamental fishes. Notable works have been carried out by different workers. (Lowrence *et.al*, 1989; Afroze and Hossain, 1990; Bhuiyan and Parveen, 1998; Borkotoki and Dey, 2002; Dobriyal *et.al*, 2003;

Mahapatra *et.al*, 2004; Dobriyal, 2005; Mitra *et.al*, 2007; Saha *et.al*, 2009 and Bahuguna *et.al*, 2010).

On the culture and maintenance of exotic ornamental fishes, important contributions were made by Plona (1962), Anderson (1962, 1963, 1965); Kaufman (1965); Fernado and Phang (1985), Andrew (1986) and Polonski (1991).

Studies have also been made by different workers on the food, nutrition and rearing of some freshwater ornamental fishes (Basavaraja *et.al*, 1988; Tekriwal and Rao, 1990; Sinha, 2000; Belsware and Naik, 2001; Mukhopadhya, 2001; Sakthivel and Ramathilagam, 2001; Sinha *et.al*, 2001; Anna Mercy, 2001; Pandian *et.al*, 2001; Swain and Das, 2001and Swain, 2008).

In recent years focus have been drawn towards the culture and breeding of ornamental fishes. Significant contributions are made by several workers (Sinha, 1972; Dixit and Agarwal, 1974; David and Rahman, 1975; Chaco and Kuriyan, 1984; Barua and Mollah, 1987; Akteruzzaman *et.al*, 1991; Mahapatra, 1999; Abidi and Thakur, 1997; Sarkar and Ponniah, 2000; Choudhury and Biswas, 2003; Dey and Sarmah 2000, 2003; Sarmah and Dey, 2004; Sarmah, 2002, 2003; Mitra, 2004; Swain, 2008; Swain and Singh, 2008).

The success of fish culture often depends on larval rearing. Efforts were made and documented by few workers (Sane and Bhide, 1992; Das and Kalita, 2003; Swain *et.al*, 2008) on embryonic, larval development and larval rearing of some freshwater ornamental fish species of NE India as well as on some food fishes by (Moyle and Cech, 1988; Reddy and Rao, 1999; Biswas, 2002).

In recent years, increased development of ornamental fish culture has necessitated to understand the disease causing factors in an aquarium. The major diseases of ornamental fishes their preventive measures and treatment have been described by many authors (Richard, 1977; Stojkovic, 1980;

Giavenni, 1981; Gratzek, 1988; Varghese, 1988; Singh and Sreedharan, 2002; Biswas, 2002; Madhumita, 2005; Swain, 2008).

No comprehensive data base on the bionomics and breeding biology of ornamental fish species of both lotic and lentic water bodies of Nagaland are worked out so far. Information on reproductive biology, embryonic development and larval rearing is very fragmentary and inadequate. Review of literature has indicated that, no work has been done on the two rheophilic ornamental fish species viz. *Danio dangila* and *Puntius chola*.

Danio dangila is a native to fresh water rivers and streams of Southeast Asia; the name "*Danio*" comes from the Bengali name *dhani*, meaning "of the rice field", probably referring to the smallness of their size or to their being found in grassy jungles in the edges of rivers and lakes.

Puntius chola is endemic to the Western Ghats and is listed as a vulnerable species by the National Bureau of Fish Genetic Resources. It inhabits freshwater ponds, streams and small canals associated with paddy fields.

Several ornamental fishes, to name some like, *Botia dario, Puntius geliues, Hara hara, Conta conta, Badis badis, Nandus nandus, Danio dangila* and *Puntius chola* etc. caught from the wild are reportedly being exported, leading to decline in their wild stock. The present trend if allowed unabated, wild ornamental fish species may be completely wiped out from nature in days to come. In this context, captive breeding and rearing of freshwater ornamental fish species can open up a new avenue.

Objectives of the study

Realizing the importance of ornamental fishes and their export potentials in overseas market, the present investigations aimed to achieve the following objectives:

1) To collect samples of *Danio dangila* and *Puntius chola* from different natural water bodies of Nagaland and study their systematic, sexual dimorphism and sex ratio.

2) To estimate the abundance trend of the two test species in both lotic and lentic water bodies of Nagaland.

3) To study the ethology of the species especially in respect of their feeding and breeding behaviours.

4) To investigate the bionomics and breeding biology of the two test species.

5) To develop in-house breeding technology and propagation of the species for the benefit of the entrepreneurs who may be involved in ornamental fish trade.

The present investigation, therefore, will depict a clear scenario of the fish *Danio dangila* and *Puntius chola* as an ornamental fish of North Eastern India especially on the technology of captive breeding and culture with their bionomics and early life history. This in turn will lead to economic benefit for entrepreneurs and aquarists engaged in the OFS trade as well as unemployed youths.

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,

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

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

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.

CHAPTER-3

RESULTS

GRAPHICS OF THE FISH SPECIES

3.1 *Danio dangila* (Hamilton, 1822)

The line diagram of the test species *Danio dangila* and its original colour photograph is depicted in **figure-1 and plate-1.**

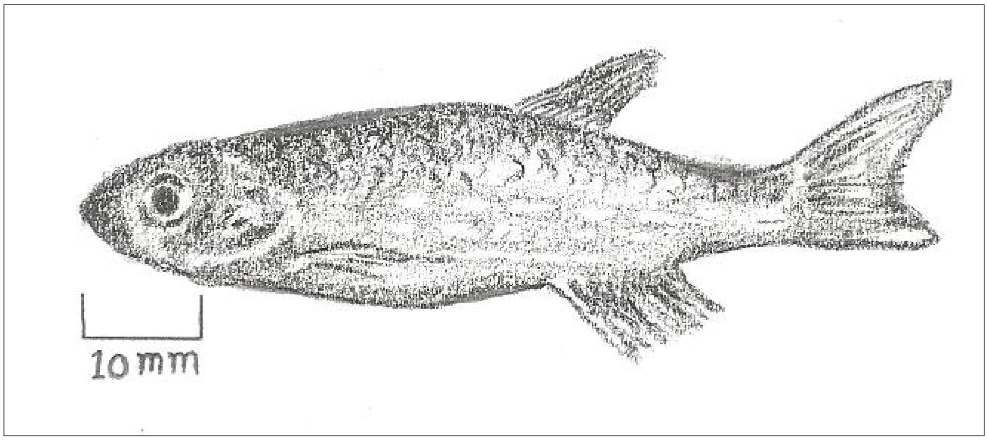

Figure-1: ***Danio dangila***

1822. *Cyprinus dangila* Hamilton-Buchanan, *Fishes of Ganges*: 321,390 (Type-locality: mountain streams of Mongher, Bihar).

3.1.1 Taxonomic account of *Danio dangila*

The species belongs to Order- Cypriniformes, Family- Cyprinidae, under Sub-family- Danioninae and Genus- Danio.

Common name:

Moustached danio (English), Lauputi (Assamese), Tapo (Adi, Arunachal Pradesh), Tapio (Nishi, Arunachal Pradesh) Nipati (Bengali), Shalynnai (Meghalaya), Nung nga (Manipur), Zer (Ao, Nagaland), Khoruno (Angami, Nagaland).

Characteristics of the species

The non-meristic characters, meristic measurements and counts and morphometrics of both male and female fishes have been described below.

Non-meristic profile of male *Danio dangila*

Body: slender, long and laterally compressed. The dorsal profile is slightly curved but ventral profile was straight. Head: moderate in size. Mouth: oblique, superior, small, lips thin and cleft extending to anterior margin of the orbit. Eyes: moderate, diameter 2.5 – 3.7 in head length. Barbels: two well developed pairs, rostral and maxillary, maxillary pairs, slightly longer than rostral pairs. Fins: the dorsal and anal fins are large, the margins of the caudal and anal fins are smooth, anal fins with two or three blue stripes. Lateral line: complete, with 36-40 scales, predorsal scales 12 to 14. Scales: small, cycloid. Colour: in live, black-olive, sides-silvery, covered with several narrow blue lines. Upper angle of gill opening with a dusky spot. Bends on the body break up anteriorly forming a mottled pattern. The body color was bright with prominent post opercular spot.

Meristic measurements and counts of male *Danio dangila*

Table-1: Measurements (cm) of 10 randomly selected males of *Danio dangila*

PARAMETER	FISH SPECIMEN										RANGE		MEAN
	I	II	III	IV	V	VI	VII	VIII	IX	X	Min	Max	
TL	7.1	5.6	5.3	5.9	5.3	6.2	8.2	6.0	4.8	5.4	4.8	8.2	5.9
HL	1.5	1.3	1.2	1.4	1.2	1.4	1.6	1.4	1.0	1.2	1.0	1.6	1.3
POL	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
PtOL	0.8	0.7	0.6	0.7	0.6	0.7	0.9	0.7	0.4	0.6	0.4	0.9	0.7
ED	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4
HD	1.2	1.0	0.9	1.1	0.9	1.1	1.4	1.1	0.8	1.0	0.8	1.4	1.1
HB	0.8	0.6	0.5	0.7	0.5	0.7	0.9	0.7	0.4	0.5	0.4	0.9	0.6
SnL	0.4	0.3	0.2	0.4	0.2	0.4	0.4	0.4	0.2	0.2	0.2	0.4	0.3
IOL	0.8	0.6	0.5	0.7	0.5	0.7	0.9	0.7	0.4	0.5	0.4	0.9	0.6
INL	0.5	0.4	0.3	0.5	0.3	0.5	0.5	0.5	0.3	0.3	0.3	0.5	0.4

GM	0.6	0.5	0.4	0.6	0.4	0.6	0.6	0.6	0.4	0.4	0.4	0.6	0.5
SL	5.8	4.5	4.2	4.8	4.2	5.0	6.6	4.9	3.8	4.3	3.8	6.6	4.8
DFL	0.8	0.7	0.6	0.7	0.6	0.7	0.9	0.7	0.5	0.6	0.5	0.9	0.7
DFH	1.2	1.0	0.9	1.1	0.9	1.1	1.3	1.1	0.8	0.9	0.8	1.3	1.0
AFL	1.3	1.0	0.8	1.1	0.8	1.2	1.4	1.1	0.6	0.7	0.6	1.4	1.0
AFH	1.0	0.9	0.7	1.0	0.7	1.0	1.1	1.0	0.5	0.8	0.5	1.1	0.9
PFL	1.6	1.2	1.0	1.4	1.0	1.5	1.7	1.4	0.9	1.0	0.9	1.7	1.3
VFL	0.9	0.8	0.7	0.9	0.7	0.9	1.0	0.9	0.6	0.7	0.6	1.0	0.8
PDL	3.3	2.6	2.5	2.7	2.5	2.8	3.8	2.8	2.3	2.6	2.3	3.8	2.8
PtDL	1.7	1.2	1.1	1.4	1.1	1.5	1.9	1.4	1.0	1.1	1.0	1.9	1.3
BD	0.9	0.7	0.6	0.8	0.6	0.8	1.0	0.8	0.5	0.7	0.5	1.0	0.7
BH	1.4	1.2	1.1	1.3	1.1	1.3	1.5	1.3	0.9	1.1	0.9	1.5	1.2
PPL	1.4	1.2	1.1	1.3	1.1	1.3	1.5	1.3	1.0	1.1	1.0	1.5	1.2
PVL	3.2	2.5	2.3	2.9	2.3	3.0	3.4	2.9	2.1	2.3	2.1	3.4	2.7
DOPV	1.8	1.3	1.2	1.6	1.2	1.7	1.9	1.6	1.1	1.2	1.1	1.9	1.5
DOVA	1.0	0.8	0.7	0.9	0.7	0.9	1.1	0.9	0.5	0.8	0.5	1.1	0.8
CFL	1.3	1.1	1.1	1.1	1.1	1.2	1.6	1.1	1.0	1.1	1.0	1.6	1.2
CPL	1.0	0.7	0.6	0.8	0.6	0.9	1.1	0.8	0.5	0.6	0.5	1.1	0.8
LDCP	0.7	0.6	0.5	0.6	0.4	0.6	0.8	0.6	0.4	0.5	0.4	0.8	0.6
HDCP	0.8	0.7	0.6	0.7	0.5	0.7	0.9	0.7	0.5	0.6	0.5	0.9	0.7

Abbr.: TL, Total length; HL, Head length; POL, Pre-orbital length; PtOL, Post-orbital length; ED, Eye diameter; HD, Head depth; HB, Head breadth; SnL, Snout length; IOL, Inter orbital length; INL, Inter nasal length; GM, Gape of Mouth; SL, Standard length; DFL, Dorsal fin length; DFH, Dorsal fin height; AFL, Anal fin length; AFH, Anal fin height; PFL, Pectoral fin length; VFL, Ventral fin length; PDL, pre dorsal fin length; PtDL, Post dorsal fin length; BD, Body Depth; BH, Body height; PPL, Pre pectoral length; PVL, Pre ventral length; DOPV, Distance between origin of pectoral and ventral, DOVA, Distance between origin of ventral and anal;

CFL, Caudal fin length; CPL, Caudal peduncle length; LDCP, Least depth of caudal peduncle; HDCP, Highest depth of caudal peduncle.

Fin Formula: D ii 8; P iv 9; V i 7; A iii 13; C 19; Ltr 6/4; Ll 39.

Morphometric Characters of male *Danio dangila*

Pre-orbital length 3.33-5.33 (4.33), Post orbital length 2.50-1.77 (1.95), Eye diameter 3.33-4.00 (3.25), Head depth 1.25-1.14 (1.18), Head breadth 2.50-1.77 (2.16), Snout length 5.00-4.00 (4.33), Inter orbital length 2.50-1.77 (2.16) Inter nasal length 3.33-3.20 (3.25), Gap of mouth 2.50-2.66 (2.60) in relation to head length.

Eye diameter, 1.33-2.25 (1.50), Snout length 2.00-2.25 (2.00) in relation to inter orbital length.

Dorsal fin length 7.60-7.33 (6.85), Dorsal fin height 4.75-5.07 (4.80), Anal fin length 6.33-4.71 (4.80), Anal fin height 7.60-6.00 (5.33), Pectoral fin length 4.22-3.88 (3.69), Ventral fin length 6.33-6.60 (6.00), Pre-dorsal fin length 1.65-1.73 (1.71), Post dorsal fin length 3.80-3.47 (3.69), Body depth 7.60-6.60 (6.85), Body height 4.22-4.40 (4.00), Pre-pectoral length 3.80-4.40 (4.00), Pre ventral length 1.80-1.94 (1.77), Distance between origin of pectoral fin and ventral fin 3.45-3.47 (3.20), Distance between origin of ventral fin and anal fin 7.60-6.00 (6.00), Caudal fin length 3.80-4.12 (4.00), Caudal peduncle length 7.60-6.00 (6.00), Least depth of caudal peduncle 9.50-8.25 (8.00), Highest depth of caudal peduncle 7.60-7.33 (6.85) in relation to the standard length.

Non-meristic profile of female *Danio dangila*

Body: body is stumpy and comperatively short, both dorsal and ventral profiles are curved. Head: moderate in size. Mouth: oblique, superior, small, lips thin and cleft extending to anterior margin of the orbit. Eyes: moderate, diameter 2.5 – 3.7 in head length. Fins: the dorsal and anal fins are comparatively small. The margins of the fins are coarse. Anal fins with two or three blue stripes. Lateral line: complete, with 36-40 scales, predorsal scales 12 to 14. Scales: small, cycloid. Colour: In live, black-olive, sides-silvery, covered with several narrow blue lines. Upper angle of gill opening with a dusky spot. Bends on the body break up anteriorly forming a mottled pattern. The body color was faint and post opercular spot was also less prominent.

Meristic measurements and counts of female *Danio dangila*

Table- 2: Measurements (cm) of 10 randomly selected female of *Danio dangila*

PARAMETER	FISH SPECIMEN										RANGE		MEAN
	I	II	III	IV	V	VI	VII	VIII	IX	X	Min	Max	
TL	8.3	5.8	5.3	6.3	5.9	7.6	7.9	5.5	5.2	6.6	5.2	8.3	6.4
HL	1.6	1.3	1.1	1.4	1.3	1.5	1.5	1.2	1.0	1.4	1.0	1.6	1.3
POL	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.3
PtOL	0.9	0.6	0.5	0.7	0.6	0.8	0.8	0.6	0.5	0.7	0.5	0.9	0.7
ED	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.4
HD	1.2	0.9	0.8	1.0	0.9	1.1	1.2	0.9	0.8	1.0	0.8	1.2	0.9
HB	0.9	0.7	0.5	0.8	0.7	0.8	0.9	0.6	0.5	0.8	0.5	0.9	0.7
SnL	0.5	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.3	0.2	0.5	0.3
IOL	0.7	0.5	0.5	0.6	0.5	0.7	0.7	0.5	0.5	0.7	0.5	0.7	0.6
INL	0.5	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.5	0.4
GM	0.6	0.5	0.4	0.5	0.5	0.6	0.6	0.5	0.4	0.5	0.4	0.6	0.5
SL	6.8	4.7	4.3	5.1	4.8	6.0	6.5	4.4	4.1	5.4	4.1	6.8	5.2
DFL	1.0	0.8	0.6	0.9	0.8	1.0	1.0	0.7	0.6	0.9	0.6	1.0	0.8
DFH	1.2	0.9	0.8	1.0	0.9	1.1	1.1	0.9	0.8	1.0	0.8	1.2	0.9
AFL	1.4	1.0	0.8	1.1	1.0	1.3	1.3	0.9	0.7	1.2	0.7	1.4	1.1
AFH	1.3	0.9	0.7	1.0	0.9	1.2	1.2	0.8	0.7	1.1	0.7	1.3	0.9
PFL	1.6	1.1	1.0	1.2	1.1	1.4	1.5	1.1	1.0	1.2	1.0	1.6	1.2
VFL	1.0	0.7	0.6	0.8	0.7	0.9	0.9	0.7	0.6	0.8	0.6	1.0	0.8

PDL	4.0	2.7	2.6	2.9	2.8	3.5	3.8	2.6	2.5	3.1	2.5	4.0	3.1
PtDL	1.8	1.2	1.1	1.3	1.2	1.5	1.7	1.1	1.0	1.4	1.0	1.8	1.3
BD	1.4	0.7	0.6	0.8	0.7	0.9	1.2	0.7	0.5	0.9	0.5	1.4	0.8
BH	1.7	1.2	0.9	1.3	1.2	1.5	1.6	1.1	0.9	1.4	0.9	1.7	1.3
PPL	1.8	1.2	0.9	1.3	1.2	1.5	1.6	1.1	0.9	1.4	0.9	1.8	1.3
PVL	3.1	2.2	1.8	2.4	2.2	2.7	2.8	2.1	1.8	2.5	1.8	3.1	2.4
DOPV	1.3	1.0	0.9	1.1	1.0	1.2	1.2	1.0	0.9	1.1	0.9	1.3	1.1
DOVA	1.4	1.1	1.0	1.2	1.1	1.3	1.3	1.0	1.0	1.2	1.0	1.4	1.2
CFL	1.5	1.1	1.0	1.2	1.1	1.4	1.4	1.1	1.0	1.2	1.0	1.5	1.2
CPL	1.1	0.7	0.6	0.8	0.7	1.0	1.0	0.7	0.6	0.9	0.6	1.1	0.8
LDCP	0.7	0.5	0.4	0.5	0.5	0.6	0.6	0.4	0.4	0.5	0.4	0.7	0.5
HDCP	0.9	0.6	0.5	0.7	0.6	0.8	0.8	0.6	0.5	0.7	0.5	0.9	0.7

Fin Formula: D ii 10; P iv 9; V i 7; A iii 14; C 19; Ltr 5; Ll 39.

Morphometric Characters of female *Danio dangila*

Pre-orbital length 5.00-5.33 (4.33), Post orbital length 2.00-1.77 (1.85), Eye diameter 3.33-4.00 (3.25), Head depth 1.25-1.33 (1.44), Head breadth 2.00-1.77 (1.85), Snout length 5.00-3.20 (4.33), Inter orbital length 2.00-2.28 (2.16) Inter nasal length 3.33-3.20 (3.25), Gap of mouth 2.50-2.66 (2.60) in relation to head length.

Eye diameter 1.66-1.75 (1.50), Snout length 2.50-1.40 (2.00) in relation to inter orbital length.

Dorsal fin length 6.83-6.80 (6.50), Dorsal fin height 5.12-5.66 (5.77), Anal fin length 5.85-4.85 (4.72), Anal fin height 5.85-5.23 (5.77), Pectoral fin length 4.10-4.25 (4.33), Ventral fin length 6.83-6.80 (6.50), Pre-dorsal fin length 1.64-1.70 (1.67), Post dorsal fin length 4.10-3.77 (4.00), Body depth 8.20-4.85 (6.50), Body height 4.55-4.00 (4.00), Pre-pectoral length 4.55-3.77 (4.00), Pre ventral length 2.27-2.19 (2.16), Distance between origin of pectoral fin and ventral fin 4.55-5.23 (4.72), Distance between origin of ventral fin and anal fin 4.10-4.85 (4.33), Caudal fin length 4.10-4.53 (4.33), Caudal peduncle length 6.83-6.18 (6.50), Least depth of caudal peduncle 10.25-9.71 (10.40), Highest depth of caudal peduncle 8.20-7.55 (7.42) in relation to the standard length.

3.1.2 Distribution trend

Nagaland: Rivers- Milak and Tsurang (Mokokchung, District), Tesuru (Phek, District), Dhansiri (Dimapur, District), Dzüza and Kehoru (Kohima, District), Lentic water bodies and Rice fields of Southern Angami Villages (Kohima, District).

N.E. India: Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Tripura.

Elsewhere: West Bengal, Bihar, Madhya Pradesh, Uttar Pradesh, Bangladesh; Nepal; Myanmar; Bhutan.

3.1.3 Ornamental precedence

Indian ornamental fishes with their brilliant colours and unique features need no introduction to the world market. The tropical ornamental fishes from North Eastern and Southern provinces of India are in great demand in the hobbyists market.

Danio dangila have brilliant coloration in live condition. The body was brownish to olive on back and silvery yellowish in belly and flanks. Three to four silvery bluish lines on flanks and the bands of the body break up anteriorly forming a mottled pattern, which make the fish more attractive and unique among the *Danio species.* Upper angle of gill opening occasionally shows a dusky spot. These characteristics put *Danio dangila* as classified ornamental fish.

3.1.4 Characteristics of the Habitat

Danio dangila mainly inhabits high altitude freshwater running streams, small water courses like pools, rice fields, ditches, and in the bed of hill streams, mountain rivulets and rivers, where the water current is low and with little aquatic vegetation such as, grassy shrubs in the edges of rivers and lakes, with depth ranging from 1-5 feet. This forms an ambient habitat for this small, colored stripes fishes. **Plate- 3**

The pH of the water bodies ranges from 6.0 - 7.5, water temperature 8 - 18°C Dissolved oxygen 6.0 - 8.5 mg/l, Hardness 4.8 - 5.5 mg/l and Total alkalinity 35 – 40 mg/l. Terrestrial plants were the dominant macro-vegetation where the test fish were collected.

3.2 *Puntius chola,* (Hamilton 1822)

Diagramatic presentation of test species *Puntius chola* and its colour photograph is presented in **figure-2 and plate-2.**

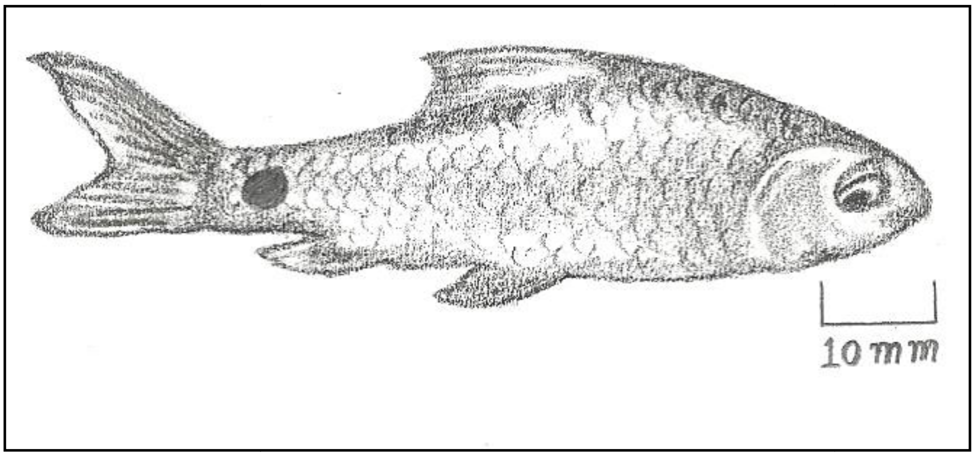

Figure-2: ***Puntius chola***

1822. *Cyprinus chola* Hamilton-Buchanan, *Fishes of Ganges*: 312, 289 (Type-locality: northeastern parts of Bengal).

3.2.1 Taxonomic account of *Puntius chola*

The species belongs to Order-Cypriniformes, Family-Cyprinidae under Sub-family-Cyprininae and Genus-Puntius.

Common name:

Swamp barb (English), Puthi (Assamese), phenugoi (Arunachal Pradesh), Kerrundi (Bengali), Shalynnai (Meghalaya), phabau nga (Manipur), Ptetsung/Tsuto zer (Ao, Nagaland).

Characteristics of the species

The non-meristic characters, meristic measurements and counts and morphometrics of both male and female fishes have been described below.

Non-meristic profile: MALE (*Puntius chola)*

Body: fairly deep and compressed, abdomen rounded, ventral profile more or less convex than dorsal side. Head: small and compressed. The operculum of adult male and female shows an orange-red mark throughout the year. Mouth: small, terminal. Eyes: moderate, iridescent orange-red. Fins: dorsal fin smooth, yellow to orange, with entire spine, often with brown spots in older individuals, other fins delicate yellowish, caudal forked. Pelvic and anal fins are tinged orange colour. Barbels: one short maxillary pair, shorter then eye diameter. Lateral line: complete, with 26-28 scales, predorsal scales 12 to 14. Scales: small, cycloid. Colour: Distinct reddish spots to conspicuous dark blotches on the body, first behind operculum and second near caudal base. In live, uniform silky silvery with a strong olive green on the back and a delicate yellowish green on flanks.

Meristic measurements and counts of male *Puntius chola*

Table- 3: Measurements (cm) of 10 randomly selected males of *Puntius chola*

PARAMETER	FISH SPECIMEN										RANGE		MEAN
	I	II	III	IV	V	VI	VII	VIII	IX	X	Min	Max	
TL	5.8	6.5	7.1	6.2	7.0	6.7	6.5	6.3	6.7	5.9	5.8	7.1	6.5
HL	1.4	1.4	1.7	1.4	1.7	1.6	1.4	1.4	1.6	1.4	1.4	1.7	1.5
POL	0.4	0.4	0.5	0.4	0.5	0.5	0.4	0.4	0.5	0.4	0.4	0.5	0.4
PtOL	0.6	0.6	0.8	0.6	0.8	0.7	0.6	0.6	0.7	0.6	0.6	0.8	0.7
ED	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HD	1.0	1.1	1.2	1.1	1.2	1.1	1.1	1.1	1.1	1.0	1.0	1.2	1.1
HB	0.6	0.7	0.9	0.7	0.9	0.8	0.7	0.7	0.8	0.6	0.6	0.9	0.7
SnL	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4
IOL	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.6	0.6
INL	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4
GM	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4
SL	4.5	5.0	5.5	4.7	5.4	5.1	5.0	4.8	5.1	4.6	4.5	5.5	4.9

DFL	0.8	0.9	1.1	0.9	1.1	1.0	0.9	0.9	1.0	0.8	0.8	1.1	0.9
DFH	1.0	1.2	1.3	1.1	1.3	1.3	1.2	1.1	1.3	1.0	1.0	1.3	1.2
AFL	0.5	0.5	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.5
AFH	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.9	0.9
PFL	0.9	1.0	1.1	1.0	1.1	1.1	1.0	1.0	1.1	0.9	0.9	1.1	1.0
VFL	0.9	1.0	1.1	1.0	1.1	1.1	1.0	1.0	1.1	0.9	0.9	1.1	1.0
PDL	2.3	2.5	2.6	2.3	2.6	2.5	2.5	2.4	2.5	2.3	2.3	2.6	2.5
PtDL	1.4	1.6	1.8	1.5	1.7	1.6	1.6	1.5	1.6	1.5	1.4	1.8	1.6
BD	0.6	0.7	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.6	0.6	0.8	0.7
BH	1.3	1.3	1.5	1.3	1.5	1.4	1.3	1.3	1.4	1.3	1.3	1.5	1.4
PPL	1.3	1.5	1.6	1.4	1.6	1.5	1.5	1.4	1.5	1.4	1.3	1.6	1.5
PVL	2.3	2.6	2.9	2.5	2.9	2.7	2.6	2.5	2.7	2.4	2.3	2.9	2.6
DOPV	1.0	1.1	1.3	1.1	1.3	1.2	1.1	1.1	1.2	1.0	1.0	1.3	1.1
DOVA	0.9	1.0	1.2	1.0	1.2	1.1	1.0	1.0	1.1	0.9	0.9	1.2	1.0
CFL	1.3	1.5	1.6	1.5	1.6	1.6	1.5	1.5	1.6	1.4	1.3	1.6	1.5
CPL	0.8	0.9	1.1	0.9	1.0	0.9	0.9	0.9	0.9	0.8	0.8	1.1	0.9
LDCP	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.6	0.7	0.6	0.6	0.7	0.6
HDCP	0.7	0.7	0.9	0.7	0.9	0.8	0.7	0.7	0.8	0.7	0.7	0.9	0.8

Fin Formula: D iii 8; P iii 13; V i 8; A ii 6; C ix 17; Ltr 6; Ll 25.

Morphometric Characters of male *Puntius chola*

Pre-orbital length 3.50-3.40 (3.75), Post orbital length 2.33-2.13 (2.14), Eye diameter 3.50-4.25 (3.75), Head depth 1.40-1.42 (1.36), Head breadth 2.33-1.89 (2.14), Snout length 4.67-4.25 (3.75), Inter orbital length 2.80-2.83 (2.50) Inter nasal length 4.67-4.25 (3.75), Gap of mouth 4.67-4.25 (3.75) in relation to head length.

Eye diameter 1.25-1.50 (1.50), Snout length 1.67-1.50 (1.50) in relation to inter orbital length.

Dorsal fin length 5.63-5.00 (5.44), Dorsal fin height 4.50-4.23 (4.08), Anal fin length 9.00-9.17 (9.80), Anal fin height 5.63-6.11 (5.44), Pectoral fin length 5.00-5.00 (4.90), Ventral fin length 5.00-5.00 (4.90), Pre-dorsal fin length 1.96-2.12 (1.96), Post dorsal fin length 3.21-3.05 (3.06), Body depth 7.50-6.88 (7.00), Body height 3.46-3.67 (3.50), Pre-pectoral length 3.46-3.44 (3.27), Pre ventral length 1.96-1.89 (1.88), Distance between origin of pectoral fin and ventral fin 4.50-4.23 (4.45), Distance between origin of ventral fin and anal fin 5.00-4.58 (4.90), Caudal fin length 3.46-3.44 (3.27), Caudal peduncle length 5.63-5.00 (5.44), Least depth of caudal peduncle 7.50-7.86 (8.17), Highest depth of caudal peduncle 6.43-6.11 (6.13) in relation to the standard length.

Non-meristic profile of female *Puntius chola*

Body: fairly deep and compressed, abdomen rounded ventral profile more or less convex than dorsal side. Head: small and compressed. The operculum of adult male and female shows an orange-red mark throughout the year. Mouth: small, terminal. Eyes: moderate, iridescent orange-red. Fins: Dorsal fin smooth, yellow to orange, with entire spine, often with brown spots in older individuals, other fins delicate yellowish, caudal forked. All fins in female are quite clear or pale yellow. Barbels: one short maxillary pair, shorter then eye diameter. Lateral line: complete, with 26-28 scales, predorsal scales 12 to 14. Scales: small, cycloid. Colour: Distinct reddish spots to conspicuous dark blotches on the body, first behind operculum and second near caudal base. In live, uniform silky silvery with a strong olive green on the back and a delicate yellowish green on flanks.

Meristic measurements and counts of female *Puntius chola*

Table– 4: Measurements (cm) of 10 randomly selected females of *Puntius chola*

PARAMETER	FISH SPECIMEN										RANGE		MEAN
	I	II	III	IV	V	VI	VII	VIII	IX	X	Min	Max	
TL	8.2	8.7	8.2	7.1	7.8	6.8	6.4	7.9	6.4	7.4	6.4	8.7	7.5
HL	2.0	2.2	2.0	1.5	1.9	1.4	1.3	1.9	1.3	1.7	1.3	2.2	1.7
POL	0.7	0.7	0.7	0.4	0.6	0.4	0.4	0.6	0.4	0.5	0.4	0.7	0.5
PtOL	0.8	0.9	0.8	0.6	0.8	0.6	0.5	0.8	0.5	0.7	0.5	0.9	0.7
ED	0.5	0.6	0.5	0.5	0.5	0.4	0.4	0.5	0.4	0.5	0.4	0.6	0.5
HD	1.4	1.5	1.4	1.3	1.4	1.3	1.2	1.4	1.2	1.4	1.2	1.5	1.4
HB	0.9	0.9	0.9	0.8	0.9	0.8	0.7	0.9	0.7	0.9	0.7	0.9	0.8
SnL	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.4	0.5	0.4	0.5	0.5

IOL	0.7	0.8	0.7	0.6	0.7	0.6	0.5	0.7	0.5	0.7	0.5	0.8	0.7
INL	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4
GM	0.5	0.6	0.5	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.4	0.6	0.5
SL	6.6	7.0	6.6	5.6	6.2	5.3	4.9	6.3	4.9	5.9	4.9	7.0	5.9
DFL	1.2	1.3	1.2	1.0	1.2	1.0	0.9	1.2	0.9	1.1	0.9	1.3	1.1
DFH	1.5	1.6	1.5	1.3	1.5	1.3	1.2	1.5	1.2	1.4	1.2	1.6	1.4
AFL	0.7	0.8	0.7	0.6	0.7	0.6	0.5	0.7	0.5	0.7	0.5	0.8	0.7
AFH	1.1	1.2	1.1	1.0	1.1	1.0	0.9	1.1	0.9	1.1	0.9	1.2	1.1
PFL	1.2	1.3	1.2	1.1	1.2	1.1	1.0	1.2	1.0	1.2	1.0	1.3	1.2
VFL	1.2	1.3	1.2	1.1	1.2	1.1	1.0	1.2	1.0	1.2	1.0	1.3	1.2
PDL	3.3	3.4	3.3	2.7	2.9	2.6	2.4	3.0	2.4	2.8	2.4	3.4	2.9
PtDL	2.1	2.3	2.1	1.9	2.1	1.7	1.6	2.1	1.6	2.0	1.6	2.3	2.0
BD	0.9	1.0	0.9	0.8	0.9	0.8	0.7	0.9	0.7	0.9	0.7	1.0	0.9
BH	1.9	2.0	1.9	1.7	1.9	1.7	1.6	1.9	1.6	1.8	1.6	2.0	1.8
PPL	1.9	2.0	1.9	1.6	1.9	1.5	1.4	1.9	1.4	1.7	1.4	2.0	1.7
PVL	3.4	3.6	3.4	2.9	3.3	2.8	2.6	3.3	2.6	3.1	2.6	3.6	3.1
DOPV	1.5	1.6	1.5	1.3	1.4	1.3	1.2	1.4	1.2	1.4	1.2	1.6	1.4
DOVA	1.4	1.5	1.4	1.3	1.4	1.2	1.1	1.4	1.1	1.4	1.1	1.5	1.3
CFL	1.6	1.7	1.6	1.5	1.6	1.5	1.5	1.6	1.5	1.5	1.5	1.7	1.6
CPL	1.3	1.4	1.3	0.9	1.1	0.9	0.8	1.2	0.8	1.0	0.8	1.4	1.1
LDCP	0.9	0.9	0.9	0.7	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.9	0.8
HDCP	1.1	1.1	1.1	0.9	1.0	0.9	0.9	1.1	0.9	1.0	0.9	1.1	1.0

Fin Formula: D iii 9; P iii 12; V ii 7; A iii 6; C ix 17; Ltr 5; Ll 26.

Morphometric Characters of female *Puntius chola*

Pre-orbital length 3.25-3.14 (3.40), Post orbital length 2.60-2.44 (2.42), Eye diameter 3.25-3.67 (3.40), Head depth 1.08-1.47 (1.21), Head breadth 1.86-2.44 (2.13), Snout length 3.25-4.40 (3.40), Inter orbital length 2.60-2.75 (2.43) Inter nasal length 3.25-4.40 (4.25), Gap of mouth 3.25-3.67 (3.40) in relation to head length.

Eye diameter 1.25-1.33 (1.40), Snout length 1.25-1.60 (1.40) in relation to inter orbital length.

Dorsal fin length 5.44-5.38 (5.36), Dorsal fin height 4.08-4.38 (4.21), Anal fin length 9.80-8.75 (8.43), Anal fin height 5.44-5.83 (5.36), Pectoral fin length 4.90-5.38 (4.92), Ventral fin length 4.90-5.38 (4.92), Pre-dorsal fin length 2.04-2.06 (2.03), Post dorsal fin length 3.06-3.04 (2.95), Body depth 7.00-7.00 (6.56), Body height 3.06-3.50 (3.28), Pre-pectoral length 3.50-3.50 (3.47), Pre ventral length 1.89-1.94 (1.90), Distance between origin of pectoral fin and ventral fin 4.08-4.38 (4.21), Distance between origin of ventral fin and anal fin 4.45-4.67 (4.54), Caudal fin length 3.27-4.12 (3.69), Caudal peduncle length 6.13-5.00 (5.36), Least depth of caudal peduncle 7.00-7.78 (7.38), Highest depth of caudal peduncle 5.44-6.36 (5.90) in relation to the standard length.

3.2.2 Distribution trend

Nagaland: Rivers- Doyang (Wokha, District), Milak and Tsurang (Mokokchung, District), Intangki (Peren District), Lentic water bodies and Rice fields of Changki Valley (Mokokchung, District)

N.E. India: Arunachal Pradesh, Assam, Manipur, Meghalaya and Tripura.

Elsewhere: West Bengal, Bihar, Madhya Pradesh, Uttar Pradesh, Bangladesh; Nepal; Myanmar; Bhutan; Sri Lanka.

3.2.3 Ornamental precedence

Live *Puntius chola* purports silvery body with olive green colour on back and a yellowish sheen on flanks; under side pale. A large, blurred–edged rosy bloch on operculum and often a black bloch behind gill cover and a deep-black bloch, occasionally framed in gold near base of caudal fin. Eyes are orange red, dorsal fin yellow to orange, often with brown spots in matured specimens, and other fins yellowish. These characteristics put *Puntius chola* as classified ornamental fish.

3.2.4 Characteristics of the Habitat.

Puntius chola inhabits freshwater ponds, streams and small canals associated with paddy fields, both lentic and lotic water bodies. The lotic habited were areas where the water current was low with muddy/loamy bottom without dominant aquatic vegetations. The lentic water bodies were commonly standing or sluggish water such as, beels, inundated fields, ditches with a depth ranging between 2– 5 ft. **Plate- 4**.

The pH of the water bodies ranges from pH 6.5 –8.0, water temperature 10 - 26°C, dissolved oxygen 6.0 - 8.6 mg/l, total alkalinity 35 – 57 mg/l, total hardness 4.9 – 6.0 mg/l.

BEHAVIOURAL PERSPECTIVES

In general, the first noticeable reaction immediate to the application of food is an initial state of excitation with rapid movement of pectoral fins. Initially the fish never responds immediately to the presence of food. After acclimatizing, the fishes start feeding immediately.The modus operandi of food capture, period of quiescence, surfacing behaviour, operculum and fins movement, postures of feeding, actual mode of movement and other related changes of the fish are precisely documented.

A schematic diagram of the overall trend of feeding when fed with supplementary feed is shown below.

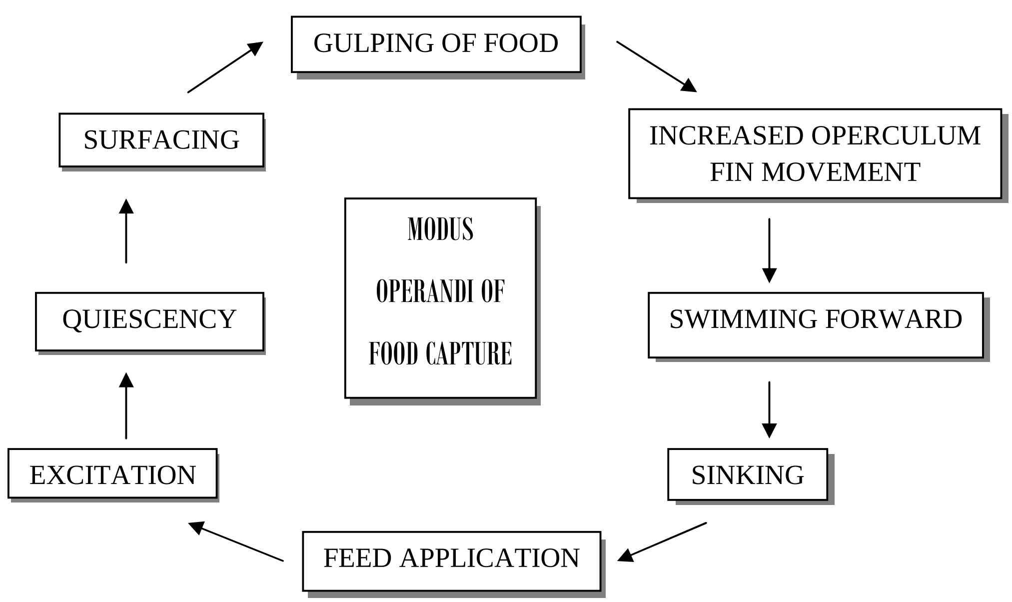

Figure-3: Feeding trend in fishes

3.3.1 Ingestive behavior of *Danio dangila*

The feeding behaviour of the test species was precisely investigated in the aquaria and its line diagram presented in **figure-4**. Responses and reactions of the test specimens towards their food elements introduced into the eco-system were visually analyzed. The feeding activities commence with the fish swiftly gulping the food elements making an angle of 70^o. The species was found to feed mostly in the surface zone. But after acclimatization, it was seen to execute at the column level, the fish rests at the bottom zone making an angle parallel to the water level with the beating of the pectoral fin, the dorsal spines are kept erect and the rays beat slowly. After gulping the food, the fish quickly returns to its initial position and conveniently swallows the gulp with slow opercular movements and with rhythmic beating of the pectoral fin. On the other hand, the fish moves down for bottom feeding from the column at an angle of 50^o.

Fin reciprocation and the opercular movement of the test species were critically studied and expressed as frequency/sec and are purported below:

Dorsal: 1.1 – 1.4 (x 1.0± 0.109); Pectoral: 1.4 – 1.8 (x 1.0 ± 0.75); Ventral: 1.0 – 1.4 (x 1.0 ± 0.134); Anal: 1.1 – 1.5 (x 1.0 ± 0.147) and Caudal: 1.9 – 2.4 (x 2.0 ± 0.174); Opercular movement: 1.7 – 2.1 (x 1.8 ± 0.136)

3.3.2 Procreatic demeanor of *Danio dangila*

Courtship: Before the actual courtship display, the male and female *Danio dangila* exhibit erratic and swift vertical and horizontal movements at the column of the aquaria, they also exhibit circular movements from time to time. The male displays itself by beating rapidly its caudel fin. Both male and female moves parallel to each other swimming too and fro in a harmonious movement. The actual courtship display starts approximately 4 hrs after inducing the pair (line diagram presented in **figure-5**).

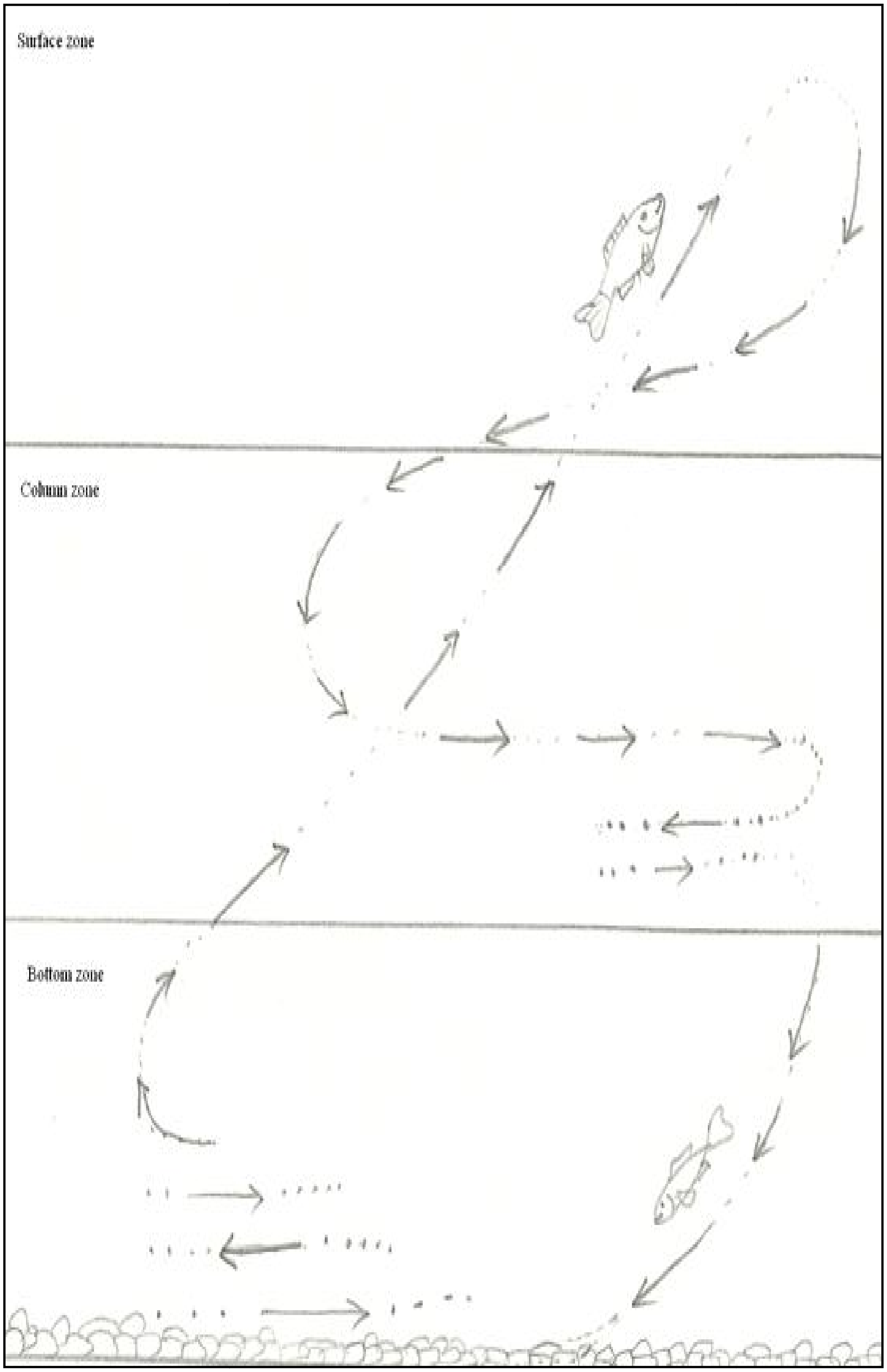

Figure-4: Schematic diagram showing lineament of feeding in *Danio dangila*.

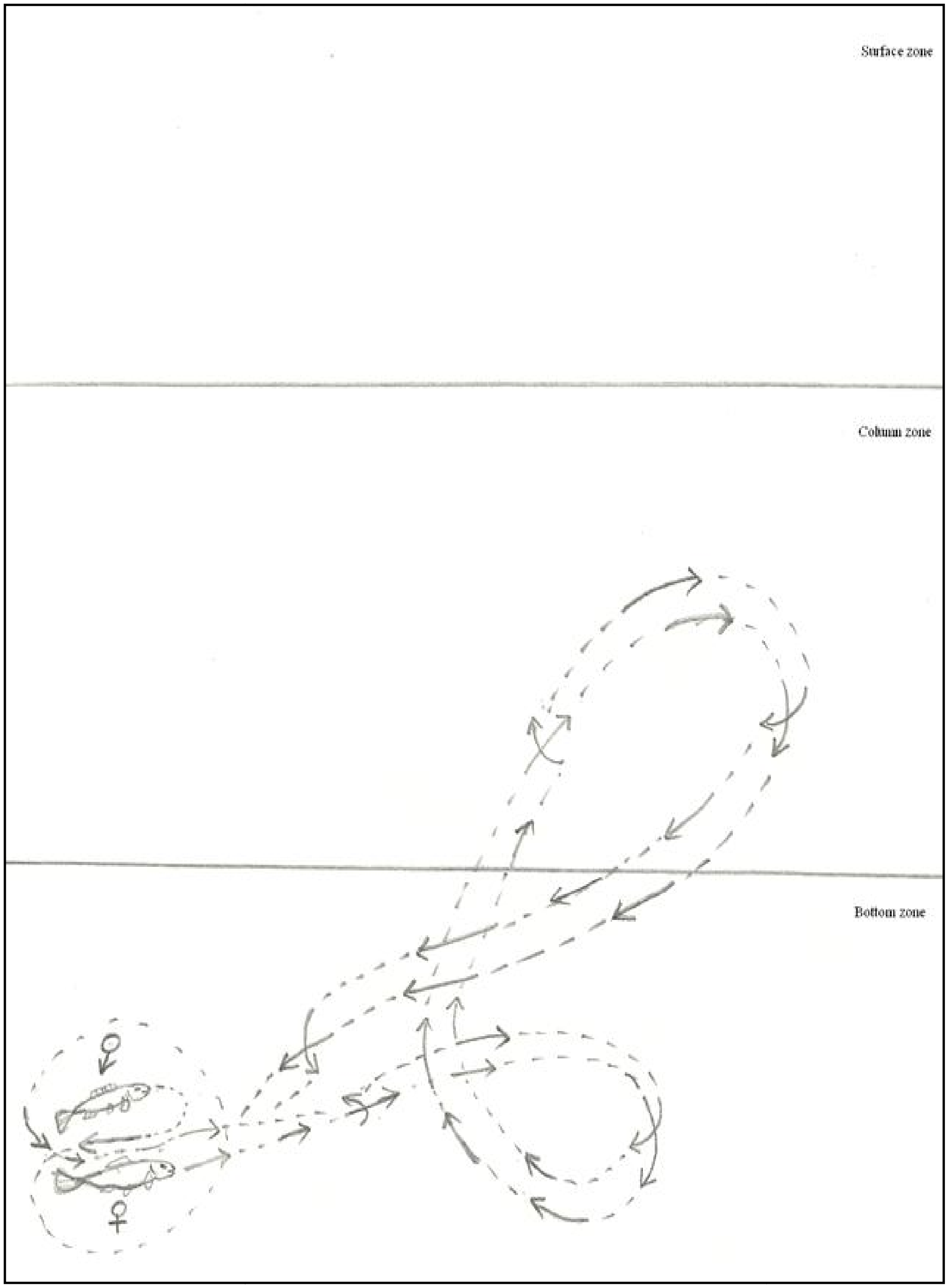

Figure- 5: Schematic diagram showing lineament of procreatic demeanor in *Danio dangila.*

The male nudges the female with the snout and pushes the female upwards and bends down and brings its genital pore in proximity with the female's genital pore enticing and interlocking the female with the pelvic and anal fin. Thereafter, the male rubs the vent of the female vigorously for 0.5 – 1 min. The display continues for 3 to 4 hours whereby the female delivers eggs in several batches and the male sheds milts over the eggs, thereby fertilizing the eggs. After the display, both male and female settles down at the bottom of the aquaria with increased opercular movement.

Parental care: Parental care was not shown by the parents towards the fertilized eggs.

3.4.1 Ingestive behavior of *Puntius chola*

The feeding behaviour of the test species was precisely investigated in the aquaria. The schematic diagram is presented in **figure-6**. Reaction and responses of the fish under study towards their food elements introduced into the eco-system were visually analysed. With a patient maneuvere to deduce 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. It was observed that, the test species do not attack food if not hungry. The test fish was found to feed mostly in the column zone. But after acclimatization, it was seen to execute at the surface level, the fish mostly rest at the bottom zone with frequent visit to the column zone. After gulping the food, the fish quickly returns to its initial position and conveniently swallows the gulp with slow opercular movements and with rhythmic beating of the pectoral fin. On the other hand, the fish moves down for bottom feeding from the column at an angle of 60^{o}.

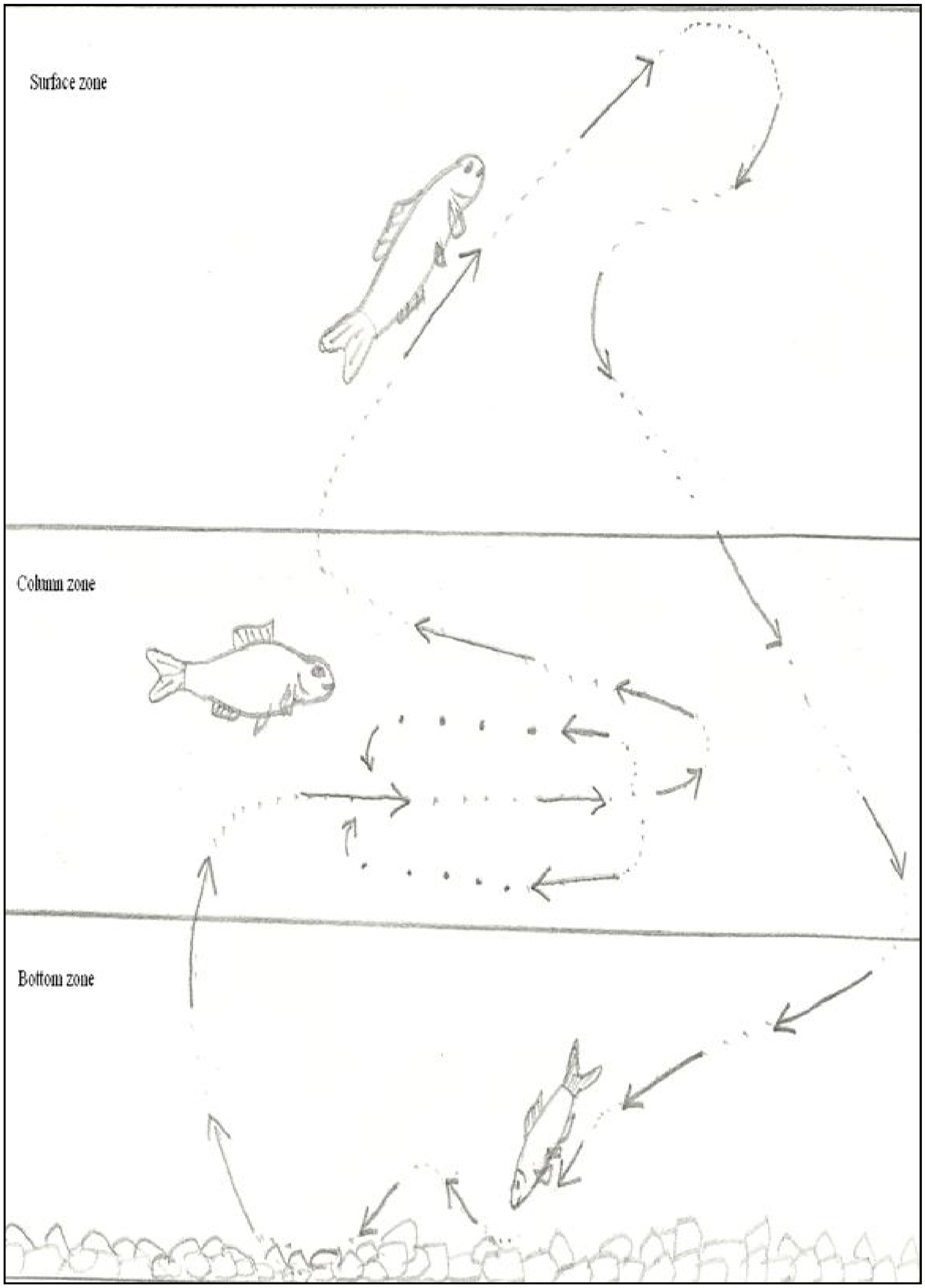

Figure- 6: Schematic diagram showing lineament of feeding in *Puntius chola*.

Dorsal: 1.1 – 1.8 (x 1.0± 0.261); Pectoral: 1.5 – 2.0 (x 1.7 ± 0.162); Ventral: 1.0 – 1.5 (x 1.0 ± 0.172); Anal: 1.1 – 1.5 (x 1.0 ± 0.356) and Caudal: 1.2 – 1.7 (x 1.0 ± 0.185); Opercular movement: 1.9 – 2.4 (x 2.0 ± 0.172).

3.4.2 Procreatic demeanor of *Puntius chola*

Courtship: Before the actual courtship display, the male and female *Puntius chola* exhibit restlessness and frequently moving from the base to surface of the aquaria. Both male and female moves parallel to each other in a rythemic movement. Schematic diagram presented in **figure-7**.The actual courtship display starts approximately 6 hrs after inducing the pair. The male nudges the female with the snout and pushes the female upwards and then, the male bends down and brings its genital pore in proximity with the female's genital pore enticing and interlocking the female with the pelvic and anal fin. Thereafter the male rubs the vent of the female vigorously for 30 seconds to 1 minute. The display continues for 3 to 4 hours whereby, the female releases eggs in several batches and the male sheds milts over the eggs, thereby fertilizing the eggs. After the act, both male and female settles down at the bottom of the aquaria with increased opercular movement.

Parental care: Parental care was not shown by any of the parents towards the fertilized eggs.

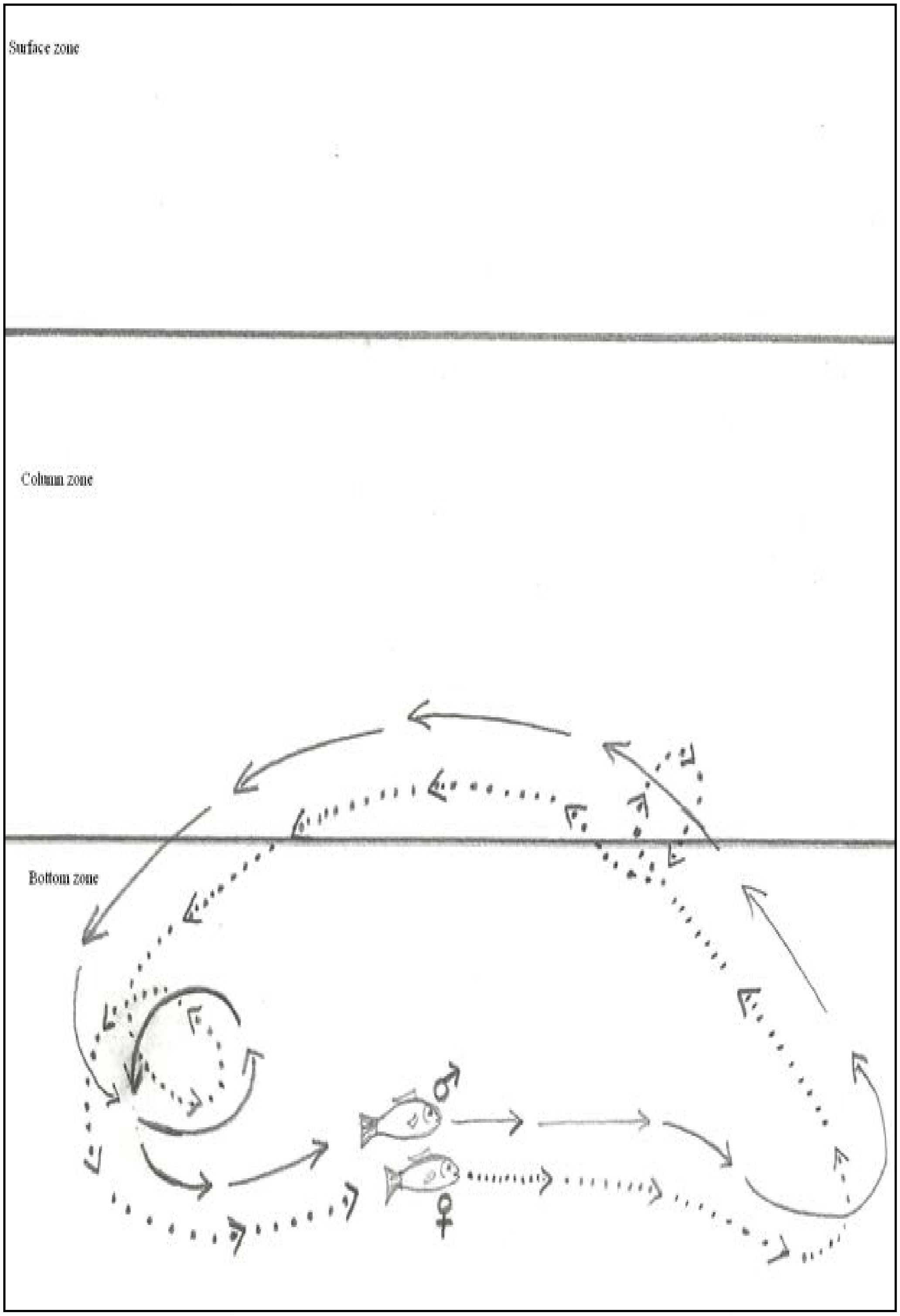

Figure- 7: Schematic diagram showing lineament of procreatic demeanor in *Puntius chola*

BIONOMICS PROFILE

Feeding is one of the most important functions of an organism. The basic functions of an organism, its growth, development, reproduction etc, all take place at the expense of the energy which enters the organism in the form of its food (Nikolsky, 1963). The details of victual spectra, lineament of Feeding, relative length of gut, hepato-somatic index, index of preponderance and gill raker exposition of *Danio dangila* and *Puntius chola* were analysed.

The food propensity enumeration of the freshwater ornamental fish species would help in their successful culture in confined water and in adopting suitable management technique to revitalize the dwindling stocks in natural lotic and lentic water.

3.5.1 Victual spectra of *Danio dangila*

On an average, 20 fishes per season were analysed for victual spectra out of which the gut of an average of 3 fishes (15%) were found empty and 17 fishes (85%) possessed filled stomach.

On the basis of index of preponderance (PI), the foods of the fish were summarized as follows during different seasons and presented in table- 5. The pie chart on the foods of the fish is given in **figure -8**.

Table- 5: Food of *Danio dangila* in different seasons.

Pre-monsoon: (March – May)	Aquatic insects (20.5 %)> Debris, mud/sand (24.5%)> Decaying plant matters (20%)> algaes (20 %)> unidentified food items (15%).
Monsoon (Jun. – August)	Aquatic insects (26.5 %)> Debris, mud/sand (32%)> Decaying plant matters (16%)> algaes (17 %)> Unidentified food items (8.5%).

Post – monsoon (Sep. – Nov.)	Aquatic insects (42%)> Debris, mud/sand (12%)> Decaying plant matters (21%)> algaes (12 %)> Unidentified food items (13%).
Winter (Dec. – Feb.)	Aquatic insects (13%)> Debris, mud/sand (31%)> Decaying plant matters (24%)> algaes (21 %)> Unidentified food items (11%).

3.5.2 Lineament of Feeding

As surface feeder the fish move to the column, stay for sometime and gulp the food swiftly from the surface → return to the bottom or retraces back among aquatic plants → moves to the column level → intake of food → returns to the bottom level → slowly returns to the column level.

3.5.3 Relative length of gut

The relative length of gut (RLG) of the test fish species have been studied separately for male and female and were purported below in Table 6 & 7.

Table- 6: RLG in different size range of male *Danio dangila*

Sl.No	SIZE RANGE (TL mm)	RLG (cm)
1.	0 – 45	0.535
2.	45 – 50	0.622
3.	50 – 55	0.640
4.	55 – 60	0.648
5.	60 – 65	0.569
6.	65 – 70	0.588
7.	70 – 75	0.611
8.	75 – 80	0.679

Table- 7: RLG in different size range of female *Danio dangila*

Sl.No	SIZE RANGE (TL mm)	RLG (cm)
1.	0 – 45	0.511
2.	45 – 50	0.600
3.	50 – 55	0.588
4.	55 – 60	0.500
5.	60 – 65	0.524
6.	65 – 70	0.567
7.	70 – 75	0.557
8.	75 – 80	0.747

3.5.4 Hepato-somatic index (HSI)

The HSI of *Danio dangila* were studied separately for both male and female and recorded below in table 8 and 9 respectively.

Table- 8: HSI in different size range of male *Danio dangila*

Sl. No	TL (mm)	HSI	HSI RANGE	HSI MEAN
1.	43	1.051	0.652 – 1.849	1.251
2.	45	1.849		
3.	50	1.535		
4.	54	1.458		
5.	58	1.514		
6.	65	1.150		
7.	68	1.238		
8.	71	0.652		
9.	72	0.826		
10.	78	1.017		

Table- 9: HSI in different size range of female *Danio dangila*

Sl. No	TL (mm)	HSI	HIS RANGE	HIS MEAN
1.	42	0.796	0.796 – 1.327	1.062
2.	50	1.327		
3.	51	0.844		
4.	54	0.875		
5.	58	0.804		
6.	60	0.830		
7.	63	0.879		
8.	67	1.083		
9.	70	0.813		
10.	75	1.128		

3.5.5 Index of preponderance

The index of preponderance (PI) of different food items was recorded in 20 specimens ranging from 4.2.-7.8 cm in TL. The PI does not differ significantly between male and female in the present species, which is why, an overall trend of PI in *Danio dangila* was studied and depicted in table-10.

Table- 10: Index of pre-ponderance in *Danio dangila*

FOOD ITEMS	OCCURRENCE O*i* (%)	VOLUME V*i* (%)	O*i* V*i*	PI
Aquatic insect parts	24.39	25.32	617.55	30.87
Algaes	21.95	18.99	416.83	20.83
Decaying plant matters	23.17	20.25	469.19	23.45
Debris (mud and sand)	19.51	12.66	246.99	12.35
Unidentified food item	10.98	22.78	250.12	12.50

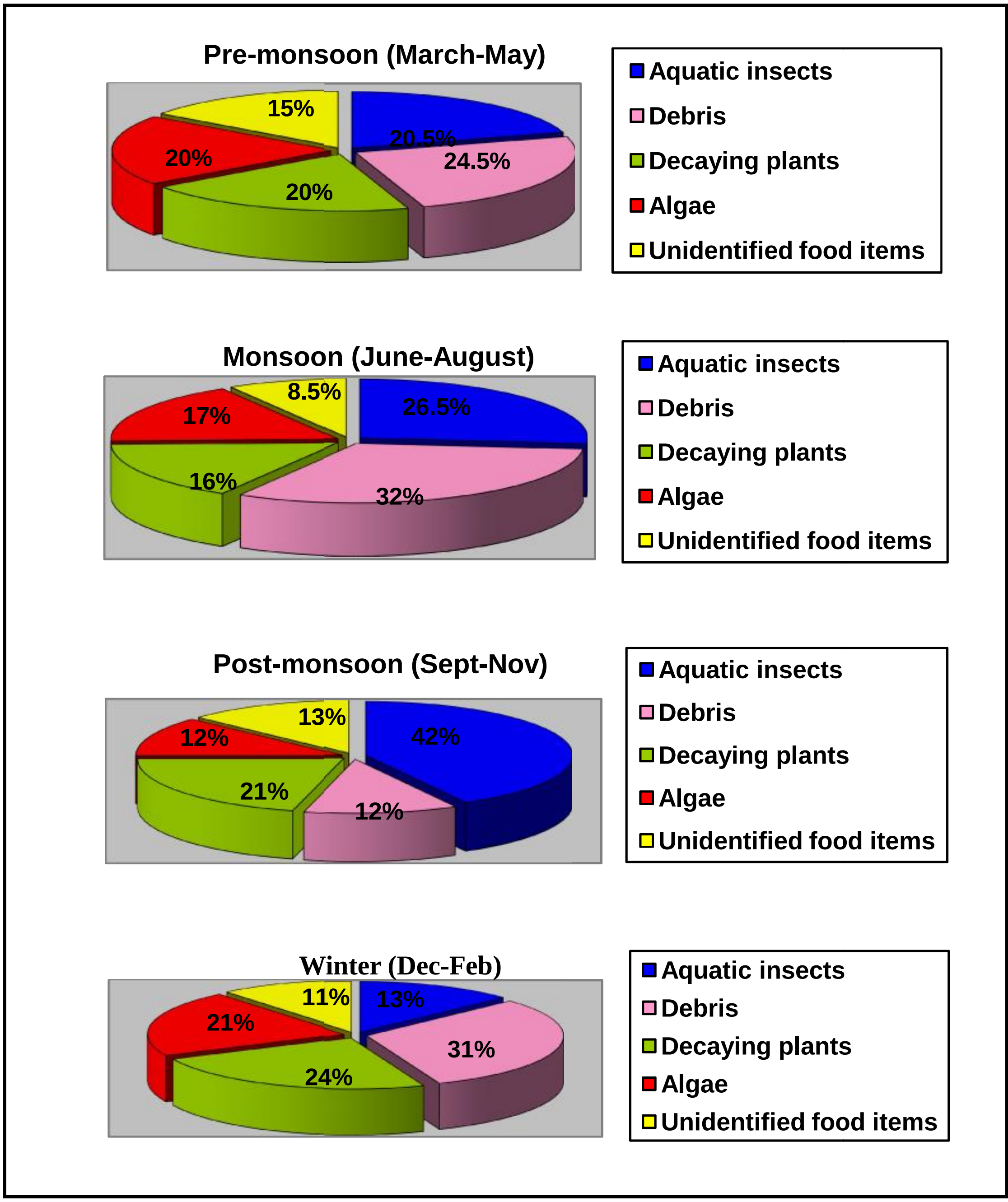

Figure- 8: Victual spectra of *Danio dangila*

3.5.6 Gill raker exposition

The anterior portion of the ventro-lateral wall of the pharyngeal cavity was perforated by five pairs of gill slit-like opening, separated by four pairs of gills. Each gill has a long lower limb and a shorter upper limb. The four gill arch separates the five gill slits in either side. Each gill arch were provided with two rows of teeth like gill rakers on the inner concave border projecting internally into the pharyngeal cavity, and two rows of comb like filament or gill lamellae on outer convex border.

The gill raker protects the delicate gill lamellae from injury apart from its principle role in feeding. They serve to prevent the food particles from escaping out through the respiratory current of water. The rakers form a very effective sieve like apparatus across the gill slits for filtering the water, in order to retain the food items in the bucco-pharynx. Gill raker was modified in relation of food and feeding habit.

The gill rakers of *Danio dangila* are short, stumpy and blunt. The gill rakers are longer in the central part of both the limb. The detail gill raker morphology of the test species is given below in table -11. The line diagram of gill raker exposition of *Danio dangila* is presented in **figure-9** and coloured photograph in **plate-5**

Table- 11: Morphology of gill raker in *Danio dangila*

Particulars	***Danio dangila***
Size range of fish	6.5 – 9 cm
No. of gill raker/gill arch	26
Size of gill raker	0.01 – 0.03 cm
Length of gill lamellae	0.2 – 0.3 cm
Length of gill arch	0.9 – 1.2 cm.

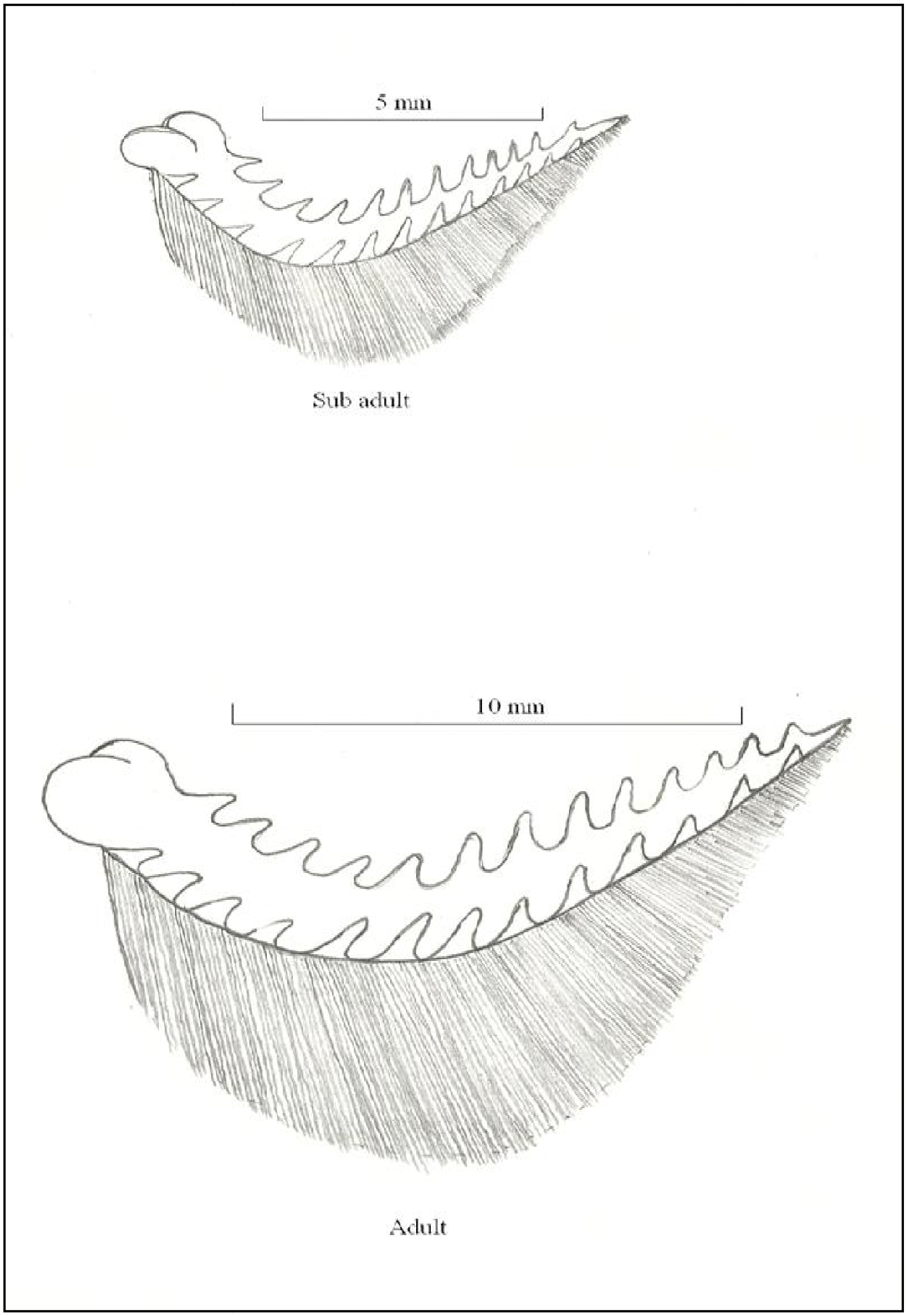

Figure- 9: Schematic diagram showing gill reker traits in *Danio dangila*

3.6.1 Victual spectra of *Puntius chola*

On an average, 20 fishes per season were analysed for victual spectra out of which the gut of no fish (0%) were found empty and 20 fishes (100%) possessed filled stomach.

On the basis of PI, the food of the test fish have been summarized as follows in different seasons and presented in table- 12. The pie chart on the foods of the fish is presented in **figure -10**.

Table- 12: Food of *Puntius chola* in different seasons.

Pre-monsoon: (March – May)	Algaes (49%)> Decaying plant matters (32%)> Debris, sand and mud (10.5 %) >Aquatic insects (5.5%) > Unidentified food items (3%)
Monsoon (Jun. – August)	Algaes (26%)> Decaying plant matters (28.5%)> Debris, sand and mud (20.3%) Aquatic insects (19.2%)> Unidentified food items (6%)
Post – monsoon (Sep. – Nov.)	Algaes (38.5%)> Decaying plant matters (29%)> Debris, sand and mud (14%) Aquatic insects (11.5%)> Unidentified food items (7%)
Winter (Dec. – Feb.)	Algaes (47.2%)> Decaying plant matters (16.9%)> Debris, sand and mud (17.4%) Aquatic insects (9.5%) Unidentified food items (9%)

3.6.2 Lineament of Feeding

As column feeder the fish move to the column, stay for sometime and gulp the food swiftly → return to the bottom or retraces back among aquatic plants → moves to the column level → intake of food → returns to the bottom level → slowly returns to the column level.

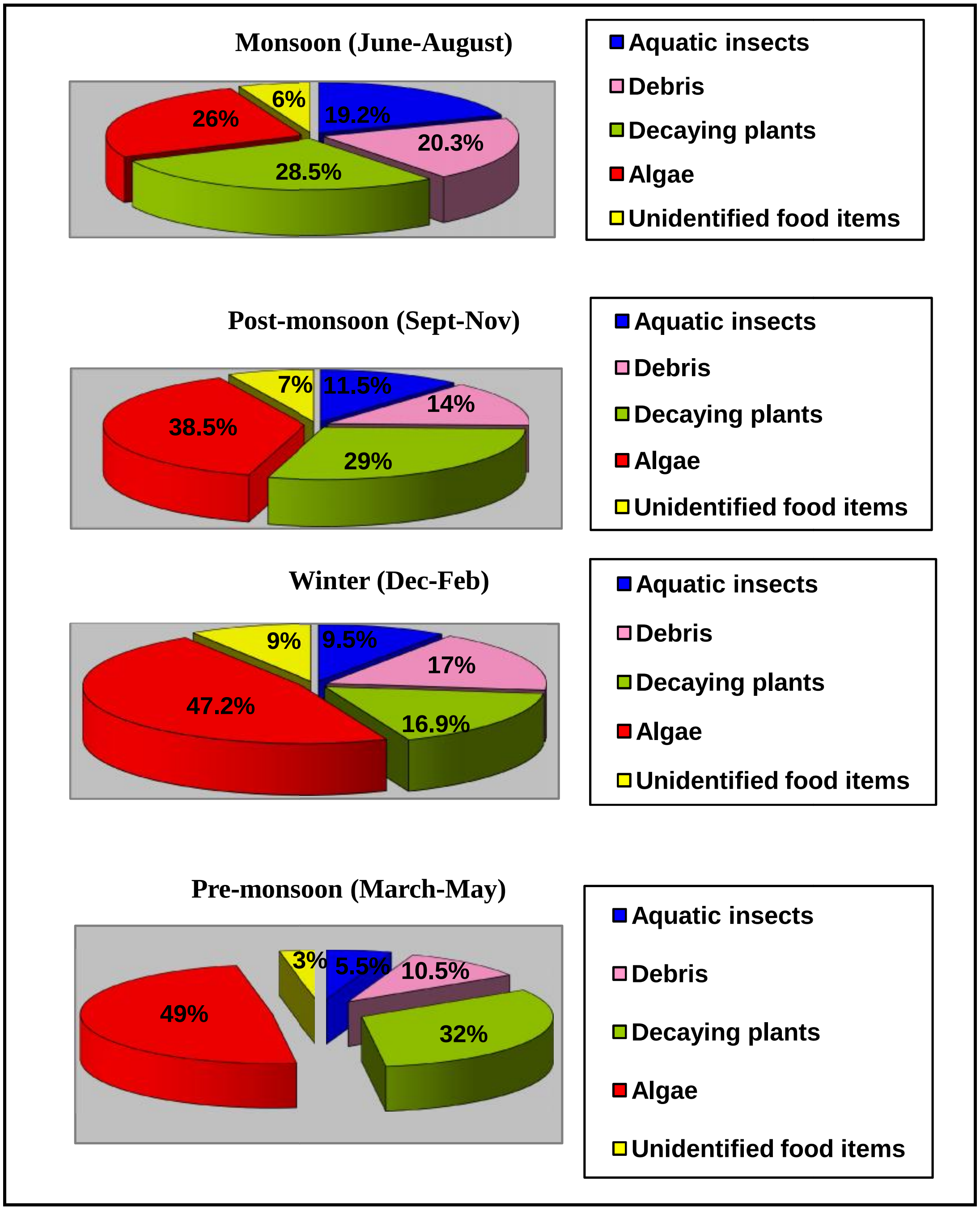

Figure- 10: Victual spectra of *Puntius chola*

3.6.3 Relative length of gut

The relative length of gut (RLG) of the test fish species have been studied separately for male and female and is purported below in Table 13 and 14.

Table- 13: RLG in different size range of male *Puntius chola*

Sl. No	SIZE RANGE (TL mm)	RLG (cm)
1.	45 - 50	3.318
2.	50 - 55	2.827
3.	55 - 60	2.655
4.	60 - 65	2.377
5.	65 - 70	2.242
6.	70 - 75	1.932
7.	75 - 80	1.842
8.	80 - 85	1.728

Table- 14: RLG in different size range of female *Puntius chola*

Sl. No	SIZE RANGE (TL mm)	RLG (cm)
1.	45 - 50	2.542
2.	50 - 55	2.222
3.	55 - 60	2.034
4.	60 - 65	2.111
5.	65 - 70	1.571
6.	70 - 75	1.667
7.	75 - 80	1.513
8.	80 - 85	1.390

3.6.4 Hepato-somatic index (HSI)

The HSI of *Puntius chola* were studied separately for both male and female and recorded below in a tabular form.

Table- 15: HSI in different size range of male *Puntius chola*

Sl.No	TL (mm)	HSI	HIS RANGE	HIS MEAN
1.	44	1.535	0.766 – 2.225	1.496
2.	46	1.374		
3.	52	1.514		
4.	55	1.809		
5.	58	1.653		
6.	61	0.766		
7.	66	2.225		
8.	74	2.213		
9.	76	1.082		
10.	81	0.987		

Table- 16: HSI in different size range of female *Puntius chola*

Sl.No	TL (mm)	HSI	HIS RANGE	HIS MEAN
1.	44	2.825	0.494 – 2.825	1.659
2.	48	2.720		
3.	54	1.088		
4.	58	1.299		
5.	63	0.714		
6.	66	1.078		
7.	70	1.442		
8.	72	0.687		
9.	76	0.494		
10.	82	0.855		

3.6.5 Index of preponderance

The index of preponderance (PI) of different food items was recorded in 20 specimens ranging from 4.5-8.5 cm in TL. The PI does not differ significantly between male and female in the present species, which is why, an overall trend of PI in *Puntius chola* has been studied and is depicted in Table-17.

Table- 17: Index of pre-ponderance in *Puntius chola*

FOOD ITEMS	**OCCURRENCE O*i* (%)**	**VOLUME V*i* (%)**	**O*i* V*i***	**PI**
Aquatic insects and parts	14.71	10.00	147.1	6.79
Algaes	26.47	28.33	749.89	34.61
Decaying plant matters	22.06	25.00	551.5	25.45
Debris (mud and sand)	25.00	21.67	541.75	25.00
Unidentified food item	11.76	15.00	176.4	8.14

3.6.6 Gill raker exposition

The anterior portion of the ventro-lateral wall of the pharyngeal cavity was perforated by five pairs of gill-slit like opening separated by four pairs of gills. Each gill has a long lower limb and a shorter upper limb. The four gill arch separates the five gill slits in either side. Each gill arch was provided with two rows of teeth like gill rakers on the inner concave border projecting internally into the pharyngeal cavity, and two rows of comb like filament or gill lamellae on outer convex border.

The gill raker protects the gill lamellae from injury apart from its principle role in feeding. They serve in preventing the food particles from escaping out through the respiratory current of water. The rakers form a very effective sieve like apparatus across the gill slits for filtering the water in order to retain the food items in the bucco-pharynx. Gill raker is modified in relation of food and feeding habit.

The gill rakers of *Puntius chola* are short, straight and pointed. The gill rakers are longer in the central part of both the limb. The detail gill raker morphology of the test species is given below in table -18. The line diagram of gill raker exposition of *Puntius chola* is presented in **figure-11** and coloured photograph in **plate-5**

Table- 18: Morphology of gill raker in *Puntius chola*

Particulars	***Puntius chola***
Size range of fish	6.6 – 8.2 cm
No. of gill raker/gill arch	52
Size of gill raker	0.03 – 0.05 cm
Length of gill lamellae	0.3 – 0.5 cm
Length of gill arch	1 – 1.2 cm.

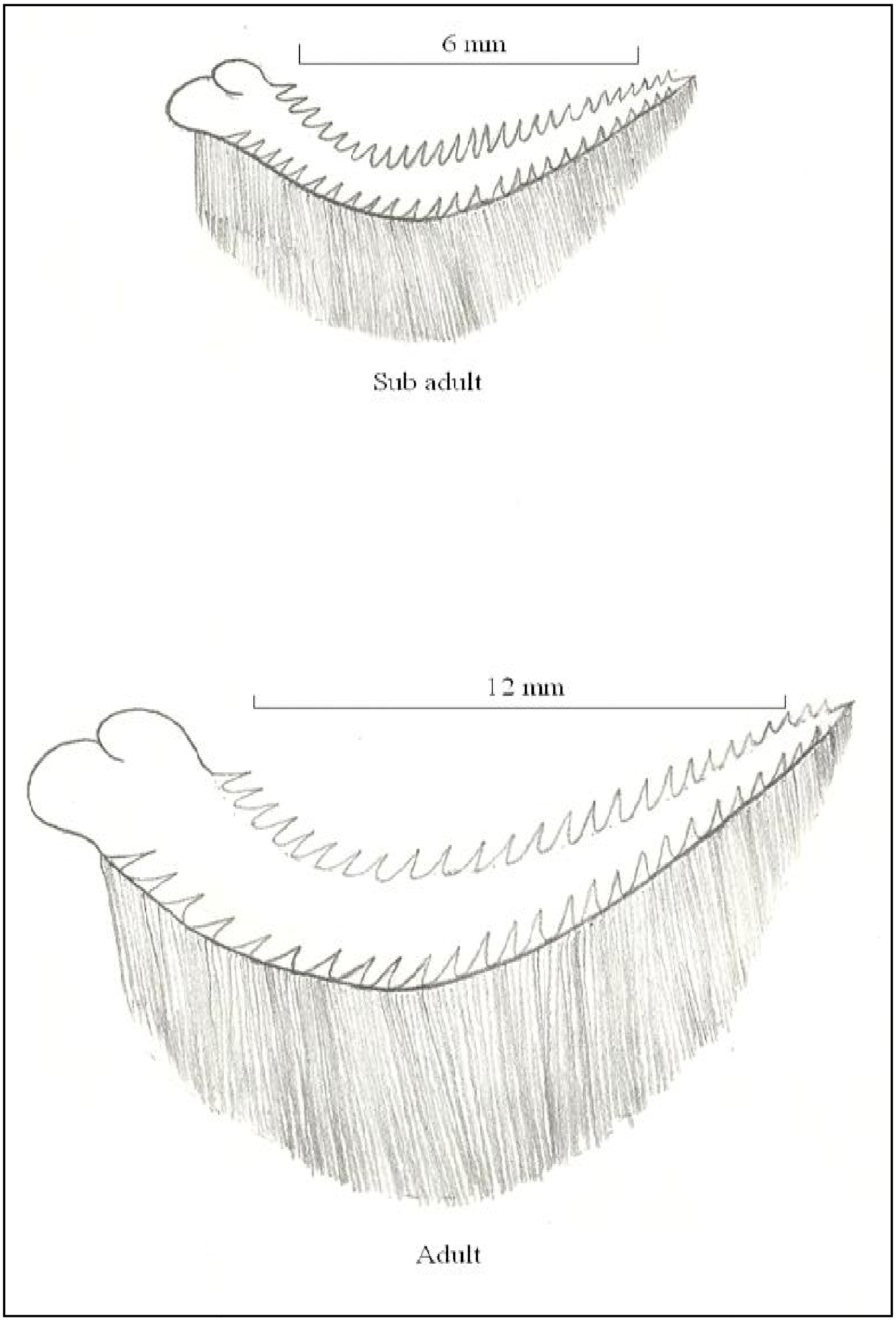

Figure- 11: Schematic diagram showing gill raker traits in *Puntius chola*

LENGTH -WEIGHT RELATIONSHIP

The weight-length relationship in the study species *Danio dangila* and *Puntius chola* have been estimated for mixed population (male and female combined), and male and female separately for both the species.

3.7.1 Length-weight relationship in mixed population of *Danio dangila*

The data on *Danio dangila* of mixed population consist of measurements of 20 specimens ranging in total length (TL) from 4.8 cm to 8.0 cm and in weight from 1.2 gm to 5.4 gm. The formula correlating the length and weight of the fish was found to be,

$$\textbf{Weight = -1.909 x Length}^{2.114}$$

The logarithmic form of the above equation is

$$\textbf{Log weight = 0.281 + 2.114 log length}$$

The coefficients of correlation between log Length and log Weight was + 0.959 and the standard error of estimate in terms of logs was ± 0.01

The test of significance of the relationship, t-test was performed, and the correlation between log length and log weight was found significant at 5% probability level.

$$\mathbf{r = +\,0.959 \pm 0.01,\ P < 0.05}$$

3.7.2 Length-weight relationship in male population of *Danio dangila*

The data on male population consist of measurements of 15 specimens ranging in total length (TL) from 4.8 cm to 8.0 cm and in weight from 1.1 gm to 5.2 gm. The formula correlating the length and weight of the fish was found to be,

$$\textbf{Weight = -1.885 x Length}^{2.103}$$

The logarithmic form of the above equation is

$$\textbf{Log weight = 0.275 + 2.103 log length}$$

The coefficients of correlation between log Length and log Weight was + 0.979 and the standard error of estimate in terms of logs was ± 0.01.

The test of significance of the relationship, t-test was performed, and the correlation between log length and log weight was found significant at 5% probability level.

$r = +0.979 \pm 0.01, P < 0.05$

3.7.3 Length-weight relationship in female population of *Danio dangila*

The data on female population consist of measurements of 15 specimens ranging in total length (TL) from 5.0 cm to 8.3 cm and in weight from 1.1 gm to 6.2 gm. The formula correlating the length and weight of the fish was found to be,

$$\textbf{Weight} = -2.022 \times \textbf{Length}^{2.055}$$

The logarithmic form of the above equation is

$$\textbf{Log weight} = 0.306 + 2.055 \textbf{ log length}$$

The coefficients of correlation between log Length and log Weight was + 0.977 and the standard error of estimate in terms of logs was ± 0.01.

The test of significance of the relationship, t-test was performed, and the correlation between log length and log weight is found significant at 5% probability level.

$r = +0.977 \pm 0.01, P < 0.05$

3.7.4 Condition Coefficient

Le Cren's condition factor was estimated separately for mixed, male and female population of *Danio dangila*. The estimated values with range (in parenthesis) and in average were:

Mixed population : Kn = 0.9416 (0.9581- 1.1319)
Male population : Kn = 0.9137 (0.9144 -1.0327)
Female population : Kn = 1.1294 (0.9865 -1.1824)

The data purport that, the well-being of the fish was comperatively better exhibited in female population than the male population.

3.8.1 Length-weight relationship in mixed population of *Puntius chola*

The data on *Puntius chola* of mixed population consist of measurements of 20 specimens ranging in total length (TL) from 5.8 cm to 8.5 cm and in weight from 1.8 gm to 6.8 gm. The formula correlating the length and weight of the fish was found to be,

$$\textbf{Weight} = -2.309 \times \textbf{Length}^{2.112}$$

The logarithmic form of the above equation is

$$\textbf{Log weight} = 0.363 + 2.112 \textbf{ log length}$$

The coefficients of correlation between log Length and log Weight was +0.977 and the standard error of estimate in terms of logs was ± 0.01

The test of significance of the relationship, t-test was performed, and the correlation between log length and log weight was found significant at 5% probability level.

$$r = +\,0.977 \pm 0.01,\ P < 0.05$$

3.8.2 Length-weight relationship in male population of *Puntius chola*

The data on male population consist of measurements of 15 specimens ranging in total length (TL) from 5.8 cm to 7.8 cm and in weight from 1.8 gm to 4.5 gm. The formula correlating the length and weight of the fish was found to be,

$$\textbf{Weight} = -2.505 \times \textbf{Length}^{2.506}$$

The logarithmic form of the above equation is

$$\textbf{Log weight} = 0.399 + 2.506 \textbf{ log length}$$

The coefficients of correlation between log Length and log Weight was + 0.979 and the standard error of estimate in terms of logs was ± 0.01

The test of significance of the relationship, t-test was performed, and the correlation between log length and log weight was found significant at 5% probability level.

$$r = +0.\,979 \pm 0.01,\ P < 0.05$$

3.8.3 Length-weight relationship in female population of *Puntius chola*

The data on female population consist of measurements of 15 specimens ranging in total length (TL) from 6.4 cm to 8.7 cm and in weight from 2.4 gm to 6.5 gm. The formula correlating the length and weight of the fish was found to be,

$$\textbf{Weight = -2.529 x Length } 6^{2.271}$$

The logarithmic form of the above equation is

$$\textbf{Log weight = 0.403 + 2.271 log length}$$

The coefficients of correlation between log Length and log Weight was + 0.980 and the standard error of estimate in terms of logs was ± 0.01.

The test of significance of the relationship, t-test was performed, and the correlation between log length and log weight was found significant at 5% probability level.

$$r = +0.980 \pm 0.01, P < 0.05$$

3.8.4 Condition Coefficient

Le Cren's condition factor was estimated separately for mixed, male and female population of *Puntius chola*. The estimated values with range (in parenthesis) and in average were:

Mixed population : Kn = 0.87395 (0.81117-0.97471)

Male population : Kn = 1.00955 (0.91211-1.15096)

Female population : Kn = 0.91266 (0.90117-1.01226)

The data purport that the well-being of the fish was comperatively better exhibited in male population than the female population.

REPRODUCTIVE BIOLOGY

To reproduce and to multiply in a geometrical ratio is the innate capacity of every organism. Among all the living species of fishes existing in the world at present, wide variety of patterns of reproduction such as unisexual, bisexuality, hermaphroditism and parthenogenesis are observed. The most predominant is bisexual reproduction. In order to evolve and standardize an efficient hatchery system for producing young fish of good quality in sufficient numbers of a given species, it is essential to know the detail reproductive biology of the fish. This involves the study of (i) sexual dimorphism and sex ratio (ii) size at first maturity (iii) ova and maturity stage of gonad (iv) fecundity (v) gonado somatic index (vi) breeding season and periodicity and (vii) spawning habitat.

Sexual dimorphism and sex ratio

Most of the fishes exhibit sexual dimorphism or secondary sexual characters by which sexes can be distinguished from each other. In few fishes secondary sexual characters are discernable throughout the life span whereas in some others they are discernable only during the breeding session. Secondary sexual characters serve several functions such as (a) Recognition of opposite sex by the members of a given sex. (b) Helping in the act of copulation such as sexual embrace (c) transfer of spermatozoa from male to female and (d) facilitating paternal care.

The maintenance of an economic ratio of the number of males to the female in the nature is called sex ratio. The sex ratio varies considerably from species to species due to the very specific breeding habits and limitations of food, space and other abiotic limiting factors of the environment. In the present study, the sexual dimorphism and sex ratio of the test fishes are studied as dependable key for the breeding programe.

3.9.1 Sexual dimorphism of *Danio dangila*

In *Danio dangila* the sexual dimorphism exhibits slightly dwarf males and large females. External morphological differences between male and female pertain to the following features is presented in table-19 and coloured photograph in **plate-6**

Table- 19: In vitro sexual dimorphism of *Danio dangila*

PARAMETER	MALE	FEMALE
BODY SHAPE	Body slender, dorsal profile slightly curved, ventral profile straight.	Body stumpy, dorsal and ventral profile curved.
FIN CHARACTERS	1. Tip of the anal fin was redish. 2. Fins with redish tinge colouration.	1. Tip of the anal fin was Whitish yellow. 2. Fins with yellowish tinge colouration.
ANAL OPENING	Oval	Round

Besides, there were some morphometrics differences between male and female and are given below:

POL in male 3.33 – 5.33 in HL (*versus* 5.00 – 5.33 in female); AFL in male 6.33 – 4.71 in SnL (*versus* 5.85 – 4.85 in female); PFL in male 4.22 – 3.88 in SnL (versus 4.10 – 4.25 in female); BD in male 7.60 – 6.60 in SnL (versus 8.20 – 4.85 in female); Distance between origin of pelvic fin and origin of anal fin 7.60 – 6.00 in SnL (versus 4.10 – 4.85 in female).

3.9.2 Sex ratio

Sex ratio was ascertained from natural stock through random selection. Out of large number of fish samples, the sex ratio was found as Male: Female that is 46 males: 112 females.

3.9.3 Size at first maturity

Size at first maturity was defined as the first attainment of ability to reproduce or sexual maturity. The onset of reproductive activity takes place with the attainment of adulthood. The attainment of sexual maturity is dependent on both intrinsic and extrinsic factors.The size at first maturity of *Danio dangila* was estimated from the parameters ova diameter and fecundity. The size at first maturity was calculated for both the sexes. For this purpose percentage occurrence of mature fishes, both male and female were shown below in table-20.

Table- 20: Size at first maturity in male and female of *Danio dangila*

SIZE GROUP (mm)	MEAN	NO. OF FISH EXAMINED	NO. OF MATURED FISH		% OF MATURED FISH	
			MALE	**FEMALE**	**MALE**	**FEMALE**
30 – 40	35	10	–	-	–	-
40 – 50	45	10	4	2	40%	20%
50 – 60	55	10	7	5	70%	50%
60 – 70	65	10	10	10	100%	100%
70 – 80	75	10	10	10	100%	100%

3.9.4 Ova and maturity stage of gonad of *Danio dangila*

The ovarian morphology in fresh water teleost consists of a pair of ovaries, their oviduct and accessory glands. The ovaries are symmetric, elongated sac-like structure and yellowish brown in colour. Each ovary at its posterior end gives rise to a wide oviduct. The two oviducts from the ovary get united to form a common oviduct.

The mature ovary of *Danio dangila* occupies the greater part of the coelom with two different sized lobes – a larger and a shorter. The length of the larger lobe ranges from 2.2 – 2.7 cm (x 2.45 cm) and the smaller lobe 1.6 – 2.1 cm (x 1.85 cm). A mature ovary contains pale yellowish coloured ova, ranging from 1.0 mm – 1.6 mm (x1.3) in diameter.

Salient characteristics of ovary belonging to stages I to VII recorded were as follows.

Stage I (Immature)

Ovaries were very small thread–like structure, whitish in colour, ova was minute, not visible to naked eye.

Stage II (Developing)

Ovaries were slightly enlarged, transparent, light yellowish in colour. Ova Minute, fibrous and spongy, Mean ova diameter (MOD) 0.11 – 0.15 mm.

Stage III (Maturing I)

Ovaries were marginally enlarged, yellowish in colour MOD ranges from 0.18 – 0.20 mm.

Stage IV (Maturing II)

The Ovaries at this stage were yellowish in colour. Ova partially yolked and MOD vary from 0.25 – 0.28mm.

Stage V (Matured)

Ovaries were yellowish. Ova were fully yolked and enlarged, translucent. MOD lies between 0.32 – 0.38 mm.

Stage VI (Ripe)

The Ovaries cover the entire coelom, which extrude out even by slight pressure, ova was spherical, large. MOD lies between 0.95 – 1.05 mm.

Stage VII (Spent)

Ovaries were shrunken, flaccid with blood vesicle prominent over the surface and with very few ripe ova in the lumen.

3.9.5 Fecundity

Fecundity refers to the number of eggs a female lays in a specified period of time and this depends on the number of eggs per spawning and the number of spawnings.

A good many specimens of *Danio dangilla* of total length (TL) ranging from 5.0cm to 6.8cm, total body weight (TW) ranging from 3.012gm to 3.257gm and total ovary weight (OW) ranging from 0.282 gm to 0.373 gm were taken for fecundity study.

Fecundity and Total fish length (TL)

It was observed that, for a fish measuring 6.8 cm in length, the maximum number of ova was estimated as 1105, while the minimum was 948 ova for a fish of length 5.0 cm. The co-efficient of correlation between the variables, Fecundity and the Total fish length with standard error was computed as follows.

$$r = +\ 0.834 \pm 0.103,\ P < 0.05$$

The linear regression equation between Fecundity and Total fish length was estimated as,

$$F = 1699.914 + 120.70\ TL$$

Fecundity and Total fish weight (TW)

It was observed that, for a fish weighing 3.257 gm, the maximum number of ova was estimated as 1145, while the minimum was 880 ova for a fish weighing 3.012 gm. The co-efficient of correlation between the variable, fecundity and the total fish weight with standard error was computed as follows.

$$r = +\ 0.820 \pm 0.108,\ P < 0.05$$

The linear regression equation between bivariates was estimated as

$$F = 1615.75 + 63.392\ TW$$

Fecundity and Total ovary weight (OW)

It was observed that, in an ovary of 0.373 gm weight, the number of ova was estimated as 1180, while the minimum was 910 ova for an ovary of weight 0.282 gm. The co-efficient of correlation between the variable, fecundity and the total ovary weight with standard error was computed as follows.

$$r = +\ 0.842 \pm 0.\ 101,\ P < 0.05$$

The linear regression equation between Fecundity (F) and Ovary weight (OW) was estimated as

$$F = 1261.456 + 6.345\ OW$$

3.9.6 Gonado–somatic index of *Danio dangila*

Gonado–somatic index (GSI) is considered as an indicator of spawning frequency and the index is also used to study the maturity of ovaries.

The monthly trend of the GSI ranges with mean ± SD of *Danio dangila* of TL 5.0– 6.8 cm was given below in table-21 as well as graphical presentation in **figure-12.**

Table-21: Gonado-somatic index in *Danio dangilla.*

Months	**Ranges GSI**	**Mean GSI ± SD**
January	1.44 – 2.12	1.78 ± 0.35
February	2.33 –3.94	3.13 ± 0.42
March	4.16 – 6.30	5.23 ± 0.58
April	7.60 – 8.52	8.06 ± 0.65
May	9.12 – 10.78	9.95 ± 0.18
June	6.61 – 7.21	6.91 ± 0.22
July	5.59 – 6.02	5.81 ± 0.33
August	1.21 – 1.42	1.31 ± 0.48
September	0.78 - 0.92	0.85 ± 0.44
October	0.37 – 0.49	0.43 ± 0.46
November	0.27 – 0.15	0.21 ± 0.14
December	1.28 – 1.94	1.61 ± 0.53

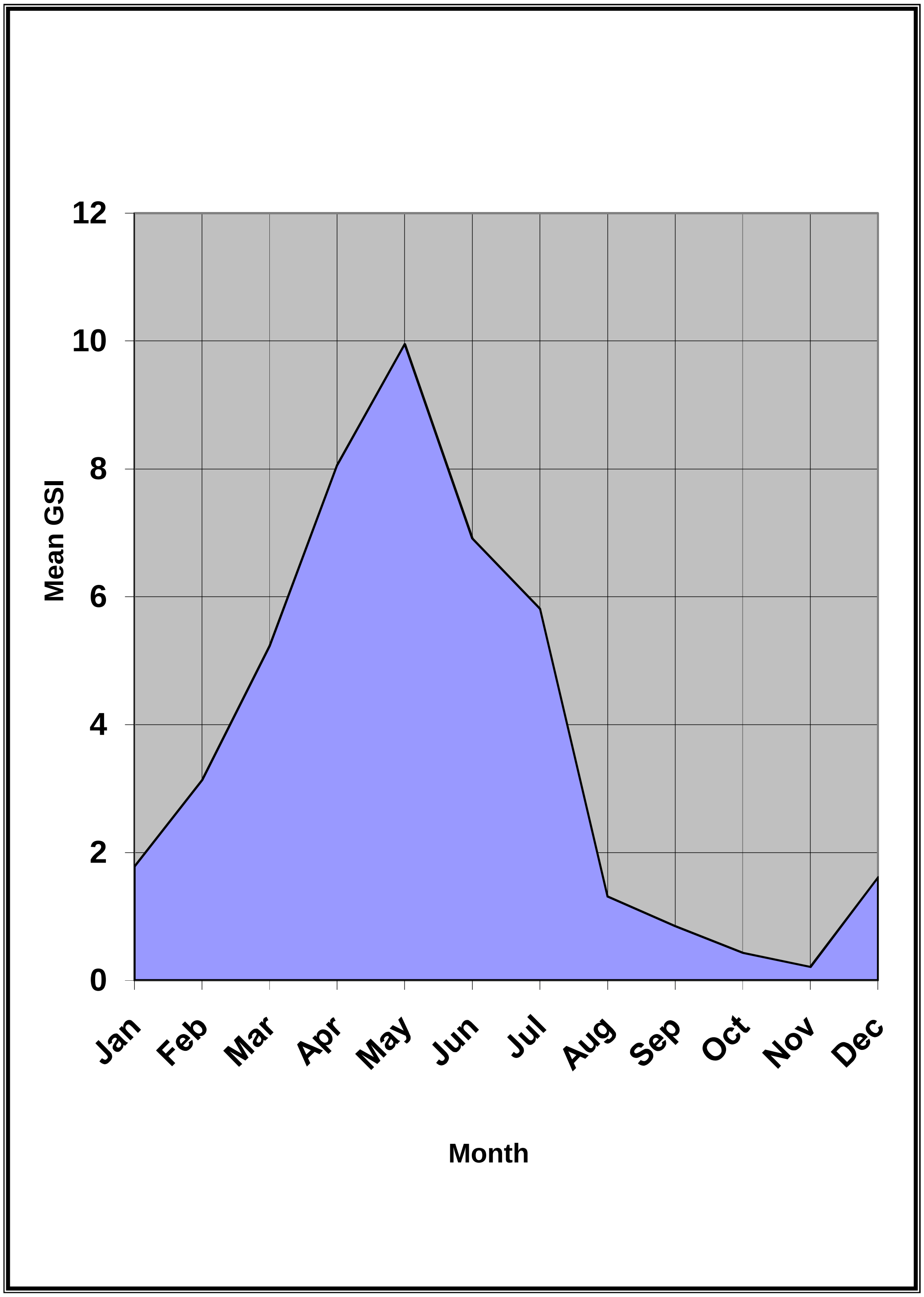

Figure- 12: Gonado-somatic index of *Danio dangila*

The GSI trend in *Danio dangila* gradually increases from March and the peak was observed in the month of May which drops down slightly by June and shows a receding phase from the month of August.

3.9.7 Breading season and periodicity

In general fishes can be divided into two categories.

Category I: - Fishes that breed once a year.

Category II: - Fishes that breed twice a year.

Danio dangilla belongs to category - I, i.e. the female spawns once during a given breeding season, with an extended breeding season which commences from beginning of April to end of July with receding phase from August. The month of May have been ascertained the climax of the breeding season.

3.9.8 Spawning habitat

For successful ex-situ reproduction of fish, the plausible natural habitat of the fish is of utmost importance.

Danio dangila is a lotic water fish and their breeding grounds were inhabited predominantly with pebbles, sand and debris. Physico-chemical parameter of the spawning ground was estimated after APHA (1998). The habitats of the species have been meticulously studied in the field. An account of the characteristics of the habitat of *Danio dangilla* is explicated in table-22.

Table- 22: Habitat of *Danio dangilla*

HABITAT				
PARAMETERS	**PREMONSOON**	**MONSOON**	**POSTMONSOON**	**WINTER**
Water colour	Light and transparent	Muddy and turbid	Light muddy and turbid	Transparent
Transparency (ft)	1.1	Nil	0.5	1.8
Water depth (ft)	2.1	4.1	3.2	1.8
Bottom condition	Muddy and scarse gravel	Muddy gravel and debris	Sandy mud, debris and gravel	Gravel and pebbles
Vegetation Type	Nil	Nil	Nil	Nil
Velocity of water (m/Sec)	0.28	1.5	0.74	0.20
Water Temperature (^{o}C)	8 – 12	10 – 18	12 – 15	2 - 4
pH	7.0	7.4	7.4	7.2
DO (mg/l)	6.2 – 7.6	7 – 8.4	7.1 – 7.8	6 - 7

3.10.1 Sexual dimorphism of *Puntius chola*

In *Puntius chola* the sexual dimorphism exhibits slightly dwarf females, and large males. External morphological differences between male and female pertain to the following features is presented in table-23 and coloured photograph in **plate-7.**

Table- 23: In vitro sexual dimorphism of *Puntius chola*

PARAMETER	MALE	FEMALE
BODY SHAPE	Body fairly deep and compressed, dorsal slightly humped.	Body deep and compressed, dorsal not humped.
FIN CHARACTERS	1. Pelvic and anal fins have tinged orange colour. 2. Intense red colouration along the lateral line during breeding season	1. All fins in female were quite clear or pale yellow.
ANAL OPENING	Oval	Round

Besides, there were some morphometrics differences between male and female and are given below:

INL in male 4.67 – 4.25 in HL (*versus* 3.25 – 4.40 in female); AFL in male 9.00 – 9.17 in SnL (*versus* 9.80 – 8.75 in female); PFL in male 5.00 – 5.00 in SnL (versus 4.90 – 5.38 in female); BD in male 7.50 – 6.88 in SnL (versus 7.00 – 7.00); Distance between origin of pelvic fin and origin of anal fin in male 5.00 – 4.58 in SnL (versus 4.45 – 4.67 in female).

3.10.2 Sex ratio

Sex ratio was ascertained from natural stock through random selection. Out of large number of fish samples, the sex ratio was found as Male: Female, that is 52 males : 145 female.

3.10.3 Size at first maturity

Size at first maturity is defined as the first attainment of ability to reproduce or sexual maturity. The onset of reproductive activity takes place with the attainment of adulthood. The attainment of sexual maturity is dependent on both intrinsic and extrinsic factors.The size at first maturity of *Puntius chola* was estimated from the parameters ova diameter and fecundity. The size at first maturity was calculated for both the sexes. For this purpose percentage occurrence of mature fishes, both male and female were shown below in table-24.

Table- 24: Size at first maturity in male and female of *Puntius chola*

SIZE GROUP (mm)	MEAN	NO. OF FISH EXAMINED	NO. OF MATURED FISH		% OF MATURED FISH	
			MALE	**FEMALE**	**MALE**	**FEMALE**
30 – 40	35	10	–	-	–	-
40 – 50	45	10	4	2	40%	20%
50 – 60	55	10	8	7	80%	70%
60 – 70	65	10	10	10	100%	100%
70 – 80	75	10	10	10	100%	100%

3.10.4 Ova and maturity stage of gonad of *Puntius chola*

The ovarian morphology in fresh water teleost consists of a pair of ovaries, their oviduct and accessory glands. The ovaries were symmetric, elongated sac-like structure and in some brownish yellow in colour. Each ovary at its posterior end gives rise to a wide oviduct. The two oviducts from the ovary get united to form a common oviduct.

The mature ovary of *Puntius chola* occupies the greater part of the coelom with two different sized lobes – a larger and a shorter. The length of the larger lobe ranges from 2.4 – 3.0 cm (x 2.7 cm) and the smaller lobe 2.0 – 2.5 cm (x 2.25 cm). A mature ovary contains pale yellowish coloured ova, ranging from 1.5mm – 1.8mm (x1.65mm) in diameter.

Salient characteristics of ovary belonging to stages I to VII recorded were as follows.

Stage I (Immature)

Ovaries were small, spongy and fibrous thread–like structure, whitish in colour, ova was minute, not visible to naked eye.

Stage II (Developing)

Ovaries were slightly swollen, transparent, light yellowish in colour. Ova Minute, Mean ova diameter (MOD) lies between 0.15 – 0.17 mm.

Stage III (Maturing I)

Ovaries were marginally enlarged, yellowish in colour MOD ranges from 0.18 – 0.22 mm.

Stage IV (Maturing II)

The Ovaries at this stage were yellowish in colour. Ova partially yolked and MOD vary from 0.24 – 0.28 mm.

Stage V (Matured)

Ovaries were yellowish. Ova were fully yolked, enlarged light lemon yellow in colour, translucent. MOD lies between 0.33 – 0.38 mm.

Stage VI (Ripe)

The Ovaries cover the entire coelom, which extrude out even by slight pressure, ova was spherical, large. MOD lies between 1.0 – 1.5 mm.

Stage VII (Spent)

Ovaries were shrunken, with very few ripe ova in the lumen.

3.10.5 Fecundity

Fecundity refers to the number of eggs a female lays in a specified period of time and this depends on the number of eggs per spawning and the number of spawnings.

A good many specimens of *Puntius chola* of total length (TL) ranging from 5.5 cm to 7.8 cm, total body weight (TW) ranging from 4.41 gm to 4.98 gm and total ovary weight (OW) ranging from 0.331 gm to 0.398 gm were taken for fecundity study.

Fecundity and Total fish length (TL)

It was observed that, for a fish measuring 7.8 cm in length, the maximum number of ova was estimated as 1605, while the minimum was 1500 ova for a fish of length 5.5 cm. The co-efficient of correlation between the variables, Fecundity and the Total fish length with standard error was computed as follows.

$$r = + 0.882 \pm 0.094, P < 0.05$$

The linear regression equation between Fecundity and Total fish length was estimated as,

$$F = 1729.233 + 132.80 \, TL$$

Fecundity and Total fish weight (TW)

It was observed that, for a fish weighing 4.98 gm, the maximum number of ova was estimated as 1580, while the minimum was 1450 ova for a fish weighing 4.41 gm. The co-efficient of correlation between the variable, fecundity and the total fish weight with standard error was computed as follows.

$$r = + 0.811 \pm 0.107, P < 0.05$$

The linear regression equation between bivariates was estimated as

$$F = 1764.226 + 99.157 \, TW$$

Fecundity and Total ovary weight (OW)

It was observed that, in an ovary of 0.398 gm weight, the number of ova was estimated as 1600, while the minimum was 1360 ova for an ovary of weight 0.331 gm. The co-efficient of correlation between the variables, fecundity and the total ovary weight with standard error was computed as follows.

$$r = + 0.833 \pm 0. 101, P < 0.05$$

The linear regression equation between Fecundity (F) and Ovary weight (OW) was estimated as

$$F = 1524.203 + 7.404 \, OW$$

3.10.6 Gonado–somatic index

Gonado–somatic index (GSI) is considered as an indicator of spawning frequency and the index is also used to study the maturity of ovaries.

The monthly trend of the GSI ranges with mean ± SD of *Puntius chola* of TL 5.5 – 7.8 cm was given below in Table-25 and graphical representation in **figure-13.**

Table- 25: Gonado-somatic index in *Puntius chola.*

Months	Ranges GSI	Mean GSI ± SD
January	1.44 – 2.12	1.78 ± 0.14
February	3.33 –4.44	3.88 ± 0.10
March	4.76 – 5.30	5.03 ± 0.18
April	6.60 – 7.12	6.86 ± 0.15
May	7.32 – 7.78	7.55 ± 0.11
June	1.98 – 2.21	2.09 ± 0.22
July	3.59 – 4.02	3.08 ± 0.13
August	4.81 – 5.42	5.12 ± 0.20
September	7.58 - 7.72	7.65 ± 0.24
October	1.57 – 1.89	1.73 ± 0.18
November	1.27 – 0.95	1.11 ± 0.16
December	0.38 – 0.54	0.46 ± 0.15

The GSI trend in *Puntius chola* gradually increases from February and attains the first peak in the month of May, thereafter it drops down slightly by June and shows a second peak during the month of September, with receding phase from the month of October.

3.10.7 Breading season and periodicity

In general fishes can be divided into two categories.

Category I → Fishes that breed once a year.

Category II → Fishes that breed twice a year.

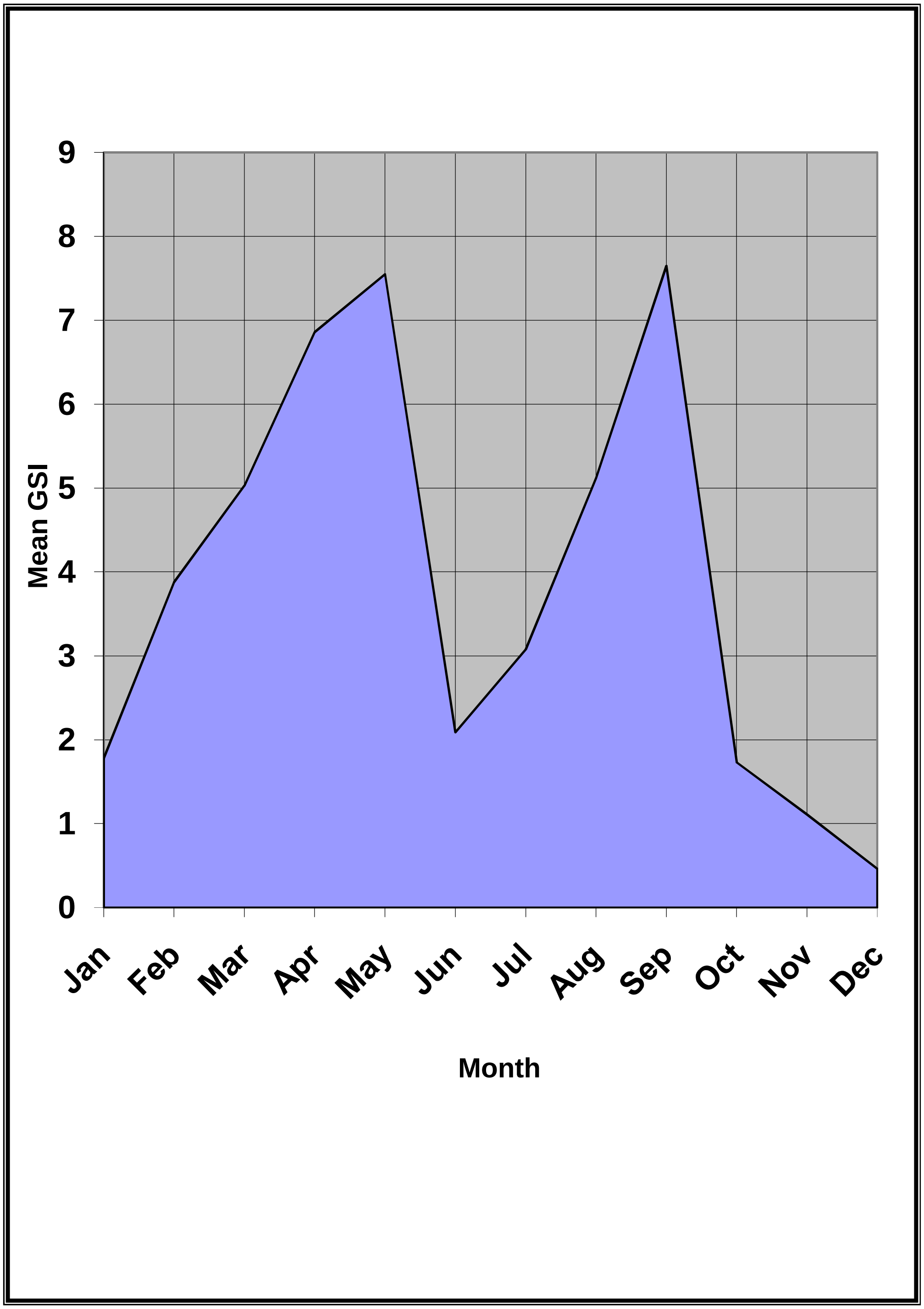

Figure- 13: Gonado-somatic index of *Puntius chola*

Puntius chola spawns once during a given breeding season. The test species is a category I fish with an extended breeding season which commences from March – May with receding phase during June – July and begins during the month of August – September. The months of May and September were recorded the climax of the breeding season.

3.10.8 Spawning habitat

For successful ex-situ reproduction of fish, the plausible natural habitat of the fish is of utmost importance. To ascertain the natural spawning niche of *Puntius chola*, intensive and periodic field survey were made during breeding season and the ecology of the spawning ground in the nature were ascertained. Physico-chemical parameters of the spawning ground were estimated after APHA (1998). The habitats of the species have been meticulously studied in the field.

An account of the characteristics of the habitat of *Puntius chola* is explicated in table-26.

Table- 26: Habitat of *Puntius chola*

HABITAT				
PARAMETERS	**PREMONSOON**	**MONSOON**	**POSTMONSOON**	**WINTER**
Water colour	Light and transparent	Muddy and turbid	Light muddy and turbid	Transparent
Transparency (ft)	1.5	Nil	Nil	1.8
Water depth (ft)	3.0	4.8	4.0	2.6
Bottom condition	Sand, mud and scarse gravel	Muddy gravel and debris	Sandy mud, debris and gravel	Sand, gravel and pebbles
Vegetation Type	Nil	Nil	Nil	Nil
Velocity of water (m/Sec)	0.24	1.8	0.74	0.22
Water Temperature (^{o}C)	10 – 12	15 – 22	18 – 24	2 - 6
pH	7.0	7.4	7.4	6.8
DO (mg/l)	6.5 – 7.2	7.3 – 8.6	7.0 – 7.6	6 - 7

LABORATORY PROPAGATION

Laboratory propagation for breeding of the two test species *Danio dangila* and *Puntius chola* includes selection of brood stock, maintainence of brood stock, stocking density, breeding set-up and breeding technique.

Feeds were the major components for the maintenance of brooders. Live feed such as planktons, formulated feed and powder form dry shrimps and fish were used for feeding the brooders.

3.11.1 Selection of brood stock of *Danio dangila* and *Puntius chola*

Good many healthy brood fish were selected of the size range

a) *Danio dangila*: Female: 5.5 – 7.3 cm TL. Male: 5.2 – 7.8 cm TL

b) *Puntius chola*: Female: 5.8 – 7.8 cm TL. Male: 5.6 – 8.5 cm TL

3.11.2 Maintenance of brood stock *Danio dangila* and *Puntius chola*

Physico-chemical characteristics of brooders tank, feeds, feeding schedule and rate of feeding were the main criteria for the maintainence of brooders. The physico-chemical characteristics of the brooders tank are depicted in the table-27.

Table- 27: Physico-chemical characteristics of the brooders tank

Air temperature °C	Water temperature °C	pH	DO (mg/l)	TA (mg/)	Water volume (lit)	Water depth (cm)	Bottom condition
22-28	20-25	7-7.5	5.2-5.8	53-58	250-300	40-50	Small pebbles/ empty

3.11.3 Feed, rate of feeding and feeding schedule of *Danio dangila* and *Puntius chola*

Feed is the major components for the maintainence of brooders. Live feed (planktons) were supplied for rearing and rising of brooders. The feeds and rate of feeding of *Danio dangila* and *Puntius chola* are shown below in table 28 and 29 for male and female. The feeds were supplied regularly at 24 hr interval without any break till the fishes breed.

Table- 28: Per diem in–house food and feeding rate of *Danio dangila*

TYPES OF FEED	FEED SUPPLIED (% BW)		FOOD CONSUMPTION (% BW)		FOOD PREFERENCE TREND
	MALE	FEMALE	MALE	FEMALE	
LIVE FOOD	20	18	8.5	7.8	Live> Formulated> Manufactured
FORMULATED FEED	10	10	5	3.5	
MANUFACTURED FEED	10	8	2	1.5	

Table- 29: Per diem in–house food and feeding rate of *Puntius chola*

TYPES OF FEED	FEED SUPPLIED (% BW)		FOOD CONSUMPTION (% BW)		FOOD PREFERENCE TREND
	MALE	FEMALE	MALE	FEMALE	
LIVE FOOD	10	10	6	4	Formulated > Live > Manufactured
FORMULATED FEED	20	20	9.5	9.6	
MANUFACTURED FEED	10	8	4	2.5	

3.11.4 Stocking density of brood stock of *Danio dangila* and *Puntius chola*

The suitable stocking density level of the two test species were given in the table below:

Table- 30: Stocking density level of *Danio dangila* and *Puntius chola*

Species	**Surface area (sq. cm)**	**Maximum recommended number**	**Present average stocking density level**	**Rate of stocking**
Danio dangila	28800	1440	100 Fishes	1 Fish / 288 sq. cm
Puntius chola	28800	1440	80 Fishes	1 Fish / 360 sq. cm

3.11.5 Breeding set up of the two test species *Danio dangila* and *Puntius chola*.

The basic requirements of breeding setup for successful breeding of the test species were size and type of breeding tank, spawning habitat and water chemistry of the breeding tank.

Size and type of breeding tank

Two different size of breeding tank were set-up for the breeding of the two test species *Danio dangila* and *Puntius chola*, which is purported below in the tabular from.

Table- 31: Size and type of breeding tank of *Danio dangila* and *Puntius chola*

Size of breeding tank (cm)	**Type of tank**	**Water depth (cm)**	**Water volume (lit)**
75 × 30 × 35	All glass	26 – 28	16 - 18
90× 35× 40	All glass	30 – 33	20 - 25

3.11.6 Breeding technique of the two test species *Danio dangila* and *Puntius chola*.

For the purpose of breeding, a breeding enclosure was designed with aluminium frame and perforated net of mesh size 0.25 cm. the breeding tanks were all glass aquarium, a feeble aeration through corner filter was regulated.

Administrations of synthetic hormone have been attempted successfully in induce-breeding the two test species *Danio dangila* and *Puntios chola*. Detailed composition of male to female hormone, dosage, timing of injection and spawning were purported below in table 32, 33 and coloured photographs presented in **plate-8**.

Table-32: Administration of synthetic hormone in *Danio dangila*

Composition	Injection Number	Hormone	Dosage to Male	Dosage to Female	Time of injection
2 M : 1 F	First	Ovaprim	Nil	0.2µgm/gm BW	Evening
	Second	Ovaprim	0.2µgm/gm BW	0.3µgm/gm BW	7 hrs after first injection
Spawning occurs eight hrs after second injection					

Table-33: Administration of synthetic hormone in *Puntius chola*

Composition	Injection Number	Hormone	Dosage to Male	Dosage to Female	Time of injection
2 M : 1 F	First	Ovaprim	Nil	0.3µgm/gm BW	Evening
	Second	Ovaprim	0.3µgm/gm BW	0.5µgm/gm BW	7 hrs after first injection
Spawning occurs six hrs after second injection					

Administration of synthetic hormone (ovaprim) was attempted successfully for spawning in the two test species.

EMBRYONIC AND LARVAL DEVELOPMENT

The embryonic development commences with the penetration of sperm into the ova. In teleost, fertilization is external followed by absorption of water by the egg and hardening of the chorion. Hatchling is the tiny free swimming larva. The larval development begins with the external feeding of fry till the formation of juvenile. In the present treatise the entire development process have been grouped into three phases (a) Embryonic phase (b) Hatchling phase and (c) Larval phase.

3.12.1 Embryonic phase in *Danio dangila*

Schematic diagram of embryonic developmental stages in *Danio dangila* are shown in **Figure- 14** and their coloured micrographs are depicted in **plate-9**.

(A) **Fertilized egg**: The fertilized egg of *Danio dangila* was non-adhesive, transparent with pale brownish colour. Tiny stings cover the surface of the egg capsule. Water hardening of the egg was completed within 10-12 minute after fertilization. The egg size was 0.8 mm to 1.0 mm (x0.90mm). The diameter of embryo proper was 0.43 mm to 0.48 mm (x.45 mm). Plate-9 (i)

(B) **Cleavage:** Plate-9 (ii)

(a). The first cleavage plane was noticeable 20 mins after fertilization in the cap of cytoplasm on the animal pole, dividing it into two equal blastomeres. The second cleavage was perpendicular to the first and divides into 4-celled blastomeres at 40-45 mins after fertilization and it forms 8 blastomeres after 1 hour 20 mins after fertilization. The 16 blastomeres stage was reached at 2 hours after fertilization and 32 blastomeres stage at 2 hours 45 mins.

(b) Morula: At 3 hours 15 mins after fertilization, cleavage consists of cohering, sticky and cluster of blastomeres above the yolk mass thus, attaining morula stage.

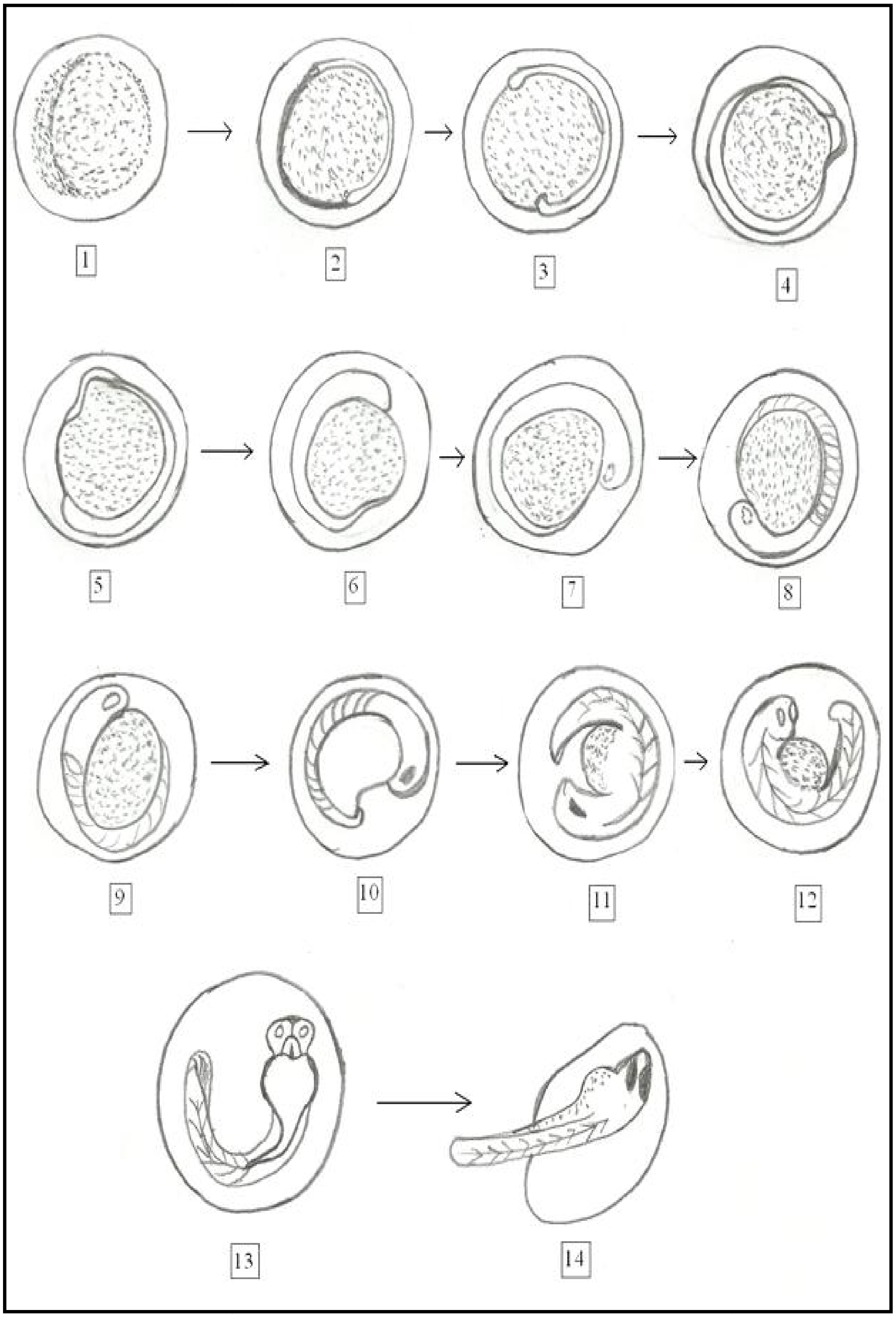

Figure- 14: Schematic diagram showing embryonic developmental stages in *Danio dangila*

(c) Blastula formation:

Early blastula: The blastoderm cells seen to expand over the yolk mass after 4 hours of fertilization.

Blastula: The central area of blastoderm was continuously seen to expand over the yolk after 4 hrs and 50 mins.

(C) **Gastrulation:** Plate-9 (iii)

(a) Early gastrula: The expanding blastoderm covers three-fourth of the yolk sac 6 hours after fertilization. The central germ ring becomes thickened as soon as gastrulation begins.

(b) Gastrula: The yolk was nearly covered by blastodermal layer in gastrula stage at 8 hrs 30 mins after fertilization. The embryonic shield developed to form embryo, generally known as yolk plug stage.

(c) Elongation of yolk mass: The earliest indication of the embryo was observed with the elongation of yolkmass at 9 hrs 45 mins after fertilization.

(D) **Organogenesis:** Plate-9 (iv)

(a) At 10.50 hrs after fertilization, the cephalic region becomes distinguished. The indication of mesodermal segmentation begins.

(b) 12.20 hrs, after fertilization, 4 somites were noticed. Plate-9 (v)

(c) Optic cup and notochord with 10 somites were noticed at 14.55 hrs after fertilization. Plate-9 (vi)

(d) 12 somites were noticed at 17 hrs after fertilization. Plate-9 (vii)

(e) 18 hrs after fertilization, the caudal region getting free from embryo was recorded. Plate-9 (viii)

(f) 19 hrs, first tail twitching movement of the embryo was noticed. The caudal region becomes free from the yolk mass and most of the yolk was covered by the embryo. Plate-9 (ix)

(g) At 20 hrs, elongation of embryo was noticeable with feeble contraction and expansion. Embryonic fin folding was observed and somite number increased up to 20. Plate-9 (x)

(h) After 22 hrs of fertilization the caudal region of the embryo becomes completely free from yolkmass. Rudimentary heart was visible, which lies distally with yolksac. Movement of embryo gradually increases and somite number was 24. Plate-9 (xi)

(i) At 24 hrs, gill organization is noticed. Eyes prominent and heartbeat observed. No further significant development noticed upto 29 hours. Plate-9 (xii)

(j) 29 hrs after fertilization the embryo occupies three fourth of the egg capsule. Pectoral fin bud was visible. Somite number was 30. Plate-9 (xiii)

(k) At 31 hrs after fertilization the embryo occupies almost all parts of the egg, embryo movement was restricted

(l) After 34 hrs of fertilization vigorous twitching movement was noticeable with rapid heart beat. Blood circulation clearly visible. Plate-9 (xiv)

(m) 36 hr after fertilization, the embryo moves vigorously inside the egg capsule, as a result the vitelline membrane burst and the embryo wriggles out with tail first. Plate-9 (xv)

3.12.2 Hatchling phase

(A) **Free embryo stage:** Plate-9 (xvi)

(a) Newly hatched pro-larva: The newly hatched pro–larva was transparent and measures about 1.8 – 2.4 mm (x 2.1 mm) in length. Yolk sac segmented, elliptical in shape, slightly broader anteriorly and narrow posteriorly. The cephalic portion was broader with prominent optic lobes. The larva remains motionless but shows uncoordinated movement (spiral movement with head region pointing downwards and the caudal region was spirally rotated, keeping at an angle of 90^o) for a short period. Four horizontal dark bands were noticed on its transparent body. Pectoral fin with rudimentary fin rays was observed.

(b) 12 hrs after hatching: The total length was 2.1 –2.8 mm (x 2.45 mm). The dark bands present become broader. The hatchlings swims swiftly when distrubed and gets settled at the bottom. Pectoral fin was fully developed with rudimentary fin rays. Yolk sac still persists. Dark pigmentation was observed in the cephalic region.

(c) Clinging stage: 24 hrs (1 day) after hatching most of the larvae get clinged to the glass aquaria with their suctorial mouth. The length of the larva was 3.0 – 3.5 mm (x 3.25 mm). Guts start to develop between yolk sac and notochord.

(B) **Mouth formation stage:**

Mouth formation of the larva was noticed after 48 hrs (2days) with feeble lip movement. The length of larva was 3.4 – 3.8 mm (x 3.6 mm). Gradual absorption of yolk mass was seen. Mouth was protrusible. Larva yet to swim freely. Eye pigment was dark in colour and head slightly free from yolk sac.

3.12.3 Larval phase

(Schematic diagram showing larval developmental stages in *Danio dangila* **Figure- 15** and coloured micrographs in **plate-10**)

(A) **Free swimming stage:**

The larva mostly remains at the bottom of the aquarium but can swim freely. Gut formation was visible in between yolk sac and fin fold. The length of larva was 3.5mm – 4.0mm (x 3.75 mm). The larva was 3 day old.

(B) **Fin formation stage:**

(a) Dorsal fin: The first indication of dorsal fin formation was observed at 9^{th} day and completed with fin rays at 20^{th} day after hatching. Few crests were observed at the primordial fin fold region. 2 dorsal spines were observed on 10^{th} day and 4 dorsal spines on 11^{th} day after hatching. Dorsal fin rays were observed at 15th day after hatching and by 20^{th} day after hatching the dorsal fin formation was completed.

(b) Anal fin: The anal fin bud was observed at 8^{th} day after hatching. On 9^{th} day after hatching 2 anal rays were noticed. 15 days after hatching, anal fin formation was completed with the formation of 3 spines and 8 rays. Anal fin was transparent with pigmentation at the base.

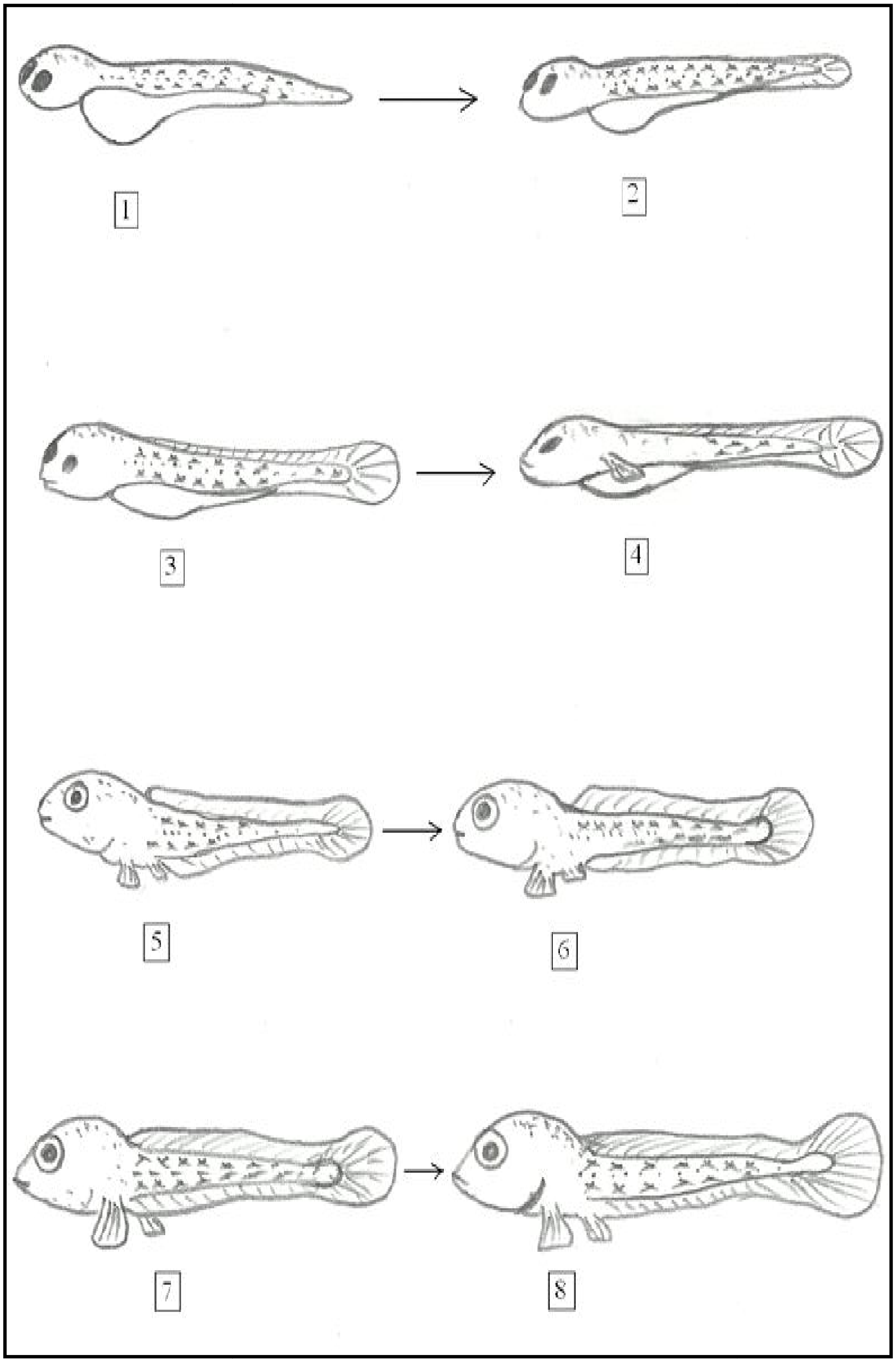

Figure- 15: Schematic diagram showing larval developmental stages in *Danio dangila*

(c) Pectoral fin: The pectoral fins develope about 10 hrs after hatching with rudimentary fin rays. 4 fin rays – 2 unbranched and 2 branched were observed on 3rd day after hatching. Pectoral fin formation was completed on 5th day after hatching. Pectoral fin was transparent with pigmentation at the base of the pectoral fin.

(d) Pelvic fin: The pelvic fin bud was observed at 7th day after hatching with rudimentary fin rays noticeable and was completed by 12th day-after hatching with the formation of 1 spine and 5 rays. Pelvic fin was transparent with dark pigmentation at the base.

(e) Caudal fin: At 1st day, the caudal fin starts differentiating from the primordial fin fold by caudal fin constriction. The caudal fin formation was completed on 5th day after hatching with 20 rays. The caudal fin was transparent.

(C) **Colour formation stage:**

The colour pigments were observed at 40 hr 45 min after fertilization. 24hrs after hatching aggregation of star- shaped melanophores were observed in the cephalic region. Faint dark bands were observed on the 4th day after hatching. On 10th day after hatching, distinct silvery blue, green and yellowish horizontal bands were noticed. Pigmentation was also noticed at the head region.

3.13.1 Embryonic phase in *Puntius chola*

(Schematic diagram showing embryonic developmental stages in *Puntius chola* **Figure- 16** and coloured micrographs in **plate-11**)

(A) **Fertilized egg:** The fertilized egg of *Puntius chola* was semi-adhesive, transparent with pale yellowish colour. Water hardening of the egg was completed within 8-10 minute after fertilization. The egg size was 0.9 mm to 1.2 mm (x1.05mm). The diameter of embryo proper was 0.45 mm to 0.51 mm (x0.48 mm). Plate-11 (i)

(B) **Cleavage:** Plate-11 (ii)

(a). The first cleavage plane was noticeable 15 mins after fertilization in the cap of cytoplasm on the animal pole, dividing it into two equal blastomeres. The second cleavage was perpendicular to the first and divides into 4-celled blastomeres at 30-35 mins after fertilization, it forms 8 blastomeres after 55 mins-1 hour after fertilization. The 16 blastomeres stage was reached at 1 hour 25 mins after fertilization and 32 blastomeres stage at 2 hours.

(b) Morula: At 2 hours 35 mins after fertilization, cleavage consists of cohering, sticky and cluster of blastomeres above the yolk mass, thus attaining morula stage.

(c) Blastula formation:

Early blastula: The blastoderm cells seen to expand over the yolk mass at 3 hours and 15 mins after fertilization.

Blastula: The central area of blastoderm was continuously seen to expand over the yolk after 4 hours.

(C) **Gastrulation:** Plate-11 (iii)

(a) Early gastrula: The expanding blastoderm covers three-fourth of the yolk sac 5 hours after fertilization. The central germ ring thickens soon after gastrulation begins.

(b) Gastrula: The yolk was nearly covered by blastodermal layer in gastrula stage at 5 hrs 45 mins after fertilization. The embryonic shield developed to form embryo, generally known as yolk plug stage.

(c) Elongation of yolk mass: The earliest indication of the embryo was observed with the elongation of yolkmass at 6 hrs 50 mins after fertilization.

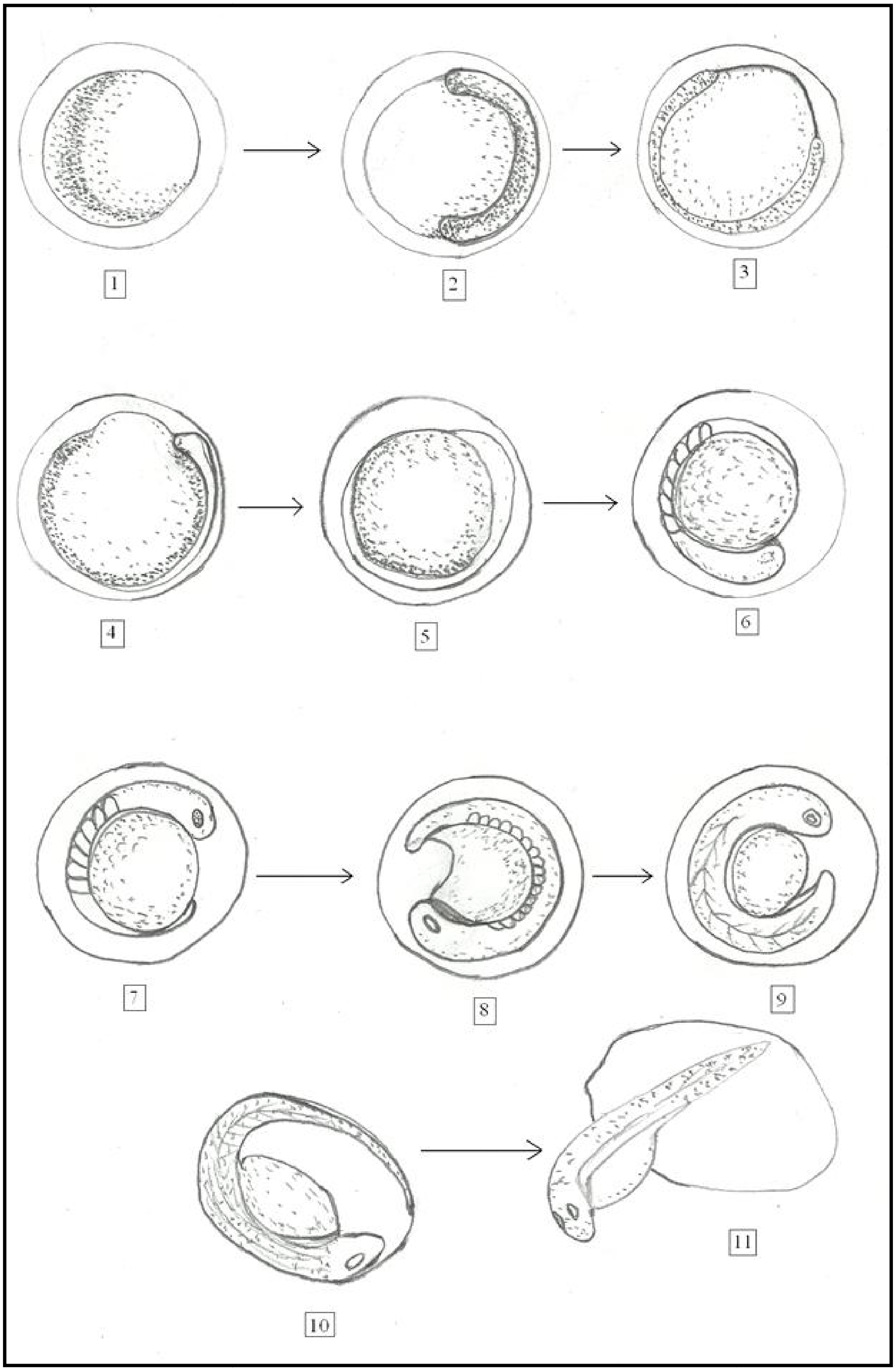

Figure- 16: Schematic diagram showing embryonic developmental stages in *Puntius chola*

(D) **Organogenesis:** Plate-11 (iv)

(a) At 7.40 hrs after fertilization, the cephalic region becomes distinguished. The indication of mesodermal segmentation begins.

(b) 8.50 hrs, after fertilization, 4 somites were noticed.

(c) Optic cup and notochord with 8 somites were noticed at 10 hrs after fertilization. Plate-11 (v)

(d) 12 somites were noticed at 11.50 hrs after fertilization. Plate-11 (vi)

(e) 12.50 hrs after fertilization, the caudal region getting free from embryo was recorded. Plate-11 (vii)

(f) 13.20 hrs, first tail twitching movement of the embryo was noticed. The caudal region becomes free from the yolk mass and most of the yolk was covered by the embryo. Plate-11 (viii)

(g) At 14.30 hrs, elongation of embryo was noticeable with feeble contraction and expansion. Embryonic fin folding was observed and somite number increased up to 20.

(h) After 15.45 hrs of fertilization the caudal region of the embryo becomes completely free from yolkmass. Rudimentary heart visible, which lies distally with yolksac. Movement of embryo gradually increases and somite number increased to 24. Plate-11 (ix)

(i) At 17 hrs, gill organization was noticed. Eyes prominent and heartbeat observed. No further significant development noticed upto 19 hours.

(j) 19.30 hrs after fertilization the embryo occupies three fourth of the egg capsule. Pectoral fin bud was also visible. Somite number was 30.

(k) At 20.45 hrs after fertilization the embryo occupies almost all parts of the egg, embryo movement was restricted

(l) After 23 hrs of fertilization vigorous twitching movement was notice with rapid heart beat and blood circulation clearly visible. Plate-11 (x)

(m) 26 hr after fertilization, the embryo moves vigorously inside the egg capsule, as a result the vitelline membrane burst and the embryo wriggles out head first. Plate-11 (xi)

3.13.2 Hatchling phase

(A) **Free embryo stage:** Plate-11 (xii)

(a) Newly hatched pro-larva: The newly hatched pro–larva was opaque and brownish in colour and measures about 2.0 – 2.5 mm (x 2.25 mm) in length. Yolk sac segmented, elliptical in shape, broad anteriorly and narrows down posteriorly. The cephalic portion was broader with prominent optic lobes. The larva remains motionless but shows uncoordinated movement (spiral movement with head region pointing downwards and the caudal region is spirally rotated keeping at an angle of 90^o) for a short period. Pectoral fin with rudimentary fin rays was observed.

(b) 12 hrs after hatching: The total length was 2.8 –3.2 mm (x 3 mm). The hatchlings swims swiftly when distrubed and gets settled at the bottom. Pectoral fin was fully developed with rudimentary fin rays. Yolk sac still persists. Dark pigmentation was observed in the cephalic region.

(c) Cling stage: 24 hrs (1 day) after hatching most of the larvae get clinged to the glass aquaria with their suctorial mouth. The length of the larva was 3.5 – 3.8 mm (x 3.65 mm). Guts start to develop between yolk sac and notochord.

(B) **Mouth formation stage:**

Mouth formation of the larva was noticed after 36 hrs with feeble lip movement. The length of larva was 4.0 – 4.5 mm (x 4.25 mm). Gradual absorption of yolk mass observed. Mouth was protrusible. Larva can not swim freely. Eye pigment was dark in colour and head slightly free from yolk sac.

3.13.3 Larval phase

(Schematic diagram showing larval developmental stages in *Puntius chola* **Figure- 17** and coloured micrographs in **plate-12**)

(A) **Free swimming stage:**

The larva mostly remains at the bottom of the aquarium but can swim freely. Gut formation was visible in between yolk sac and fin fold.

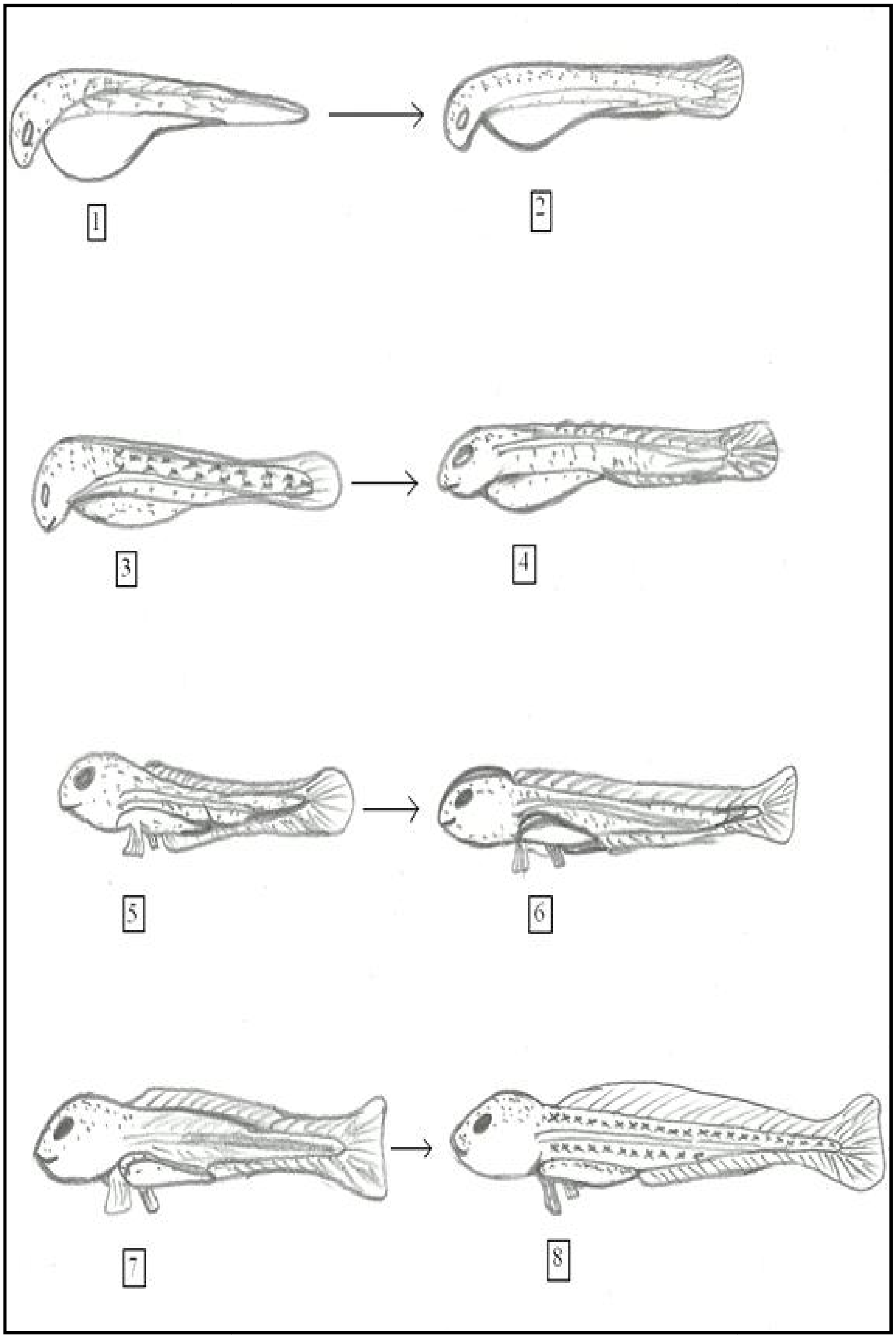

Figure- 17: Schematic diagram showing larval developmental stages in *Puntius chola*

The larva was 3 day old and measures about 4.6mm – 4.8mm (x 4.7 mm) in length.

(B) **Fin formation stage:**

(a) Dorsal fin: The first indication of dorsal fin formation was observed at 8^{th} day and completed with fin rays at 18^{th} day after hatching. Few crests were observed at the primordial fin fold region. 2 dorsal spines were observed on 9^{th} day and 4 dorsal spines on 11^{th} day after hatching. Dorsal fin rays were observed at 14^{th} day after hatching and by 18^{th} day after hatching the dorsal fin formation was completed.

(b) Anal fin: The anal fin bud was observed at 7^{th} day after hatching. On 8^{th} day after hatching 2 anal rays were noticed. 14^{th} day after hatching, anal fin formation was completed with the formation of 4 spines and 10 rays. Anal fin was transparent with pigmentation at the base.

(c) Pectoral fin: The pectoral fins developed about 9 hrs after hatching with rudimentary fin rays. 4 fin rays – 2 unbranched and 2 branched were observed on 3rd day after hatching. Pectoral fin formation was completed on 4^{th} day after hatching. Pectoral fin was transparent with pigmentation at the base.

(d) Pelvic fin: The pelvic fin bud was observed at 6^{th} day after hatching with rudimentary fin rays noticeable and was completed by 11^{th} day-after hatching with the formation of 2 spines and 6 rays. Pelvic fin was transparent with dark pigmentation at the base.

(e) Caudal fin: on the first day after hatching, the caudal fin starts differentiating from the primordial fin fold by caudal fin constriction. The caudal fin formation was completed on 5^{th} day after hatching with around 22 rays. The caudal fin was transparent.

(C) **Colour formation stage:**

The colour pigments were observed at 36 hrs 30 minutes after fertilization. 24 hrs after hatching aggregation of star- shaped melanophores were observed in the cephalic region. Dark spots with yellowish white bands were observed on the 3^{rd} day after hatching. On the 8^{th} day after hatching, distinct dark horizontal bands and a dark blotch at the base of caudal fin was noticed. Pigmentation was also noticed at the head region.

LABORATORY REARING OF FRY

Early larval stages are the most crucial and vulnerable stage in the life-cycle of a fish. During this period, the young ones are defenseless against and sensitive fluctuation in the environmental factors such as dissolved oxygen, temperature, alkalinity etc. and to handling stress. Hence the survival at these stages depends on the maintenance of optimum water quality, availability of adequate choice food and predator free aquatic environment.

At present, the national average survival rate from hatchling to fry stage is only 14% which is indicative of the lacuna that exists in our present day larval care protocol followed in the seed production centers (Thomas *et.al*, 2003).

3.14.1 In-house tank set up of *Danio dangila* and *Puntius chola*

Several sets of aquarium are essential for rearing the fry of *Danio dangila* and *Puntius chola*. Hence aquarium setup is an important exercise for rearing. Freshly hatched larvae are called spawn or pro-larvae. They do not feed on exogenous food for about 56 hrs. During this period they utilize the yolk present in the yolk sac. By the time the yolk sac is fully absorved i.e. 3rd day, it is referred to as post larvae. The post larvae were then accordingly reared in three different tank conditions termed as, (a) nursery aquaria (b) rearing aquaria (c) grow out aquaria and were depicted below in table-34.

Table- 34: Types of tanks for nursery, rearing and grow out.

Tanks	Size of tanks (cm)	No. of aquaria	Type of tanks	Water volume (lit)
Nursery tanks	75 × 30 × 35	6	All glass	12-15
	90 × 35 × 40	6	All glass	16-20
Rearing tanks	90 × 35 × 40	3	All glass	18-22
	105 × 40 × 45	3	All glass	25-30
Grow out tanks	240 × 120 × 60	6	FRP	40-70
	240 × 120 × 60	6	Cement cisterns	50-80

(a) Nursery aquaria

The nursery aquaria were required for rearing 3 days old pro- larvae for a period of 25 days to one month. 6 aquaria each (3 each of two sizes) were set up for rearing of the pro- larvae of the two test species, the required water depth was maintained at 25 cm with feeble filtration by corner filter. The aquariums bottom / floor were covered with small river stone. The temperatures of the aquaria were maintained by a thermostat mechanism.

(b) Rearing aquaria (Advanced fry aquaria):

The rearing aquaria were for rearing of advanced fry. The fry beyond 25 days old stage of both the test species were reared up to 55 days. Feeble aeration was supplied through corner filter. Small smooth stone or chips cover bottom of the aquaria. Thermostat was maintained for temperature flactuation. The water depth was maintained at 30-35cm.

(c) Grow out aquaria:

The grow out aquaria were Fibre Reinforced Plastic tubs (FRP) and cement cisterns, the water depth of the FRP tubs and cement cisterns were maintained at 80cm and 100 cm respectively. The sub adult and advanced fries of both the tests species were reared in these grow out aquaria.

The physico-chemical condition of nursery, rearing and grow out aquaria were depicted below in table-35.

Table- 35: Physico-chemical condition of nursery, rearing and grow out aquaria

Parameters	Nursery	Rearing	Grow out
pH	7.5	7.5	7.8
DO (mg/l)	5.6-6.1	5.4-5.6	5.5-5.6
TA (mg/l)	38.3-41.3	42.3-48.7	44.7-52.4
TH (mg/l)	15.2-17.5	18.2-21.5	16.9-20.1
Temperature (^{o}C)	25-27	21-23	17-20

Fries stocking density in nursery aquaria of *Danio dangila* and *Puntius chola*

The fries stocking density of *Danio dangila* and *Puntius chola* in nursery tank were determined through series of trial experiments. In the present studies, four different stocking densities were maintained, they are 60 fries/aquarium, 80 fries/aquarium, 100 fries/aquarium and 120 fries/aquarium. Significantly the survival rate were found 85% *(Danio dangila)* and 72% *(Puntius chola)* in 60 fries/aquarium; 90% *(Danio dangila)* and 83% *(Puntius chola)* in 80 fries/aquarium; 92% *(Danio dangila)* and 88% *(Puntius chola)* in 100 fries/aquarium and 77% *(Danio dangila)* and 79% *(Puntius chola)* in 120 fries/aquarium, with highest survival rate at 100 fries/aquarium in both the cases.

The highest and viable stocking density of the test species fries at the rearing (advanced fry stage) and grow out aquaria obtained in the present study were depicted below.

Table- 36: Viable stocking density in rearing and grow out aquaria.

Species	**Rearing aquaria**		**Grow out aquaria**	
	Density/tank	Survivability (%)	Density/tank	Survivability (%)
Danio dangila	80	90	100-120	95
Puntius chola	80	95	100	98

3.14.2 Food and feeding of fries of *Danio dangila* and *Puntius chola*

Feeding is the most important criteria for successful survival of the fry. For commercial mass culture of fresh water ornamental fish, identification of food of the fry is of utmost importance. In absence of food, there is every possibility of mass mortality. Both live food and supplementary feed are experimented in the present investigation along with the culture of live feed.

The live/natural feed used for fry of *Danio dangila* and *Puntius chola* were primarily, infusoria, planktons & mosquito larva. While egg yolk suspension, manufactured feed, formulated feed and dry fish & prawn powder were supplemented.

Preparations of the supplementary feed for the fries of test species are as follows:

(i) Infusoria: Infusoria such as *Paramaccium* and *Stylonychia* form an ideal starter food for newly hatched spawn after the yolk sac absorption. It was prepared by adding kitchen waste such as banana peels, bruised lettuce leaves, hydrilla etc. in boiled and cooled water.

(ii) Egg yolk suspension: For the preparation of egg yolk suspension, a hard-boiled egg yolk was minced in a dish and properly mixed in 500ml of water. This suspension was allowed to settle for 5-10 minutes.

(iii) Live feed: Live feed such as; *moina*, *daphnia*, *tubifex* etc. were collected by plankton net of fine mesh size from the ponds.

(iv) Formulated feed: Feed such as, Rice bran, oil cake, minced Fish/prawn power were formulated for feeding.

(v) Manufactured feed: Feeds such as Tokyo and tubifex etc. were minced and use for feeding advanced fry and sub - adult of the test fish species.

Table- 37: Fries feeding schedule of *Danio dangila*.

Food type	Days of fry	Rate (ml/day)	Feeding time (hrs)	Duration (days)
Infusoria	Up to 10 day larva	50 ml diluted to 1 : 1	8.30 a.m. 1.00 p.m. 7.00 p.m.	7 days
Mixture of Infusoria and yolk suspension	10 days onwards	70 ml	8.30 a.m. 1.00 p.m. 7.00 p.m.	15 days
Planktons	25-30 days onwards	150 ml	9.00 a.m. 5.00 p.m.	1 month
Formulated feed Minced Fish/prawn power	30 days onwards	100 mg	9.00 a.m. 5.00 p.m.	1 month
Mosquito larva Formulated feed	60 days onwards	Adequate	Once daily	Till grow out

Table- 38: Fries feeding schedule of *Puntius chola*.

Food type	Days of fry	Rate (ml/day)	Feeding time (hrs)	Duration (days)
Infusoria	Up to 10 day larva	50 ml diluted to 1 : 1	8.30 a.m. 1.00 p.m. 7.00 p.m.	7 days
Mixture of Infusoria and yolk suspension	10 days onwards	70 ml	8.30 a.m. 1.00 p.m. 7.00 p.m.	15 days
Powderd manufactured feed/Planktons	25-30 days onwards	150 ml	9.00 a.m. 5.00 p.m.	1 month
Formulated feed Rice bran, oil cake, minced Fish/prawn power	30 days onwards	100 mg	9.00 a.m. 5.00 p.m.	1 month
Manufactured and Formulated feed	60 days onwards	Adequate	Once daily	Till grow out

3.14.3 Rearing duration and trait of *Danio dangila* and *Puntius chola*

The fries were reared in glass aquarium with initial feeding through infusoria and thereafter with mixture of infusoria and yolk suspension, followed by planktons and minced fish/prawn powder/formulated feed, till they attain a comfortable size and were being transferred to cemented cisterns and FRP tubs, where they were fed with Mosquito larva/Formulated feed. Adequate care was taken during the period to avoid overcrowding and infection from allochthonous sources. The grown up fries of *Danio dangila* and *Puntius chola* were finally stocked in stocking pond prepared for the species.

FISH MALADY AND RESTRAINTS

Fish are susceptible to various diseases. The major symptoms of disease of fish are loss of appetite, abnormal swimming behaviour, poor reflex reaction, increase in the rate of ventilation, clumping of fins, remaining in the surface water, inactive in the bottom, production of excessive mucous and changes in colouration (Varghese, 1988)

In the present investigation, the diseases of the two test fishes were classified into two major groups from the causative point of view. (I) Pathogenic diseases caused by (a) Fungi, (b) Bacteria, (c) Protozoa, (d) Worms (e) Crustacea (II) Non-pathogenic diseases caused by (a) Asphyxation (b) Injuries caused due to rough handling (c) Poor water quality (d) Environmental stress (e) Malnutrition (f) Miscellaneous reasons.

Ornamental fish farmers in temperate region have already practiced several treatments that are listed in the present study. However, these treatments were not effective and hence in the present study the treatments were modified to attain optimum effectiveness.

3.15.1 Zymosis types and traits

The nosology of common aquarium fish species so far reported by earlier workers along with those accounted in the present test fish species were depicted in Table 39.

Table– 39: Nosology of *Danio dangila* and *Puntius chola*

Diseases	Occurrence	Species	
A. BACTERIAL			
i) Fin and Tail Rot	+	*Danio dangila*	*Puntius chola*
ii) Ulcer disease	+	*Danio dangila*	*Puntius chola*
iv) Cotton wool disease	+	*Danio dangila*	*Puntius chola*
B.FUNGAL INFECTION			
i) Body fungus	+	*Danio dangila*	*Puntius chola*
ii) Eye fungus	+	*Danio dangila*	-
iii) Gill rot	+	*Danio dangila*	*Puntius chola*
C. PARASITIC INFECTION			
i) Argulosis	+	*Danio dangila*	*Puntius chola*

A. Protozoan infection

(i) *Myxosporidian*

The causative myxosporidian genera reported are mainly *Leptotheca, Chloromyxum, Myxobolus, Hennegunya, Myxidium, Thelohanellus* and *Leptospera*.

Symptom: The parasite produces cysts on different regions of the body especially in the opercular region, dorsal part of the body and caudal region.

Heavy infection can lead to bursting of the cysts and ulceration and raising of the scales along the posterior margins and falling of scales.

Stage of infection: Infection found in adults.

Treatment: Cloromycetin 125 mg dissolved in 300 ml of water and spread over the aquarium. Second dose of 125 mg Chloromycetin in 300 ml of water was administered after 4 days. After 7 days of initial infection, small cysts were observed at the places of initial infection. A mixture of 250 mg Tetracycline, 250 mg chloromycetin, 2 mg K MnO_4, 2 mg Riboflavin and 2 mg malachite green was prepared. Initially a bath in 3 ml/l of the above mixture for ½ hr was given daily until the disappearance of all the cysts. The cysts re-occur after a lapse of 15 days Bath in 0.5 mg NaCl / litre of water was given daily in the morning and a bath in 2 mg/litre malachite green for 15 mins, was administered daily after 5 hours of first bath, until the cysts disappear. For mild infection, dip treatment in 0.5 mg/litre water daily twice for 15 mins was effective.

Period and duration: The duration of the disease was 7 – 30 days.

B. Fungal infection

Body Fungus The causative fungus for this disease as reported by earlier worker is *Saprolegnia* spp.

Symptom: Tufts of minute white hair like outgrowths occur in the affected area of the body.

Stages of infection: Infection was found in both adult and sub-adult fish.

Treatment: In case of mild infection in fin rays, manual removal of the fungal hyphae with forcep and dipping the infected fish in 1 mg/ liter $KMnO_4$ for 5 mins was effective. Chloromycetin 125 mg dissolved in 300 ml water and spread over the hospital tank was found effective. In case of heavy infection a second dose of 125 mg Chloromycetin dissolved in 300 ml of water was administrated and found effective.

Duration of treatment: 2 days in case of mild infection, 7 – 12 days in case of heavy infection.

Remark: The disease mostly occurs in the dorsal portion of the body, beneath the dorsal fin and also in the opercular region. In case of severe infection, it may pierce the opercular bone and expose the gills.

C. Bacterial infection

(i) ***Tail and fin rot***: The causative bacteria for this disease as reported by earlier worker are mainly Aeromonas spp, Pseudomonas spp and Vibrio spp.

Symptom: The first signs of the disease was the appearance of a white line on the margin of the fin, spreading and imparting frayed appearance to the appendage which eventually putrifies and disintegrates.

Stage of infection: Infection was found mainly in adult as well as sub-adult stages.

Treatment: Dip treatment in 1– 2 mg malachite green/litre of water was found effective

Period and duration: 7 –10 days treatment was necessary depending on the intensity of infection.

(ii) ***Ulcer disease***

The causative bacterium for this disease reported by earlier workers is *Flexibactor columnaris*.

Symptoms: The symptom of this disease is occurrence of raised, white plagues, often with reddish peripheral zone leading to hemorrhagic ulcers.

Stage of infection: Infection was found in adult stage.

Treatment: Chloromycetin 125 mg dissolved in 300 ml water and spread over the hospital tank was found effective. In case of no improvement a second dose of 125 mg Chloromycetin was administered and found effective with 50% change of water. Bath treatment in 1 mg KMnO4/litre along with 250 mg Chloromycetin dissolved in 1 litre water was also found effective.

Period and duration: Duration of the diseases was 7 –12 days.

D. Helminth infection

The helminth infection is found negligible in the studied fish mostly caused by nematodes. The fish is not much affected by the parasites.

3.15.2 Prophylactic and control measures

It is well known that, prevention is better than cure. This also applies in the case of fish. With the observance of this principle most of the losses can be avoided from the very outset, particularly when disease are often difficult to cure or cannot be cured at all once they break out. Prevention measures and practices should be economical and should cover as far possible all fish disease. The origin of many fish disease may be due to, deficiencies in the environment and the maintainence and on the other hand, to the general condition attained by fish or inherited or acquired resistance.

The general prophylactic measures adopted in the present experiment for the prevention of disease of test fishes were.

(1) **Avoid over crowding**: This may not be an actual cause of disease but contributes to the rapid spread of any infection.

(2) **Avoid over feeding**: Over feeding contributes to invitation of diseases, because uneaten food settled on the bottom of the tank will rot and pollute the water.

(3) **Avoid supply of inadequate diet**: The supply of poor quality diet is one of the factors of gross imbalance in protein –carbohydrate fat ratio, which leads to a variety of infection.

(4) **Partial water exchange weekly**: Approximately 10-12 % of the water should be changed weekly under ideal condition. This prevents the building up of excess nitrogenous condition and helps in keeping the entire tank in good condition.

(5) **Maintenance of water quality**: Rapid changes of the physico-chemical properties of the aquarium water will have weakening effect on fish. The water quality like dissolve oxygen, water temperature, pH value and hardness are maintained in permissible limit for prevention of disease.

(6) **Precaution on new addition**: The introduction of new fish, plants or accessories into the established aquaria may cause a sudden onslaught of disease. Therefore, precautionary measures are taken before introducing any new addition.

(7) **Quarantine process**: The precautionary measure for preventing disease is the quarantine process. All new fishes caught from the wild are kept in the quarantine cemented tanks and aquarium for 2 –4 weeks.

(8) **Avoid contamination**: Contamination takes place due to unhygienic handling and management. Use of unsterilize nets and unwashed hand. Therefore, proper sterilization method is followed after handling of diseased fish and their tanks.

(9) **Proper cleanliness and filtration**: Filtration of water is a must in order to keep the aquaria glowing. Lack of cleanliness lead to, unhealthy water condition and growth of algae and moss, thereby, boost harmful organisms which may help disease outbreak. The utmost care is taken for cleanliness and filtration of the aquaria.

CHAPTER-4

DISCUSSION

Graphics of the fish species

The present investigation exposes the comprehensive account of the systematic of the two test species *Danio dangila* and *Puntius chola* hitherto remained unattended from the drainages of Nagaland. Variations in some morphometric, meristic and morphological characters have been observed in the species, *Danio dangila* and *Puntius chola* from earlier worker (Talwar and Jhingran, 1991) and are purported below.

Danio dangila

Morphometrics:

BD 7.60 – 6.60 (male) and 8.20 – 4.85 (female) in relation to SL (*versus* 3.2 – 3.7 Talwar and Jhingran (TG), 1991); SnL 5.00 – 4.00 (male) and 5.00 – 3.20 (female) in relation to HL (*versus* 3.3 – 4.3 TG); ED 3.33 – 4.00 (male) and 3.33 – 4.00 (female) in relation to HL (*versus* 2.5 – 3.7 TG).

Meristic:

D ii 8 (male) D ii 10 (female) (*versus* D ii 9-11 Talwar and Jhingran (TG), 1991); P iv 9 both male and female (*versus* P i 11-12 TG); A iii 13 (male) A iii 14 (female) (*versus* A ii-iii 12-15 TG).

General characteristics

Danio dangila possess attrective body coloration in live condition. The fish species is rather small in size, easily adapted to aquarium life and display majestic movements. These characteristics put *Danio dangila* as classified ornamental fish. Similarly Dey *et.al*, (2002), also placed *Danio dangila* as classified ornamental fish in the global market.

Puntius chola

Morphometrics:

BD 7.50 – 6.88 (male) and 7.00 – 7.00 (female) in relation to SL (*versus* 2.8 - 3.1 Talwar and Jhingran, 1991).

Meristic:

D iii 9 (female) (versus D iii 8 Talwar and Jhingran, 1991. (TG); P iii 13 (male) P iii 12 (female) (versus P i 14 TG); V ii 7 (female) (versus V i 8 TG); A ii 6 (male) A iii 6 (female) (versus A ii 5 TG).

General characteristics

Because of beautiful colour pattern on the body, orange red eyes and deep-black blotch near base of caudal fin, *Puntius chola* is put under classified ornamental fish, which is also in accordance with Dey *et.al*, (2002).

Behavioural perspectives

Danio dangila

Ingestive behaviour of the test species have been precisely investigated in the aquaria. Normally, *Danio dangila* is a surface feeder but it is well competent to feed both at column as well as bottom level when food is supplied in the aquaria.

The males of *Danio dangila* are not territorial and do not display aggressiveness towards other males, as reported in daniids (Sarmah, 2002). Courtship behaviour or courtship dance was found in the test species as was reported in *Brachydanio rerio* (Guthric and Mutz, 1993), *Danio aequipinnatus* (Kharbuli *et.al,* 2004) and *Puntius chola* (Vincent and Thomas, 2008). The male initiates the motivation for courtship by poking the female. The female remains indifferent while the male entices the female during spawning and this is in accordance with the view of Leigh (1977) and Fischer (1980) in small sea basses of the genus *Thypoplectrus* of Caribbean coral reefs.

Puntius chola

Ingestive behaviour of the test species have been precisely investigated in the aquaria. The feeding activities commence with the fish swiftly gulping the food elements making an angle of 70^o. Normally, *Puntius chola* is a column feeder but after acclimatization, it was seen to execute at the surface level.

Courtship behaviour or courtship dance was found in the test species as was in conformity with the findings of Vincent and Thomas, (2008). Similar observations were also made by Gathrie and Muntaz, (1993) in *Brachydanio rerio.* Before the actual courtship display, the male and female of *Puntius chola* exhibit restlessness and frequently moving at the base of the aquaria. Both male and female exhibits swimming movements in pairs, circling and pushing the female on the abdomen during courtship. The male initiates the motivation for courtship and in the process nudges the female with the snout and pushes the female upwards and then bends down and brings its genital pore in proximity with the female's genital pore enticing and interlocking the female with the pelvic and anal fin.

Bionomics profile

Danio dangila

The present study portrayed that, the basic food of *Danio dangila* was of animal origin, such as aquatic insects, earthworm, tadpole, mosquito larva and planktons, showing variation in their percentage of consumption during different seasons. Besides this, the study further revealed that, the secondary food of the test species comprises of decaying plant matters and algae which were found to occur in maximum percentage throughout the year whereas mud and sand are found to be incidental food.

The overall victual spectrum was almost similar in both male and female species. The gut content of the test species was found to vary in different seasons with the availability of the food items. The most intensive feeding takes place during the season, when the food consisting mostly of the young aquatic insects or other food of animal origin were found abundent. This was found to be relevant in the present test species. Therefore, changes in the supply of food and the seasonal rhythm of feeding were connected with the availability of feed itself throughout the year. Similar observations on food and feeding habit of fishes was also reported by Galis *et.al,* (1993).

The relative gut length (RLG) is closely related to the food habit (Das, 1958). The length of the gut varies not only with the food habit but also in relation to size and age of the species (Sinha and Moitra, 1975 a, 1975 b and Sinha, 1972). Low and high RLG indices purport for the carnivores and herbivores respectively with an intermediate value prevailing for the omnivores. This is for the fact that, long intestine of the herbivores helps in the digestion and absorption of the vegetable matters than the carnivores whose diet is easily assimilated consisting of animal matter.

The present findings revealed specific changes in RLG of the species. It showed every 5.0 mm increment in body length from sub adult to adult. In females at maximum size range of 75 – 80 mm, the RLG was 0.747 whereas in males at size range of 75 – 80 mm, the RLG was only 0.679, indicating that, males of the test species are more carnivorous than the females. Comparative analysis between male and female of *Danio dangila* revealed that, the males have shorter alimentary canal than the females. Fluctuation in the RLG may also be attributed to the condition of food supply, the amount of indigestible matter and age of individual fish irrespective of its body size (Kapoor *et.al,* 1975) which were in accordance with the present empirical investigation.

The hepato somatic index (HSI), considered as an exponent of feeding intensity showed direct relationship with size range in the test species. The present study showed that, HSI changes in relation to food supply and seasonal rhythm in food consumption through adaptive change in the abiotic condition. However, during breeding season, the HSI in the female of the test species showed decreasing trend than the overall HSI. This was attributed to the development of gonads during spawning season, which occupy the major space of the abdominal cavity. Similar observation on HSI in relation to food supply and seasonal rhythm in food consumption was also made by (Jhingran, 1961). Therefore, it can be inferred that the intensity of feeding shows a downward trend in the approach of the maturation phase and ingestion improve only when the spawning period was over.

The gill rakers of fishes are variously modified according to feeding habits of the fish (Das and Moitra. 1956, 1963). The structure of the gill raker is closely related to the feeding behaviour of the fish. The number and structure of the gill rakers can vary considerably, from a few small hard structures in predatory fishes to a complex network of numerous rakers in planktophagic fishes (Nikolsky, 1963). The gill raker configuration basically revealed carnivorous pattern both in structure and number of the test species. The gill rakers were short, stumpy and blunt and also were longer in the central part of both the limb.

Puntius chola

Based on the recorded variation in the type of food consumed, *Puntius chola* may reasonably be classified as marginal euryphagic feeder. Euryphagism has evolved as an adaptation to unstable supply of food. (Nikolsky, 1963). Changes in the food spectrum were observed in the same population of the test species throughout the year. However, no differences between male and female *Puntius chola* in their food spectra were observed.

The present study portrayed that, the basic food of *Puntius chola* was algae showing variation in their percentage of consumption during different seasons. Besides this, the study further revealed that, the secondary food of the test species comprises of decaying plant matters, aquatic insects and debris. Dasgupta, (2004) also found predominance of plant matter in the gut contents of *Colisa fasciata*. Certain unidentified food items were also present which were classified as incidental food. The overall victual spectra were almost similar in both male and female species. The gut content of the test species revealed that, the percentage of occurrence of algae was the highest. The most intensive feeding takes place during the months, when the food consisting mostly of algae and related food are found in abundance. Therefore, changes in the supply of food and the seasonal rhythm of feeding are connected with the condition of the fish itself throughout the year.

The present study portrayed that, for every 5.0 mm increment in body length, changes in RLG was recorded. Comparative analysis between male and female of *Puntius chola* revealed that, the males have a shorter alimentary canal than the females. In males at maximum size range of 80 – 85 mm, RLG was 1.728. Whereas, in females at size range of 80 – 85 mm the RLG was 2.365 thus, indicating that, females of the test species were more algaephagus than the males. Fluctuation in the RLG may also be attributed to the condition of food supply, the amount of indigestible matter and age of individual fish irrespective of its body size (Kapoor *et.al*, 1975). This was in accordance with the present empirical investigation.

In breeding season, the HSI in the female of the test species showed more or less decreasing trend than the overall HSI. The low feeding behaviour during breeding season in the present test species was therefore, attributed to the development of gonads during spawning season, which occupy the major space of the abdominal cavity (Jhingran, 1961). Thus it can be inferred that the intensity of feeding shows a downward trend in the approach of the maturation phase and ingestion improve only when the spawning period is over.

The gill rakers of *Puntius chola* were short, straight and pointed. The gill rakers are longer in the central part of both the limb.The gill raker configuration basically revealed omnivores pattern both in structure and number of the test species. Similarly, Das and Moitra (1963) also reported that gill rakers of fishes are variously modified according to feeding habit of the fish.

Length-weight relationship

Danio dangila

In the present study the value of regression co-efficient (n) for male was found 2.103 and for female 2.055, thus the value of '**n**' was slightly lower in case of female population, but in mixed population it showed a value of 2.114. The results indicated that, the values of '**n**' in all the cases were less than 3, and therefore it deviated from the cube law. The divergence from the cube law may be due to certain environmental factors and general condition such as, appetite and gonadal contents of fish. The growth in weight of fish, in general, is directly proportional to the cube of its length, but sometimes values of the relationship may deviate from the cube law, either due to environmental factors or condition of fish (Le Cren, 1951; Solanki *et.al*, 2004). Pervin and Mortuza (2008) cited that, the length-weight relationship is very important for proper exploitation and management of fish species population. The economic value of fish depends upon its length weight relationship.

The correlation coefficient (r) between log-length and log-weight were 0.979 for males, 0.977 for females and 0.959 for mixed population. As was evident from't' test' the coefficients of correlation were highly significant in all cases (5% probability) and the body weight positively and significantly correlated with the length. Similar observations were also made by Subba and Ghosh, (2000) and Pawar and Mane, (2006) in case of *Glyptothorax telchitta* and *Glossogobius giuris* respectively.

The ponderal index or coefficient of condition is an index of well being of the fishes. In the present study Le Cren's relative condition factor (Kn) was estimated separately for mixed, male and female population. The value of Le Cren's relative condition factor attributes to be more reliable index for the well being study of the fish. The data of present study purported that, the well-being of the fish was comparatively better exhibited in female population than the male population.

Puntius chola

In *Puntius chola*, the value of regression co-efficient (n) for male was found 2.506 and for female 2.271, thus the value of '**n**' was slightly lower in case of female population, and in mixed population it showed a value of 2.112. The results indicated that, the values of '**n**' in all the cases were less than 3, and therefore it deviated from the cube law. The divergence from the cube law may be due to certain environmental factors and general condition such as, appetite and gonadal contents of fish. Similar observation was also made in case of *Danio dangila* where the values of 'n' were found to deviate from the cube law (Angami and Ahmed, 2010).

The correlation coefficient (r) between log-length and log-weight were 0.979 for males, 0.980 for females and 0.977 for mixed population. As was evident from't' test' the coefficients of correlation were highly significant in all cases (5% probability) and the body weight positively and significantly correlated with the length. Similar observations were also made by Subba and Ghosh, (2000) and Pawar and Mane, (2006) in case of *Glyptothorax telchitta* and *Glossogobius giuris* respectively.

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Reproductive biology

Danio dangila

The present work gave a comprehensive treatise on the reproductive biology of *Danio dangila*. All the parameters within the ambit of reproductive biology of fish have been successfully covered and documented in the present study. The innovative exposition on the sexual dimorphism, size at first maturity, spawning habitat were significant.

The studied test species were sexually dimorphic and the in-vitro characteristics elucidated will immensely help in field collection and identification of male and female species.

The morphological size differences in between the sexes were significant in the present test OFS. The males are slightly smaller than the female which thus confirms the observations of Kurian and Inasu, (1997), Pal *et.al,* (2003) and Thomas *et.al,* (2003). Usually the female are found to be larger than male of the same age group (Tessy and Inasu, 1997). In case of *Danio dangila* also the femals are found to be larger than male species. Males of *Danio dangila* have reddish tinge colour fins whereas the tip of the anal fin was found to be reddish. The females have yellowish tinge colour fins and whitish yellow anal fin tip.

The sex ratio of *Danio dangila* varies considerably from one population to another and from year to year in the same population which support the proposition of Nikolsky (1963). However, sparse male population over female in nature is a significant feature, which may perhaps have relevance to low natural population of the species. This contradicts with the findings of higher male sex ratio than female in case of *Colisa* *fasciata* from the flood plain wetland of Ganga river basin as reported by Mitra *et.al,* (2007).

The individual relates the onset of maturity to the attainment of a particular size. Sexual maturity is also related to the abundance of food supply. Considerable variation exists among different populations of the same species, and also within the limits of a single population (Noikolsky, 1963) and was also evident in the present study. The male and female *Danio dangila* attain its sexual maturity at 4.5 cm in total length which is species specific since Mitra *et.al,* (2007) reported that, females of *Colisa fasciata* reaches its maturity at 5.7 cm in total length.

Danio dangila is a seasonal breeder and it breeds once in a year and exhibits rhythmic changes in the structure of the ovary in different seasons. Seven stages of ovarian cycle have been identified in the test species studied. Whereas, only four stages in the ovarian cycle of some Indian fresh water fishes including *Rasbora (Parluciosoma daniconius)* were reported by Raizada, (1971).

Fecundity is an important tool to understand the reproductive capacity of a fish species. It is a basic means of regulating the rate of reproduction to changing environmental condition. Regression analysis depicts a positive correlation between fecundity and ovary weight but negative correlation between fecundity and total length and total weight, which was a significant feature and forms a record in this OFS. Nikolsky, (1963) reported that, the individual fecundity is increased by fractional spawning which was also recorded in the present test species. Borkotoki and Dey, (2002) reported a high degree of positive correlation between fecundity and gonad weight in *Parluciosoma daniconius* indicating a significant relationship between fecundity and gonad weight.

Although fecundity is considered a phylogenetic character of a fish, it also depends upon various environmental parameters, which the fish had to face in different stages of its life history. Mahapatra *et.al*, (2004) reported that, fecundity was more dependent on body length when compared to body weight and ovary weight in *Brachydanio rario*. The fecundity aspects of population dynamics, racial characteristics and fishery management practices appear relevant for the present OFS in their mass culture and breeding, and this was also opined by Agarwal, (1996).

Nutrition and environment influence the gonadal development of a fish. Under optimum condition the gonads begin to grow rapidly and there is also an accompanying increase in the total weight of the fish. The relationship is direct and represented by the GSI. Unlike high GSI index in total spawner (Sarmah, 2002), the test species, a protracted spawner showed an ascending trend in the GSI. This indicated ovary maturation and decline for spawning. In the spent or resting female the lowest GSI was recorded. However, recovery in GSI values indicated by nominal increase of the index was observed which culminates to a new breeding cycle. Borkotoki and Dey, (2002) observed that, the GSI trend in five different size range of *Parluciosoma daniconius* showed increasing GSI with increase in total length. The GSI trend in the test species indicates a spike in the month of May. Unlike pronounced abdomen bulging of matured female in IMC and other fishes, *Danio dangila* exhibited marginal swelling of abdomen. And this feature is more discernable in medium sized fishes.

The breeding season of *Danio dangila* spans from May to mid June, with highest amplitude of spawning in the month of May synchronizing with the onset of monsoon which was also reported by Mustafa *et.al*, (1980) in other fish species. The optimal environmental conditions under which the test species breed successfully in nature have been ascertained during the field studies.

Puntius chola

The present contribution forms a comprehensive treatise on the reproductive biology of *Puntius chola*, hitherto remained unreported from NER. Similar to *Danio dangila*, all the parameters within the ambit of reproductive biology of fish have been successfully covered and completed in the present study.

The studied test species was sexually dimorphic and the in-vitro characteristics elucidated will immensely help in field collection and identification of male and female. The morphological size differences in between the sexes were significant in the present test species. The males were larger than the female in the present test fish which contradict with the findings of Thomas *et.al,* (2003) in some freshwater salmonids. Presence of rough dorsal side of pectoral fin, a common feature in male Indian Major Carp (Dey and Roy, 1991) was not discernable in this species. Further, *Puntius chola* does not exhibit rough pelvic fin and was also opined by Pal *et.al*, (2003).

The sex ratio of *Puntius chola* varies considerably from one population to another and from year to year in the same population which support the proposition of Nikolsky (1963). However, sparse male population over female in nature is a significant feature, which may perhaps have relevance to low natural population of the species.

According to Wootton (1982), the difference in sexual maturity is due to variations in genetic resources and environmental conditions of the fish habitat. The male *Puntius chola* matured much earlier than the female in the present test fish species. Considerable variation exist among different populations of the same species, and also within the limits of a single population (Nikolsky, 1963) was also observed.

In the present investigation, on the basis of the ova diameter study, seven maturity stages were determined in *Puntius chola* as was reported by (Kesteven, 1960).

Regression analysis depicts a positive correlation between F and OW, TL and TW, which was significant and forms a record in this Species. The individual fecundity was increased by fractional spawning. Although fecundity is considered a phylogenetic character of a fish, it also depends upon various environmental parameters, which the fish had to face in different stages of its life history. Bahuguna *et.al*, (2010) observed fecundity in *Barilius bendelisis* and commented that, fecundity increases with increase in all body parameters. The fecundity aspects of population dynamics, racial characteristics and fishery management practices (Agarwal, 1996) appeared relevant for the present fish species also in its mass culture and breeding.

Nutrition and environment conditions influence the gonadal development of a fish. Under optimum condition the gonads grows rapidly which also increases the total weight of the fish. The relationship is direct and represented by the GSI. Unlike high GSI index of in total spawner (Sarmah, 2002), the test species, which spawns twice in a year, exhibited two mean GSI above 17% just before spawning which is in accordance with the findings of Thomas *et.al,* (2003). As observed in *Danio dangila*, the ascending trend in the GSI was indicative of ovary maturation and decline for spawning. The lowest GSI was recorded in the spent or resting female. However, recovery in GSI values was indicated by increase of the index, which culminates to a new breeding cycle. The GSI trend in the test species indicated an initial spike in the month of May with a lull phase in June, which than recovered with the second spike in the month of September. *Puntius chola* exhibited marginal swelling of abdomen. This feature is more discernable in medium sized fishes. The GSI of the first breeding period showed a maximum peak during the month of May and the GSI of the second breeding period showed a maximum peak in the month of September. The occurance of different sizes of fries in nature indicated prolong breeding period. Thus, the breeding period may be described as primary phase of prolonged breeding season. After this spawning phase, few brooders remain unspawned and enter in the quiescent period. On the approach of favourable environmental conditions, they spawned during September.

According to Jhingran (1983), most of the freshwater fishes in Indian sub-continent breed during monsoon season. In this context the present study revealed that, *Puntius chola* has two breeding season, once in the month of May and another in the month of September with inactive period of spawning during October-April and June-August. During these two breeding season majority of the brooders were found to be ready to breed, as evident from the GSI and occurrence of large number of ripe fishes in the natural as well as in the stocking pond. The spawning grounds of *Puntius chola* was characterized by the occurrence of gravels, mud and sand in the shallow water at a depth not more than 4-4.8 feet, where typically very low water current occurs. Thus it belongs to "lithophil spawner" category (Nikolsky, 1963).

Laboratory propagation

Danio dangila

The present study forms the model for the development of the technology of captive breeding of endemic ornamental fish species of NER using synthetic hormone. The in–house breeding technology of *Danio dangila* has been successfully achieved.

Malady free, bright coloured with good finage and fast growing specimen were considered as an important criteria for the selection of brood stock. The selection of breeding stock from the wild becomes considerably easier than the cultivated strain to avoid inbreeding of the species (Kelly, 1987). In the present investigation mean ova diameter (MOD) of the brooders was found to be a favourable indicator for spawning and maturity of the species which can be correlated with the findings of Shashi and Sengupta (2002). The study further revealed that, the MOD of the effective brooder should lie between 0.95 – 1.05 mm prior to inducing. Saha *et.al,* (2009) reported that, the maximum mean diameter of ova of *Amblypharyngodon mola* was 1.82 mm obtain during March to August with a peak mean value in June.

Minor variation in the physico-chemical parameters of the brooder tank does not affect the survivability of the brooders of the test species investigated. When the physico-chemical conditions in the brooders tank were compared to the condition recorded in the wild, a significant variation was observed. However, these variations did not adversely affect the rearing of the brooders. It may, therefore, be ascertained that, the combination of factors in the brooders tank as a whole contributes to successful rearing of present OFS brooders**.**

Nutrition is another important factor and there was a distinct variation in the preference level of different feed**.** As *Danio dangila* feeds exclusively on living organisms, the feeds were disinfected prior to application to avoid contamination or outbreak of disease.

The spawning was achieved in captivity by induced breeding through hypophysation in various teleost fish in India and abroad (Turner, 1993 and Anna Mercy *et.al,* 2003). The failure of spawning after administration of gonadotropin in many species is due to the dopamine inhibitor activity (Nandeesha *et.al*, 1991).

In contrast to reports of partial spawning in *Botia dorio* (Das, 2004), induced breeding with ovaprim was successful in *Danio dangila*. This may be due to the salmon gonadotropin releasing hormone (sGnRH) and domaperidone–a dopamine inhibitor substance in the ovaprim. Significantly, male reaches oozing state one month earlier than the functional maturity of the female. This is in confirmity with the observation of Sukumaran *et.al*, (1984) in *H. molithrix* and *C. idella*.

The males of *Danio dangila* are not territorial and do not display aggressiveness towards other males, as reported in daniids (Sarmah, 2002). Courtship behaviour or courtship dance was found in the test species as was reported in *Brachydanio rerio* (Guthric and Mutz, 1993), *Danio aequipinnatus* (Kharbuli *et.al,* 2004), and *Puntius chola* (Vincent and Thomas, 2008). Turner, (1993) and Anna Mercy *et.al*, (2003) reported that, cyprinid fishes in general, exhibit some common patterns in their courtship and reproductive behavior. The male initiates the motivation for courtship by poking the female. The female remains indifferent while the male entices the female during spawning (Leigh, 1977 and Fischer, 1980).

A total of 12 sets were induced out of which 11 sets spawned successfully with a success rate of 91.67%. The negative response of one set was identified as inappropriate hormone administration. The test species was a seasonal spawner and after the courtship display, releases eggs in batches. The experimental result showed that, the eggs released in the first batch were 100% fertilized, which declines proportionately in subsequent batches. The success of fertilization in *Danio dangila* was found directly proportional to the matured stage of eggs in the ovarian cycle. First batch ova were found in sixth stage of maturity ensuing 100% fertilization. But, in subsequent batches the ova are mostly found in their fourth and fifth stages mingled with limited number of ova at sixth stage of maturity. Unlike other fishes, this trend which forms a unique feature in *Danio dangila* results in descending trend of fertilization in subsequent batches.

Puntius chola

As indicated in *Danio dangila*, the captive breeding of *Puntius chola* using synthetic hormone will thus form a model technology for induce breeding of other indigenous ornamental fish species of NER region. The present study revealed that, the MOD of the effective brooder should lie between 1.0–1.5 mm prior to extragenous administration of synthetic hormone.

The survivability of the test brooders were not adversely affected for rearing upon some minor variation in the physico-chemical parameters of the brooders tank. *Puntius chola* feeds on planktons. The plantonic feeds were disinfected prior to its application to avoid contamination or outbreak of disease.

In contrast to reports of partial spawning in *Botia dorio* (Das, 2004), induced breeding with hormone, ovaprim was successful in *Puntius chola*. Significantly, male reaches oozing state one month earlier than the functional maturity of the female which is in confirmity with the findings of Sukumaran *et.al*, (1984) in *H. molithrix* and *C. idella*.

In the present investigation 'Ovaprim' was found successful for inducing *Puntius chola* in laboratory conditions. The domaperidone potentiate the action of Gonadotropin by inhibiting the dopamine in the fish (Nandeesha *et.al*, 1991). The response of *Puntius chola* to higher doses of ovaprim during the study could be related to the high dopamine activity in this test species. *Puntius chola* does not respond actively to single dose of ovaprim, but two split doses gave better results. This could be due to the self-potentiating action of releasing hormone when given in split doses.

Courtship behaviour or courtship dance was reported in some ornamental fish species viz, *Brachydanio rerio* (Guthric and Mutz, 1993), *Danio aequipinnatus* (Kharbuli *et.al*, 2004). The male initiates the motivation for courtship by poking the female. *Puntius chola* was found to exhibit swimming movements in pairs and circling. Vincent and Thomas (2008) reported that, the sequence of behavioural patterns in *Puntius chola* is well organized and characterized by several circling movements around the female during courtship activity. After the courtship display the female releases eggs in batches.

A total of 12 sets were induced out of which 10 sets spawned successfully with a success rate of 83.33 %. The negative response of two set was identified as inappropriate hormone administration. The success of fertilization in *Puntius chola* was found directly proportional to the maturity stage of eggs in the ovarian cycle. Fertilization rate was recorded between 70 to 95 % at ambient water temperature ranged from 20 - 26.5 ^{0}c. The lower fertilization rate could be attributed to the comparatively poor quality of the eggs and milt produced by the brood stock reared in laboratory conditions.

Embryonic and larval development

Danio dangila

The pigmentation in the yolk and non sticky eggs are the chief diagnostic characters of the eggs of *Danio dangila*. The quality of sexual product (yolk amount) and environmental conditions such as temperature, dissolved oxygen etc were found significant which is in accordance with the findings of Blaxter, (1969). Swain, (2008) reported that, the period of incubation differs according to the water temperature, under ideal water temperature condition (26-28^{0} c) the hatching period of gold fish was three days. However in the present study, the incubation period was 36 hours at 20–26.5 ^{0}c water temperature. Thus, it is apperant from the present study that, the period of incubation differs in different fish species and therefore, does not dependent on the ambient water temperature.

The present findings also confirm the view of Balon, (1975) that even in the same species, which live in different water bodies among wild and domesticated races, early ontogenetic differences were evident. Several workers, (Blaxter, 1969; Suzuki and Hibiya, 1984 and Unal *et.al*, 2000) reported that, chromatophores in newly hatched free larva are absent. However, in *Danio dangila* at 29.45 hrs after hatching chromatophores were distinctly recorded. Further star shaped chromatophores were observed to aggregate at perspective band sites.

Presence of some cement organs for attachment to substratum as reported in some cyprinids, like carp and bream (Nikolsky, 1963; Blaxter, 1969 and Balon, 1975) was evident in the present test species as well. Pre-hatching latency observed in daniids for a brief period (Harrington, 1947) was also observed in *Danio dangila*. This may be attributed to its lotic habitat.

Puntius chola

The eggs of *Puntius chola* are non adhesive and non demersal with grey or black pigmentation on the yolk. The incubation period was 26 hours at 22–27^{0} c water temperature. However, Swain, (2008) reported that, the period of incubation differs according to the water temperature, under ideal water temperature condition (26-28^{0} c) the hatching period of gold fish was three days.

The present findings also confirm the view of Balon (1975) that even in the same species which live in different water bodies among wild and domesticated races early ontogenetic differences are evident. As observed in *Danio dangila* the chromatophores are distinctly recorded in *Puntius chola* at 26.35 hrs after hatching. More over, the chromatophores were also observed to aggregate at perspective band sites of the newly hatched larva.

Presence of some cement organs for attachment to substratum as reported in some cyprinids, like carp and bream (Nikolsky, 1963; Blaxter, 1969 and Balon, 1975) was evident in the present test species. Pre-hatching latency observed in daniids for a brief period (Harrington, 1947) was conspicuously absent in *Puntius chola*. This may be attributed to its lentic habitat.

Laboratory rearing of fry

Danio dangila

The success of rising of hatchlings up to the marketable size has been achieved in the present study. The most crucial and sensitive stage in the life of a fish is during early larval stages. The large-scale mortality of spawn may occur due to appreciable differences in the physico-chemical conditions of water, lack of requisite amount of choice food and predatory nature of fish. Hence, the rate of survival at these stages depends on the maintenance of optimum water quality, availability of sufficient amount of right kind of food and a predator free environment.

Approximately, 90% survivability of fry of *Danio dangila* in glass aquaria has been achieved in the present study. Whereas Pal *et al,* (2003) reported about high mortality in laboratory rearing of fries. Swain *et.al,* (2008) reported on successful larval rearing of *Ompok pabda* at CIFA, Kalyani centre, leading towards commercialization.

The fries of *Danio dangila* were carnivorous and prefer infusorians rather than egg yolk suspension and other pelleted food. Survivability of the fries was found correlated with the availability of choice food and the feeding schedule. The larvae soon changes its food habit to live crustacean larva and other zooplanktons from twenty fifth day onward after hatching, confirming similar observations in sea bass larva (Parazo *et.al*, 1998).

Puntius chola

The successful raising of hatchlings up to the marketable size of the species has been achieved in the present study.

Early larval stages are the most critical and sensitive stage in the life of a fish. The rate of survival at different stages depends on the maintenance of optimum water quality, availability of sufficient amount of right kind of food and a predator free environment.

Almost, 95% survivability of fries of *Puntius chola* in glass aquaria has been achieved in the present study. Larval rearing of some ornamental fish species endemic to North East India was also reported by CIFA, Bhubaneswar centre (Swain *et.al*, 2008).

The fries of *Puntius chola* were omnivorous but reject egg yolk suspension and other pelleted food. As in *Danio dangila* the Survivability of the fry was also found to be correlated with the availability of choice food and the feeding schedule. The early fry feeds on cultured infusorians, egg yolk and planktons but it soon accepts the formulated and manufactured feed from thirtyth day onward after hatching.

Fish malady and restraints

Danio dangila

Danio dangila was most susceptible to fungal attack caused by *Saprolegnia spp*, which manifest in the body as well as the eyes. The infection develops secondarily in areas, which were bruised by rough handling or mechanical injury. Jhingran, (1991) also reported the secondary fungal infection of fishes. Heavy fungal infection leads to ulceration or exfoliation of the skin followed by haemorrhage and blindness. Next to fungal disease, *Danio dangila* was prone to bacterial disease, tail and fin rot followed by ulceration. Overcrowding in the stocking tank could be the causative factor for the outbreak of bacterial disease and was also reported by Schaperclaus (1986). General prophylaxis measures opined by various authors (Hora and Pillay, 1962; Gopalalkrishnan, 1963; Schaperclous, 1986; Singh and Sreedharan, 2002 and Mishra and Das, 2005) were found to be relevant in the present test species. But to be precise, the present investigation recommended that, the disease specific prophylaxis measure was more effective and desirable for control of malady of *Danio dangila*.

Treatment through antibiotics like chloromycetin, tetracycline, oxytetracycline etc. at prescribed doses yield good results in the test species as, also reported by some workers (Schaperclous, 1986; Varghese, 1988; Swain, 2008). However, some modifications of doses were required to fetch better result. Body fungus treated by dip treatment @ 1mg/litre Km_nO_4 solution was found effective (Hora and Pillay, 1962 and Swain, 2008).

“Salt treatment”, a very popular among the aquarists for its cheap, easily available and non-toxic proportion was found effective for treatment of *Myxosporidian* and moderately effective in fungal disease. Malachite green @ 5 mg/litre water when spread 2–3 drops in nursery tank before spawning and hatching phase showed good result against fungal attack, which was also reported by (Srivastava, 1987).

Puntius chola

The occurrence and magnitude of various fish diseases are closely related to the sanitary conditions prevalent in the water and also the condition and general health of the fishes themselves. *Puntius chola* was also found most susceptible to fungal attack caused by *Saprolegnia spp*, which manifest in the body as well as the eyes. This fungal infection develops secondarily in areas, which were bruised by rough handling or mechanical injury (Jhingran, 1991). Next to fungal disease, *Puntius chola* was also prone to bacterial disease, tail and fin rot followed by ulceration. Overcrowding in the stocking tank probably leads to outbreak of bacterial disease (Schaperclaus, 1986). General prophylaxis measures opined by different authors (Hora and Pillay, 1962; Gopalalkrishnan, 1963; Schaperclous, 1986; Swain, 2008 and Mishra and Das, 2005) were found to be relevant in the present test species.

Treatment through antibiotics like Chloromycetin, tetracycline, oxytetracycline etc. at prescribed doses as given by various authors (Schaperclous, 1986; Varghese, 1988; Singh, 2002; Singh and Sreedharan, 2002) yield good results in the present test species.

CHAPTER-5

SUMMARY

The North Eastern Region (NER) harbours plenty of excellent verities of ornamental fish species, which are very attractive and fetch a very high price in the overseas markets. At present the ornamental fish trade in NER is based on wild capture from natural habitat. Scientific studies on captive breeding and larval rearing of important indigenous ornamental fish species of NER are very meager and inadequate. Therefore, the present investigation was taken up to study the bionomics and breeding biology of two rheophilic ornamental fish species namely *Danio dangila* and *Puntius chola*. Due Emphases was given on the following broad aspects viz. graphic of the fish species, behavioural prespective, bionomics profile, length-weight relationship, reproductive biology, laboratory propagation, embryonic and larval development, laboratory rearing of fry and fish malady and restraints.

The graphics of the two test fish species were portrayed separately with the meristic measurements and counts, morphometrics and non-meristic characters of both male and female along with their distributions pattern and characteristics of the habitat. Both the test species were attractive, peaceful in nature, small in size, exhibits brilliant colouration and display graceful movements in the aquarium. These characteristics put *Danio dangila* and *Puntius chola* as classified ornamental fish species.

Various behavioural perspectives of both the species were intricately explicated. Ingestive behaviour was elucidated during; surface, column and bottom feeding with schematic diagrammatic representation, fin reciprocation and opercular movement were observed and recorded during feeding. Procreate demeanor portrayed the courtship display and spawning behaviour of the test species.

On the bionomics profile of the test species, the victual spectra have been recorded during pre-monsoon, (March-May), monsoon, (June-August), post-monsoon, (September-November) and winter, (December-

February). Lineament of feeding was precisely explained through flow chart. Relative gut length (RLG) was explained in respect of total length. The overall hepato-somatic index (HSI) was recorded. The index of preponderance (PI) was ascertained. The morphology of gill and gill rakers were also been studied and illustrated in the two test species.

The Weight-Length relationship of *Danio dangila* and *Puntius chola* for mixed population as well as for male and female population were studied separately and ascertained. The co-efficient of condition and the well being of the species have been studied separately using Le Cren's formulae for male and female population of the two test fish species.

The study on the reproductive biology of the species include, In-vitro sexual dimorphism, sex ratio, size at first maturity, maturity stages of ova. Fecundity of the test species was estimated and the relationship between fecundity and other variables were recorded through co-efficient of correlation with t-test and Regression equation after least square method. The GSI trend elucidates the breeding season and periodicity of the test species. The category and trend of spawning period were documented. Spawning habitat of the test species were studied from field data and the trend were been reciprocated in the laboratory experiments.

Laboratory propagation was focused on the technology of induce breeding of the two test species. The general criteria for selection of brooders, maintenance of the brooder stock and stocking density of the brooders were studied. The in-house breeding techniques for mass production under control condition have been elucidated for the test species.

The embryonic and larval developments of *Danio dangila* and *Puntius chola* were studied. Three important phases' viz (i) embryonic phase (ii) hatchling phase and (iii) larval phase were highlighted with microphotographs and hand drawings.

Details accounts of in-house rearing of the fries were explicated based on present experimental findings under laboratory rearing of fry. The technique of rearing the fries in glass aquariums and advanced fries in cement cistern and fibre reinforced plastic tubs with suitable stocking densities were presented with special emphasis on survivability of the fries at different stocking rate. The food and feeding schedules of fries of the two test species have also been documented.

Fish malady and restraints of the two test species were studied. The occurrence and causative agents of the diseases have been investigated. A total of seven diseases in *Danio dangila* and six diseases in *Puntius chola* were observed and their prophylactic and treatment measures were discussed.

The findings were thoroughly analyzed statistically, where ever needed, and amalgamated under an explicated discussion. The intra and inter relationship among the different aspects of the two test species were made over the period of three years in the laboratory as well as in the fields.

The present investigation, hitherto remained unattended, depicted a clear scenario on the technology of captive breeding and culture with their bionomics and early life history of the two rheophelic ornamental fish species *Danio dangila* and *Puntius chola*. The developed breeding technology and the larval rearing of ornamental fish species when transferred from lab to land, the OFS trade will flourished unabated in the region and will contribute to the socio economic development of the state.

The treatise consists of 39 tables, 17 figures and 12 plates that amply support the findings incorporated. An elaborated list consisting of all the literatures consulted during the course of the present study have been presented under the Reference section.

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A P P E N D I X

Research paper published.

1. Length-Weight Relationship of *Danio dangila* (Ham.) from the Drainages of Nagaland. *Life Science Bulletin*, Vol. 7 (1) 2010: 59-61. (Communicated)

2. Ingestive Behaviour of *Danio dangila* (Ham.) recorded in the drainages of Nagaland. *Journal of Current Science*, 15 (1) 2010: 91-98. (Communicated)

Legends of figure- 14 and plate- 9 (Embryonic development in *Danio dangila*)

1. Fertilized egg (Zygote)
2. Clevage, morula stage 3 hrs after fertilization
3. Blastula, 4 hrs after fertilization
4. Gastrula, elongation of yolkmass- 9.45 hrs after fertilization
5. 12.20 hrs after fertilization-4 somites
6. 10 somites at 14.55 hrs after fertilization
7. 12 somites at 17 hrs after fertilization
8. 18 hrs after fertilization, caudal region getting free from embryo- 14 somites
9. At 19 hrs first tail twitching movement of the embryo observed- 18 somites
10. 20 somites at 20 hrs
11. 22 hrs after fertilization- 24 somites
12. 29 hrs after fertilization- 30 somites
13. At 31 hrs after fertilization the embryo occupies almost all part of the egg
14. 34 hrs after fertilization, vigorous twitching movement observed
15. 36 hrs after fertilization the embryo wriggles out with tail first

Legends of figure- 15 and plate- 10 (Larval development in *Danio dangila*)

1. Newly hatched pro-larva
2. Clinging stage
3. 2 days old larva
4. 3 days old larva
5. 8 days old larva
6. 10 days old larva
7. 15 days old larva
8. 25 days old larva
9. 1 month old larva

<u>Legends of figure- 16 and plate- 11 (Embryonic development in *Puntius chola*)</u>

1. Fertilized egg (Zygote)
2. Clevage 2 hrs after fertilization
3. Morula, 2. 35 hrs after fertilization
4. Blastula, at 4 hrs after fertilization
5. 6. 50 hrs after fertilization elongation of yolkmass
6. At 10 hrs after fertilization, optic cup and notochord with 8- somites
7. 12. 50 hrs the caudal region getting free from the embryo, somites- 18
8. At 15. 45 hrs after fertilization, caudal region seperated from yolkmass- 24 somites
9. At 19. 30 hrs embryo occupies three fourth of the egg, 30 somites
10. 23 hrs after fertilization, vigorous twitching movements
11. 26 hrs after fertilization the embryo wriggles out head first

Legends of figure- 17 and plate- 12 (Larval development in *Puntius chola*)

1. Newly hatched pro-larva

2. Clinging stage

3. 2 days old larva

4. 4 days old larva

5. 7 days old larva

6. 10 days old larva

7. 15 days old larva

8. 25 days old larva

9. 1 month old larva

Plate-1: Photograph of *Danio dangila*

Plate- 2: Photograph of *Puntius chola*

River Dhansiri

River Tesuru

River Kehoru

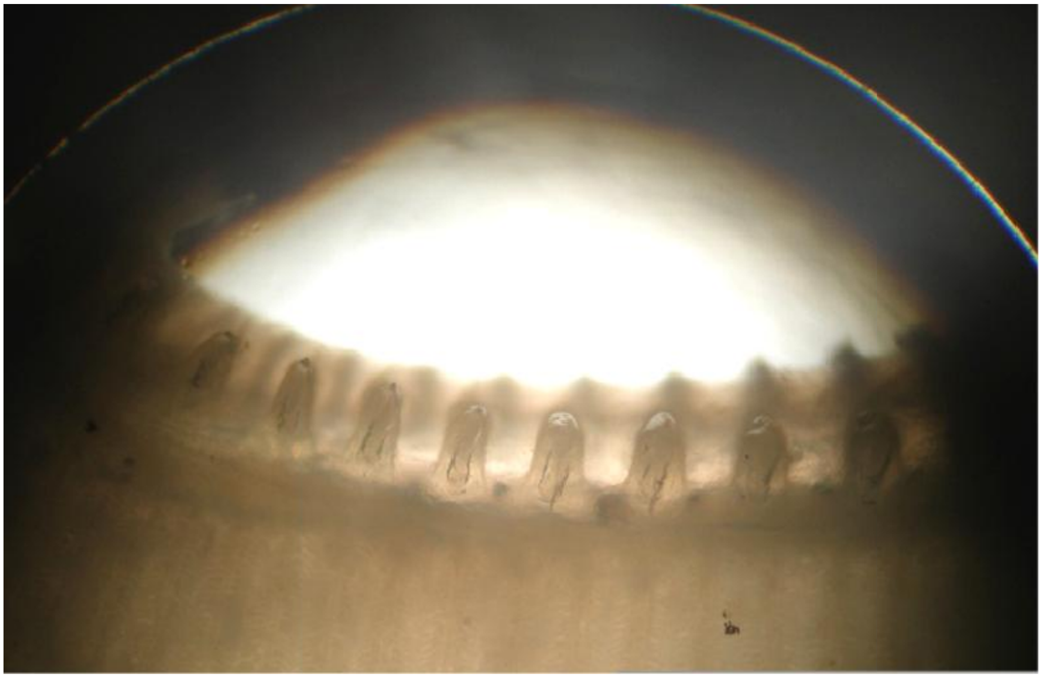
River Tsurang

River Dzuza

River Milak

Lentic water and Rice fields of Southern Angami Village (Kohima)

Plate- 3: Habitat of *Danio dangila*



Gill rakers configuration in *Danio dangila*

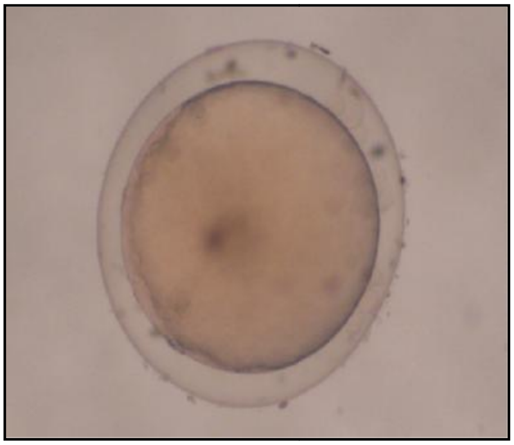
Gill rakers configuration in *Puntius chola*

Plate- 5: Micrographs showing gill rakers configuration in *Danio dangila* and *Puntius chola*

Males of *Danio dangila* Reddish colour tip of anal fin in male

Females of *Danio dangila* Whitish colour tip of anal fin in female

Plate- 6: Photograph showing sexual dimorphism in *Danio dangila*



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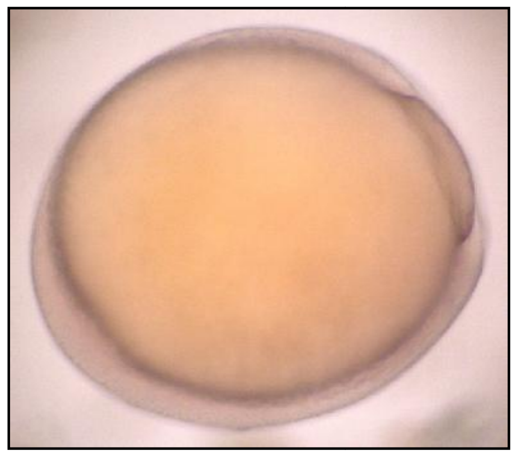

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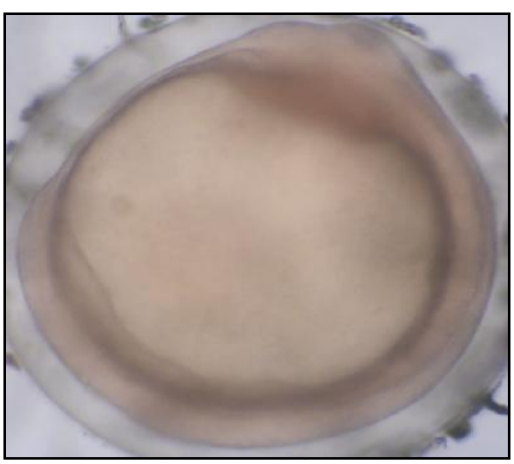

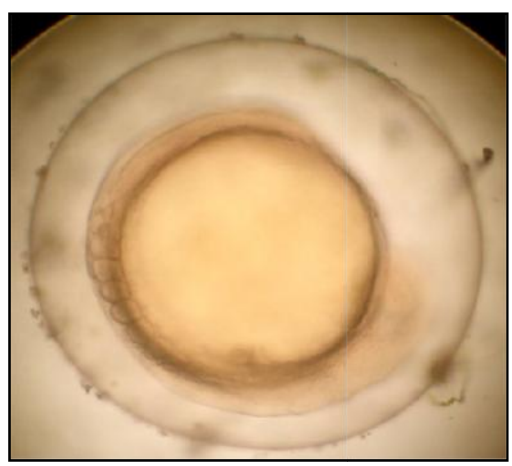

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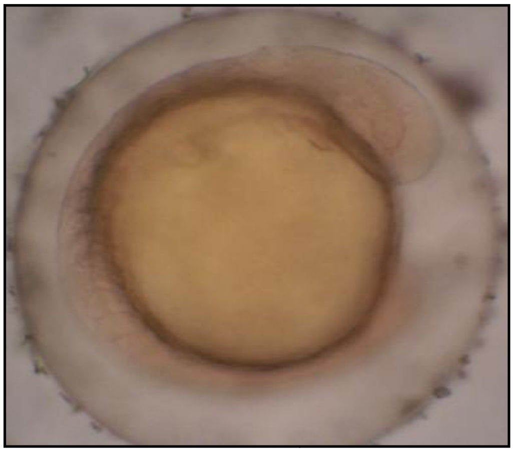

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Plate- 9: Micrographs showing embryonic developmental stages in *Danio dangila*

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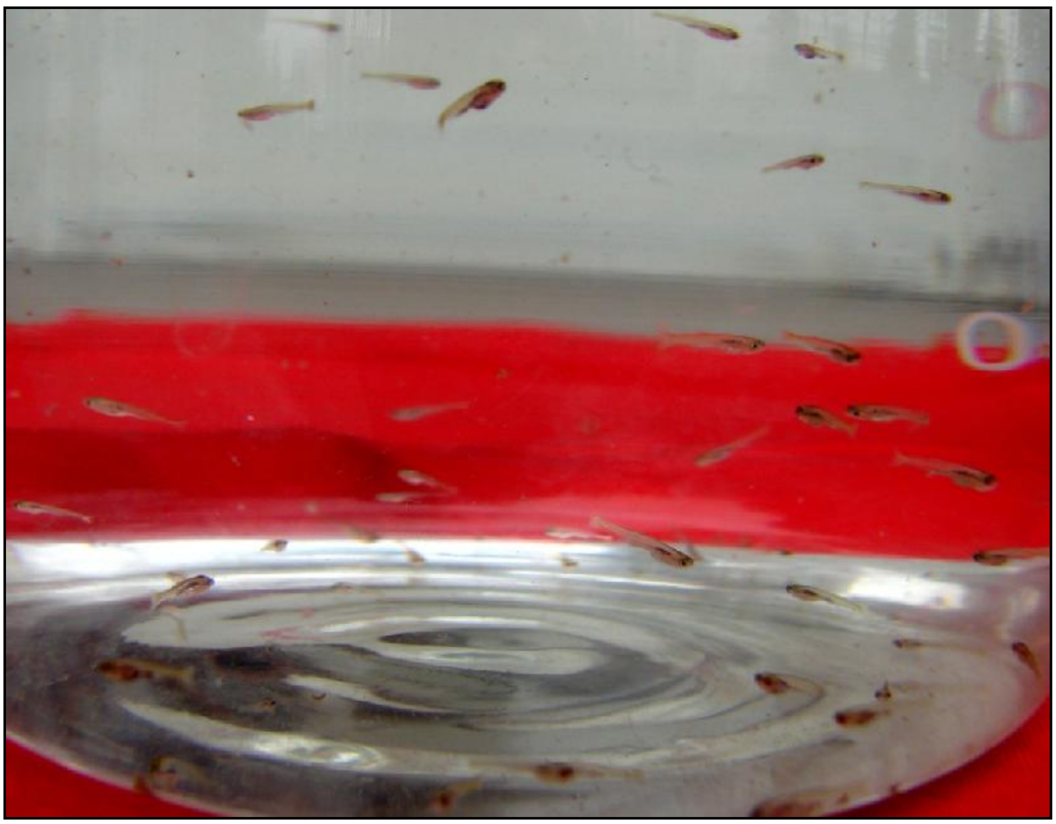

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Plate- 10: Micrographs showing larval developmental stages in *Danio dangila*

 

River Tsurang River Doyang

 

River Intangki River Milak

 

Rice fields and lentic water bodies of Changki Valley (Mokokchung)

Plate- 4: Habitat of *Puntius chola*

Males of *Puntius chola*

Reddish colour stripe and tinged orange fins in male

Females of *Puntius chola*

Pale Yellowish fins in female

Plate- 7: Photograph showing sexual dimorphism in *Puntius chola*

Hormone administration in *Danio dangila*

Breeding set up for *Danio dangila*

Hormone administration in *Puntius chola*

Breeding set up for *Puntius chola*

Plate- 8: Adminstration of synthetic hormone and breeding set up of *Danio dangila* and *Puntius chola*.

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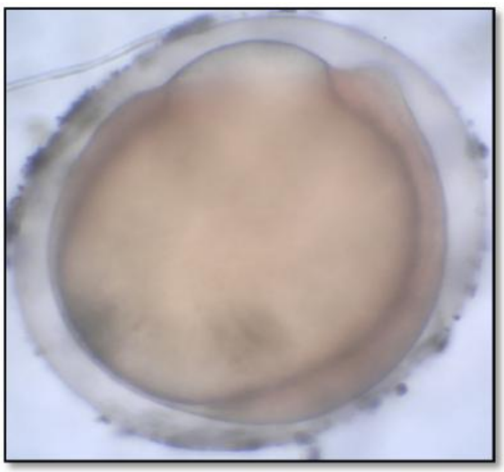

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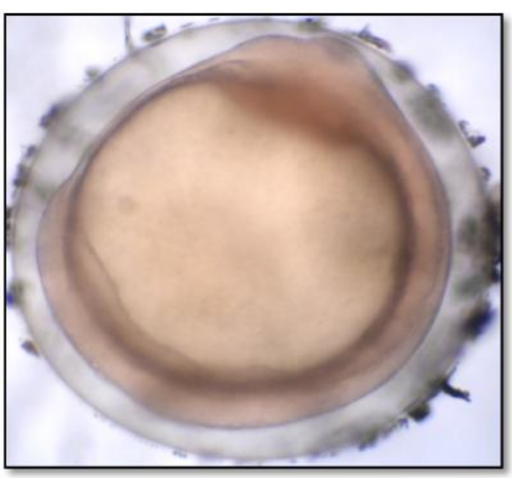

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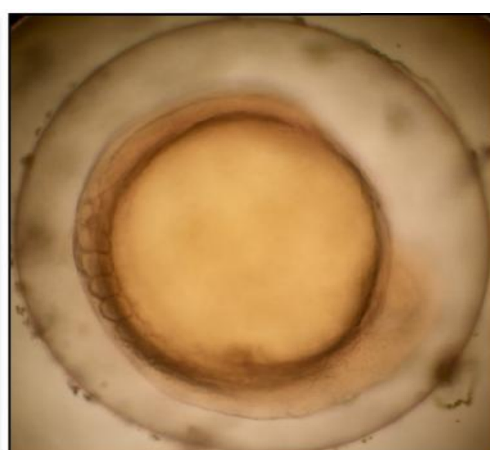

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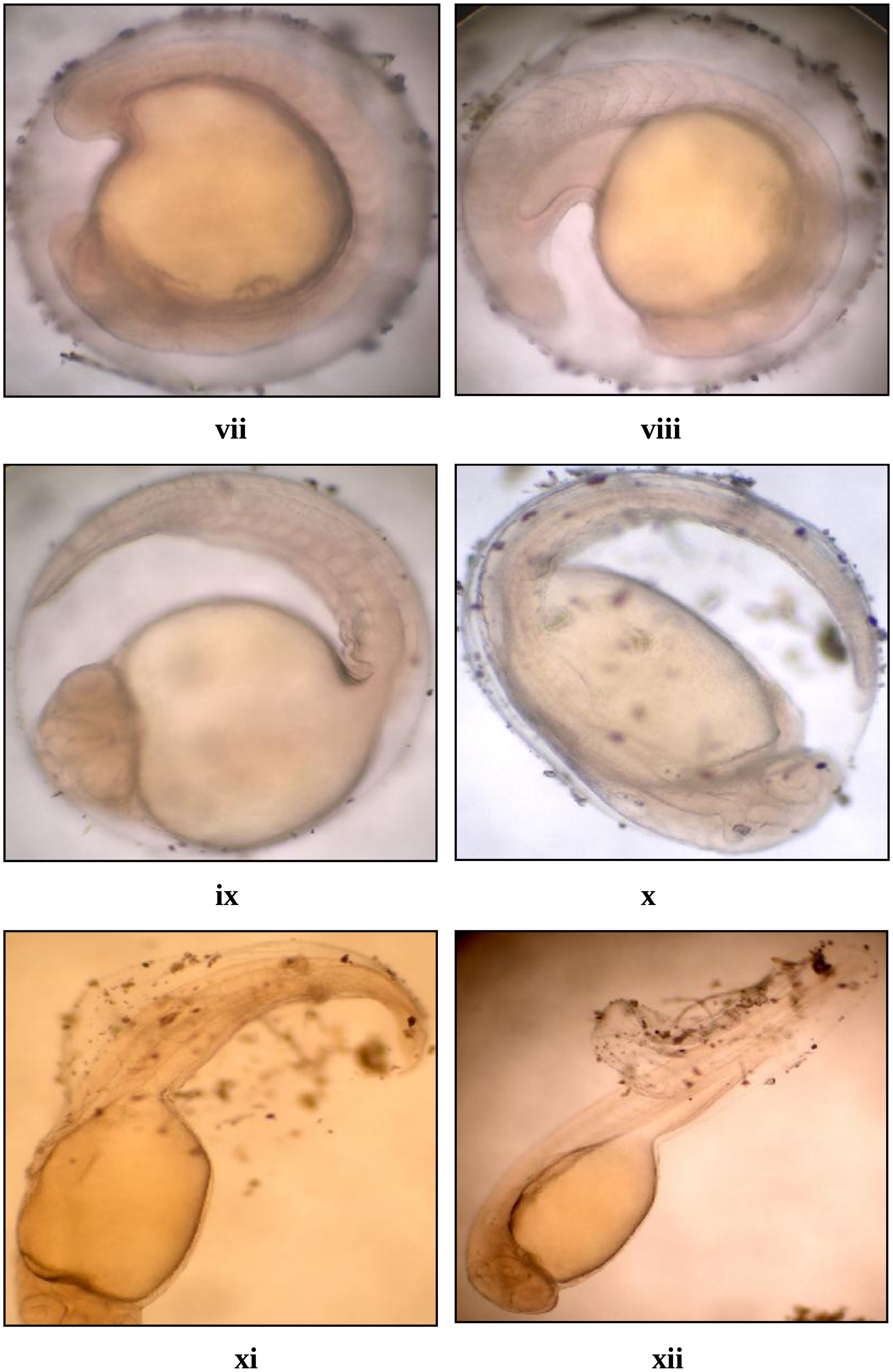

Plate- 11: Micrographs showing embryonic developmental stages in *Puntius chola*

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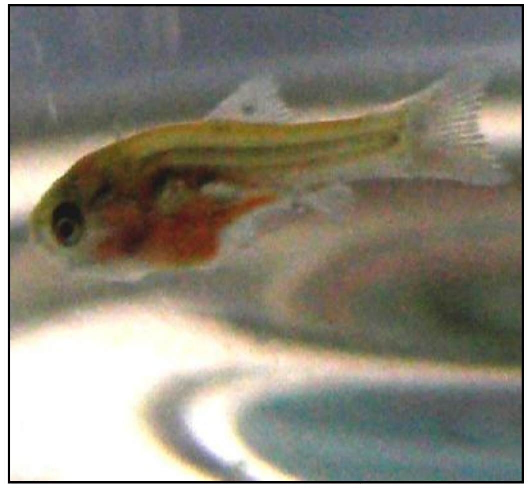

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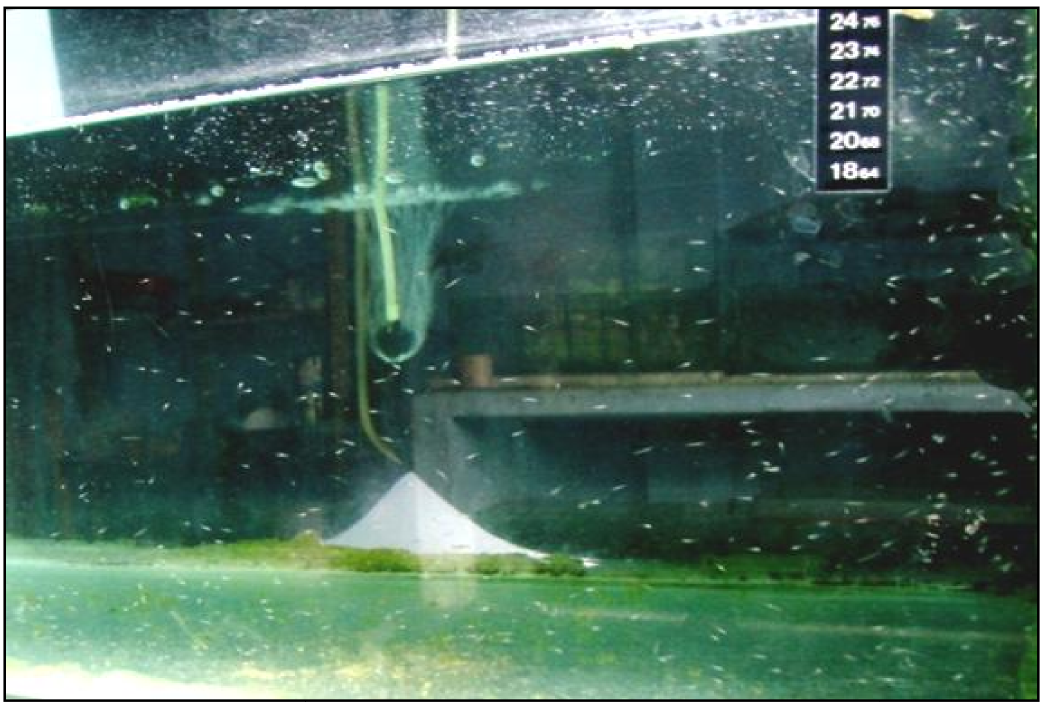

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Plate- 12: Micrographs showing larval developmental stages in *Puntius chola*

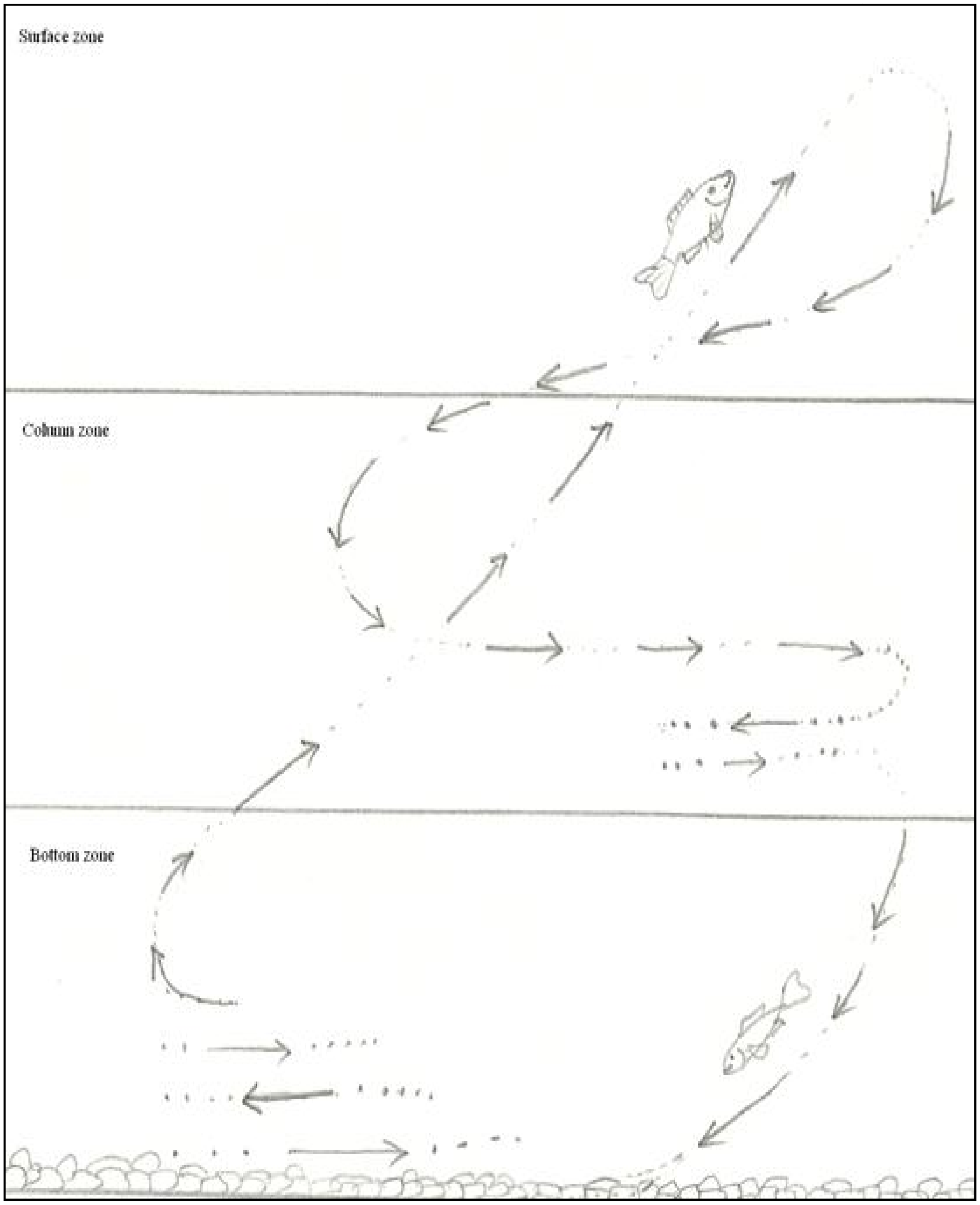

Figure-4: Schematic diagram showing lineament of feeding in *Danio dangila*.

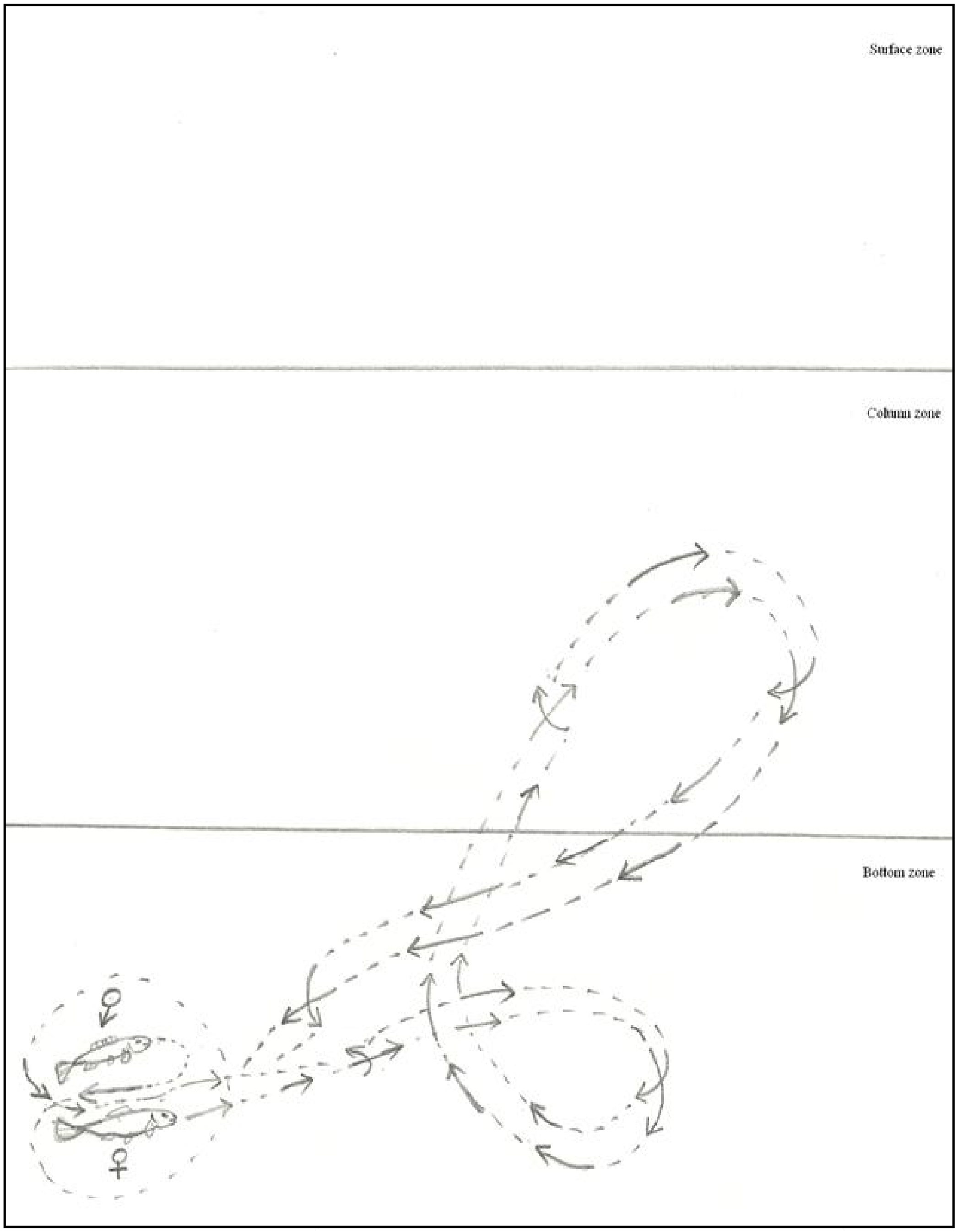

Figure- 5: Schematic diagram showing lineament of procreatic demeanor in *Danio dangila*

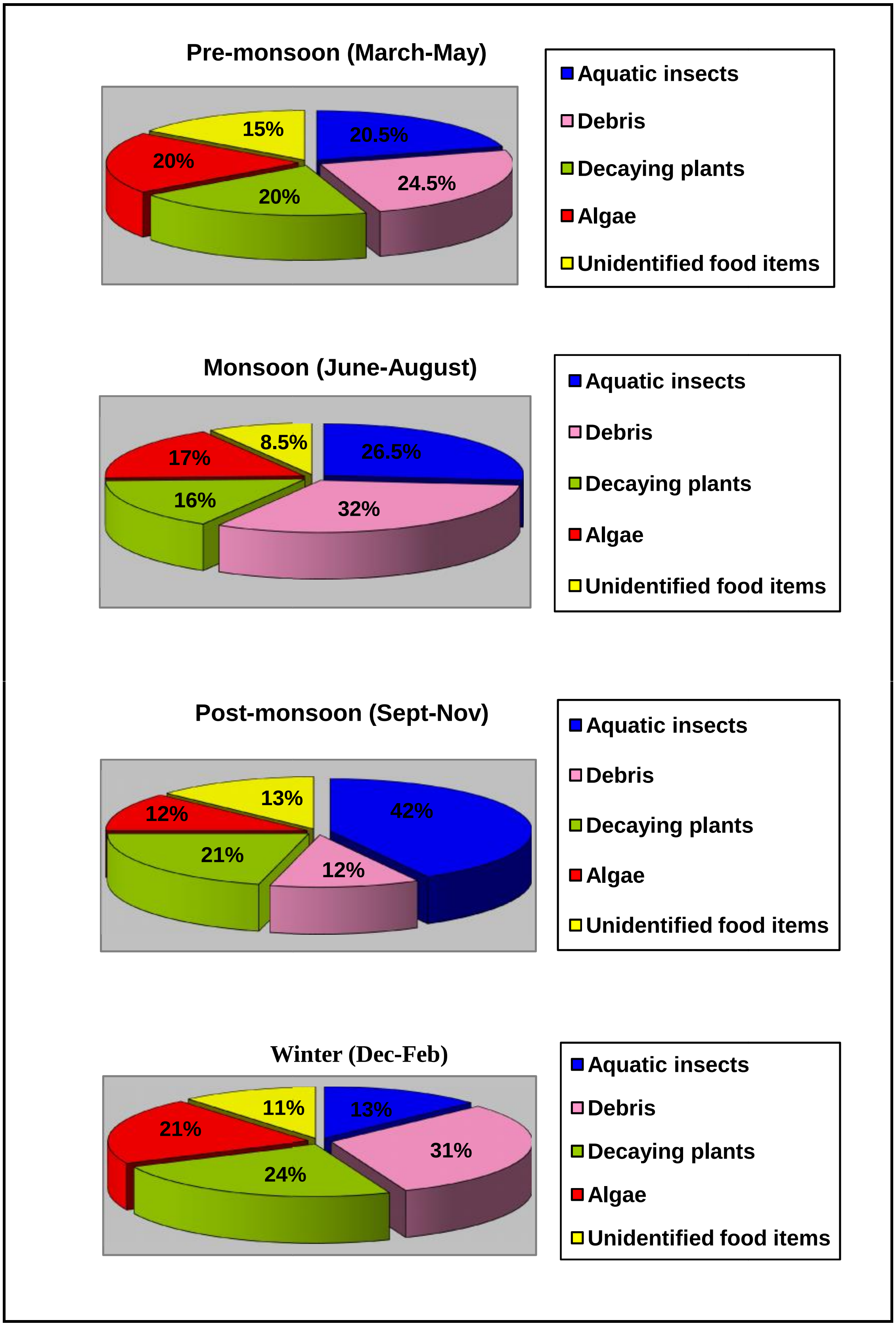

Figure- 8: Victual spectra of *Danio dangila*

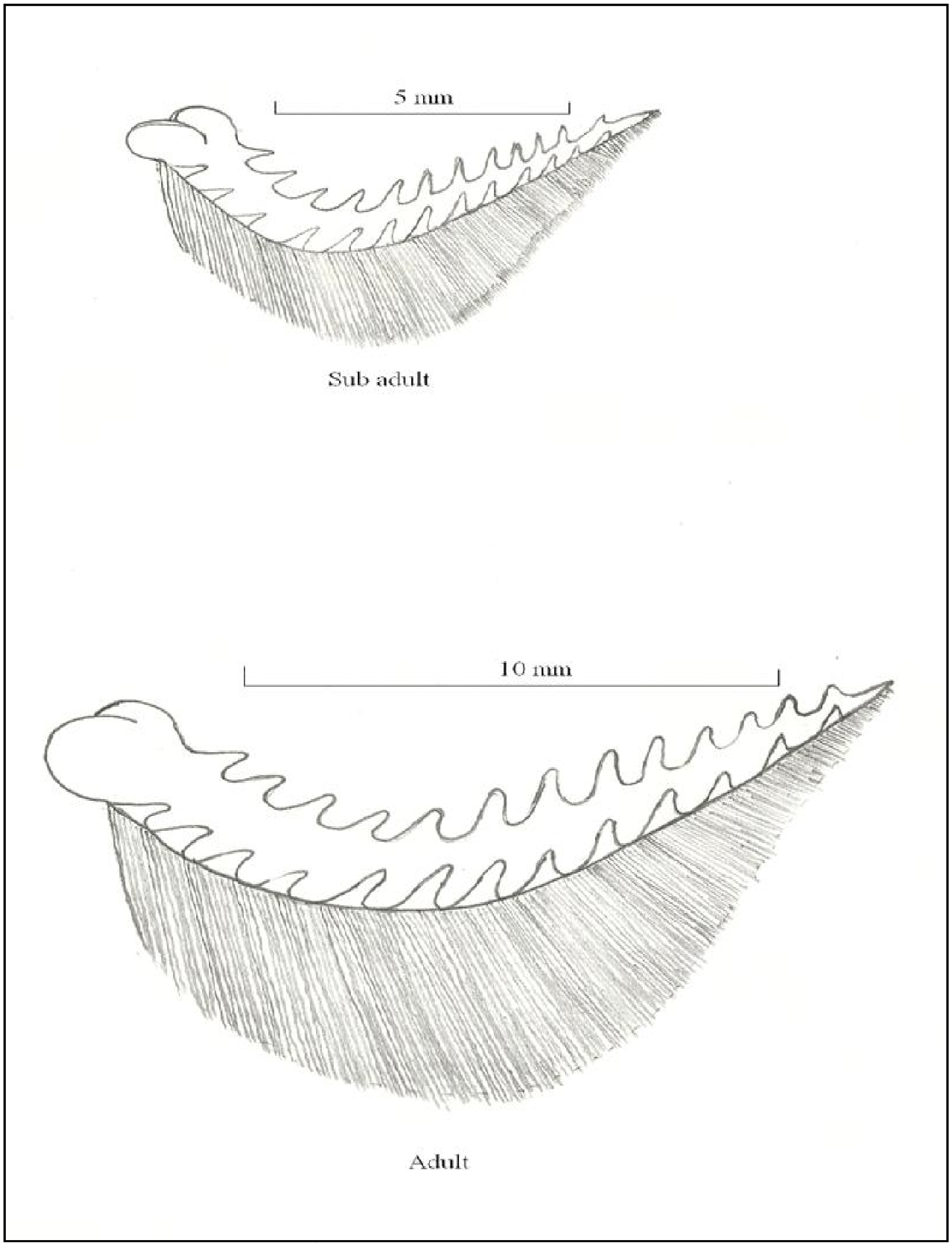

Figure- 9: Schematic diagram showing gill reker traits in *Danio dangila*

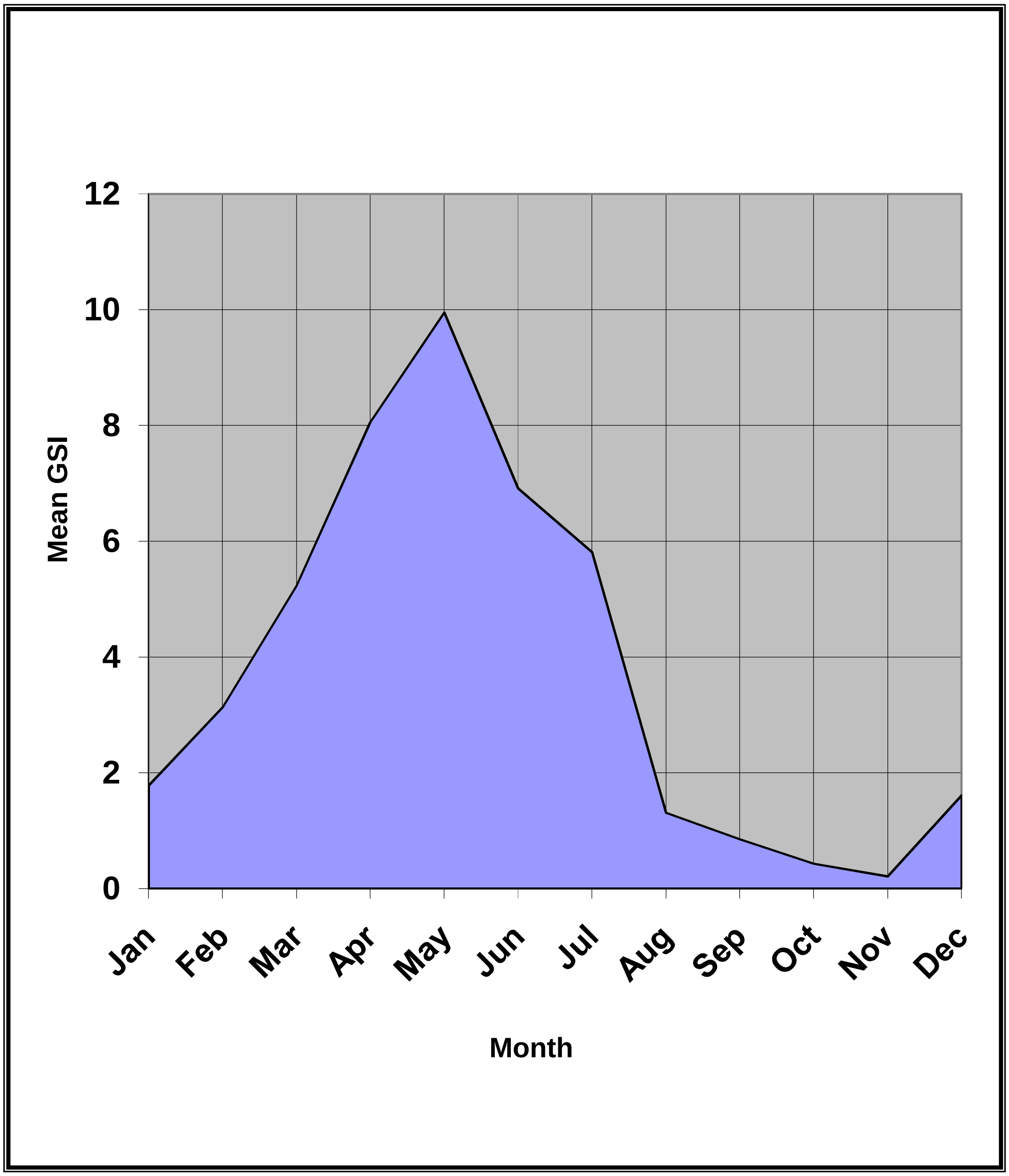

Figure- 12: Gonado-somatic index of *Danio dangila*

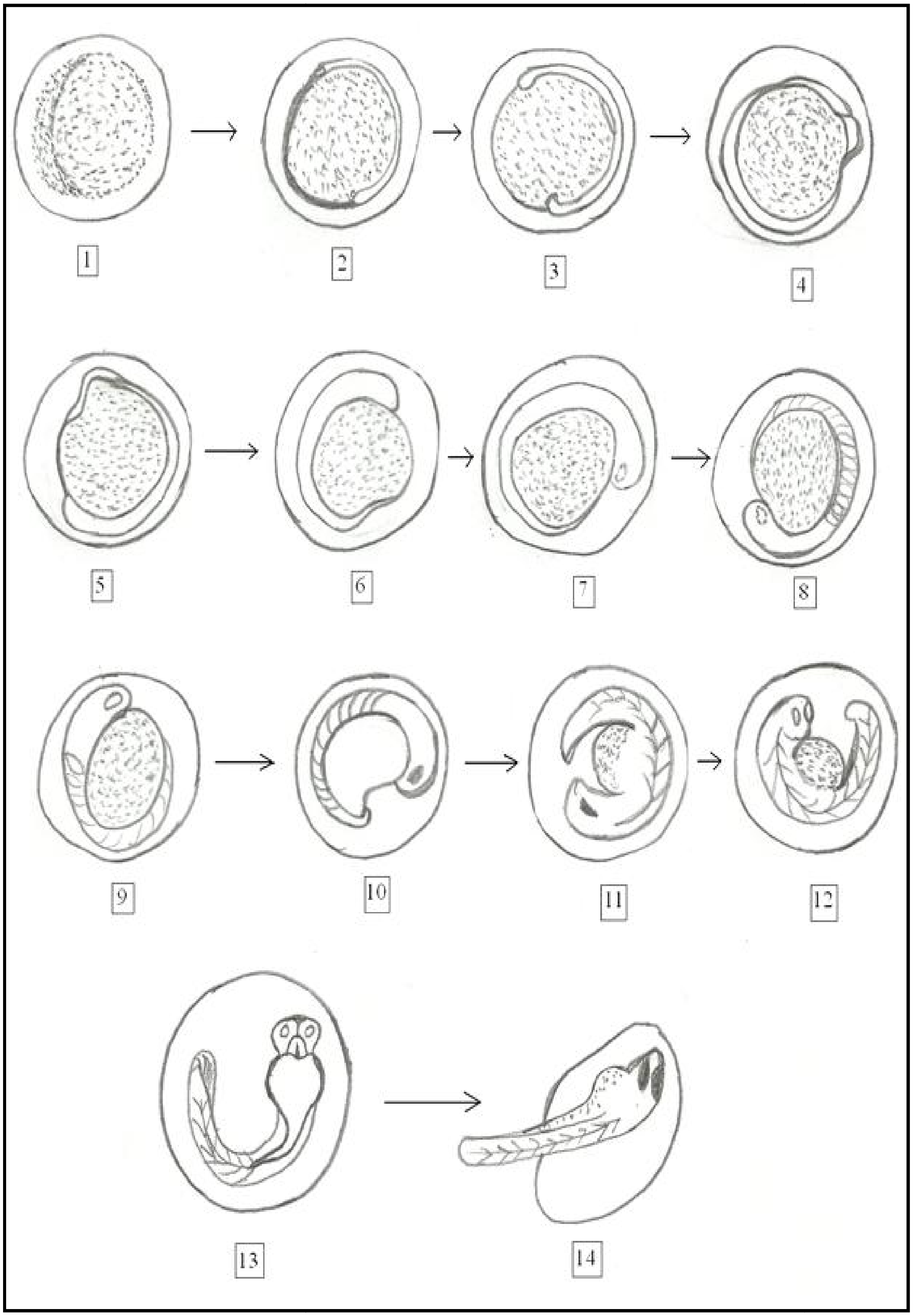

Figure- 14: Schematic diagram showing embryonic developmental stages in *Danio dangila*

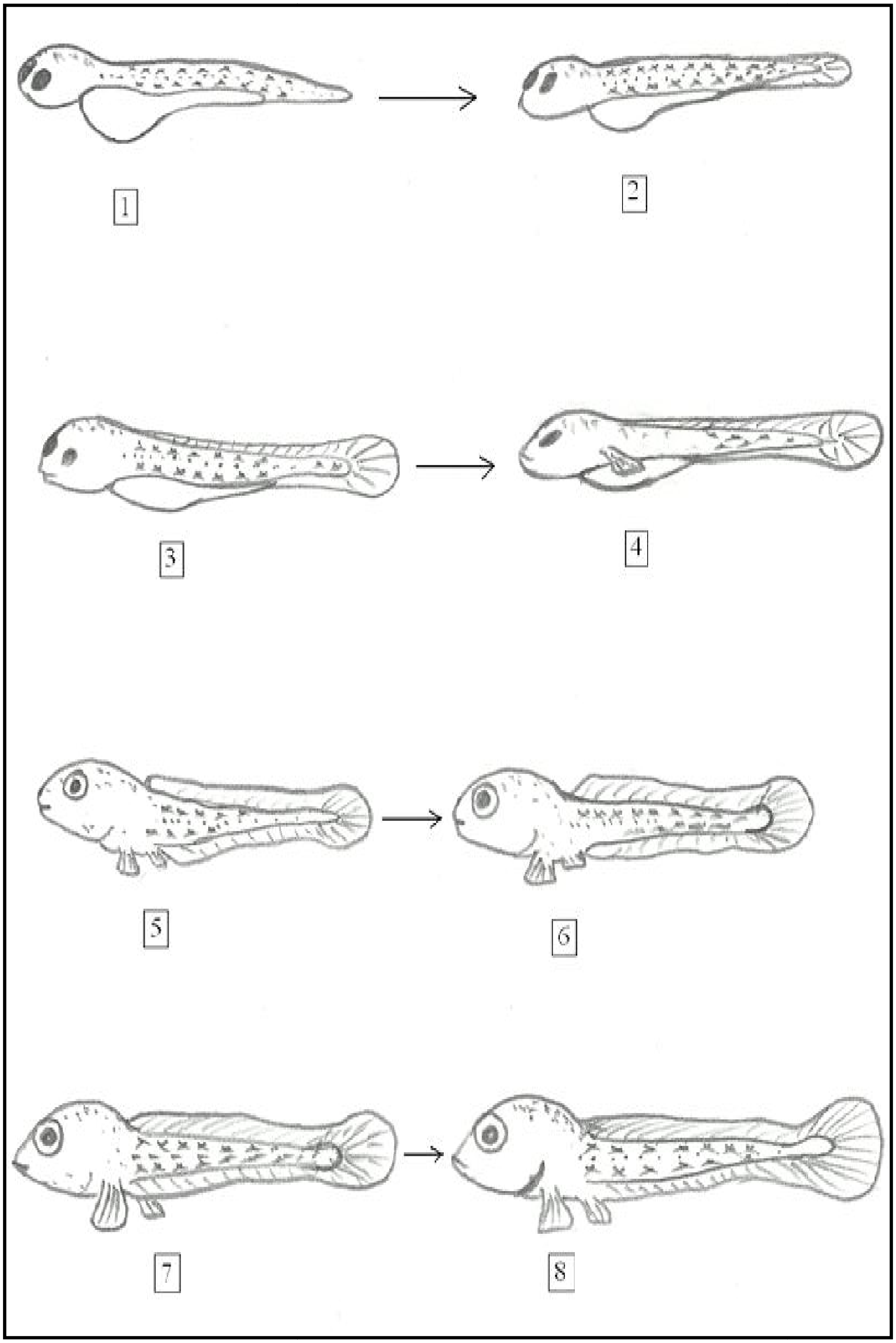

Figure- 15: Schematic diagram showing larval developmental stages in *Danio dangila*

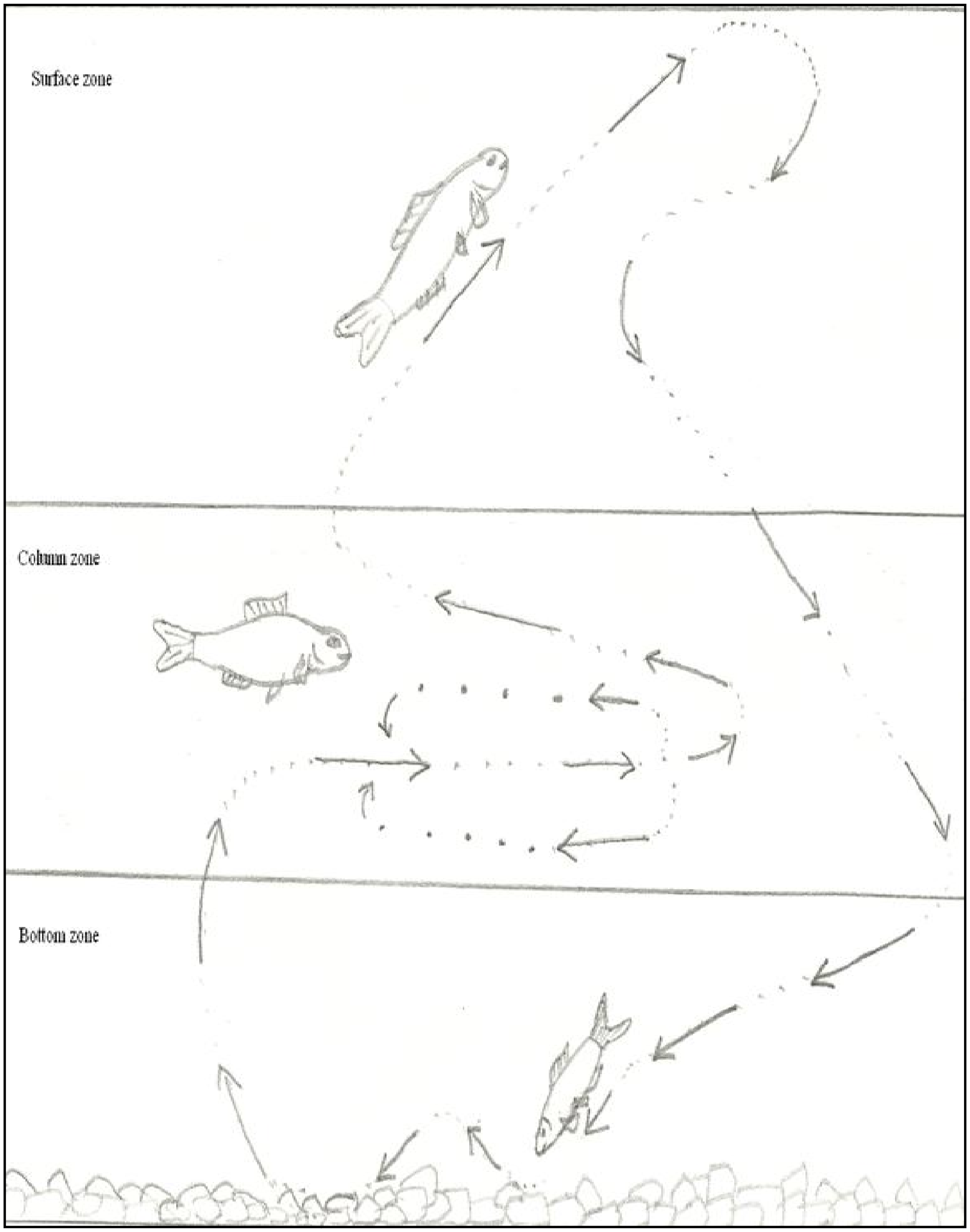

Figure- 6: Schematic diagram showing lineament of feeding in *Puntius chola*.

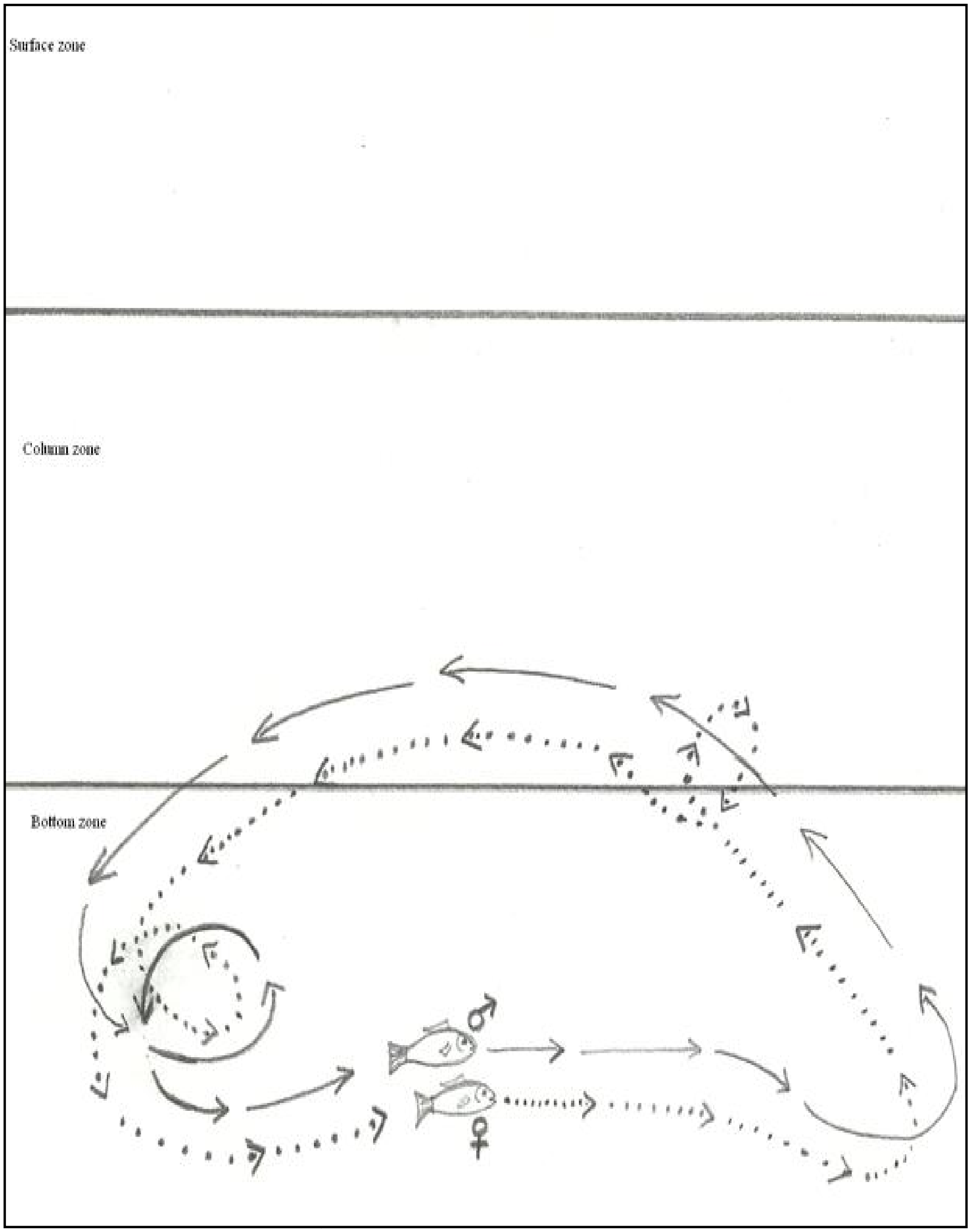

Figure- 7: Schematic diagram showing lineament of procreatic demeanor in *Puntius chola.*

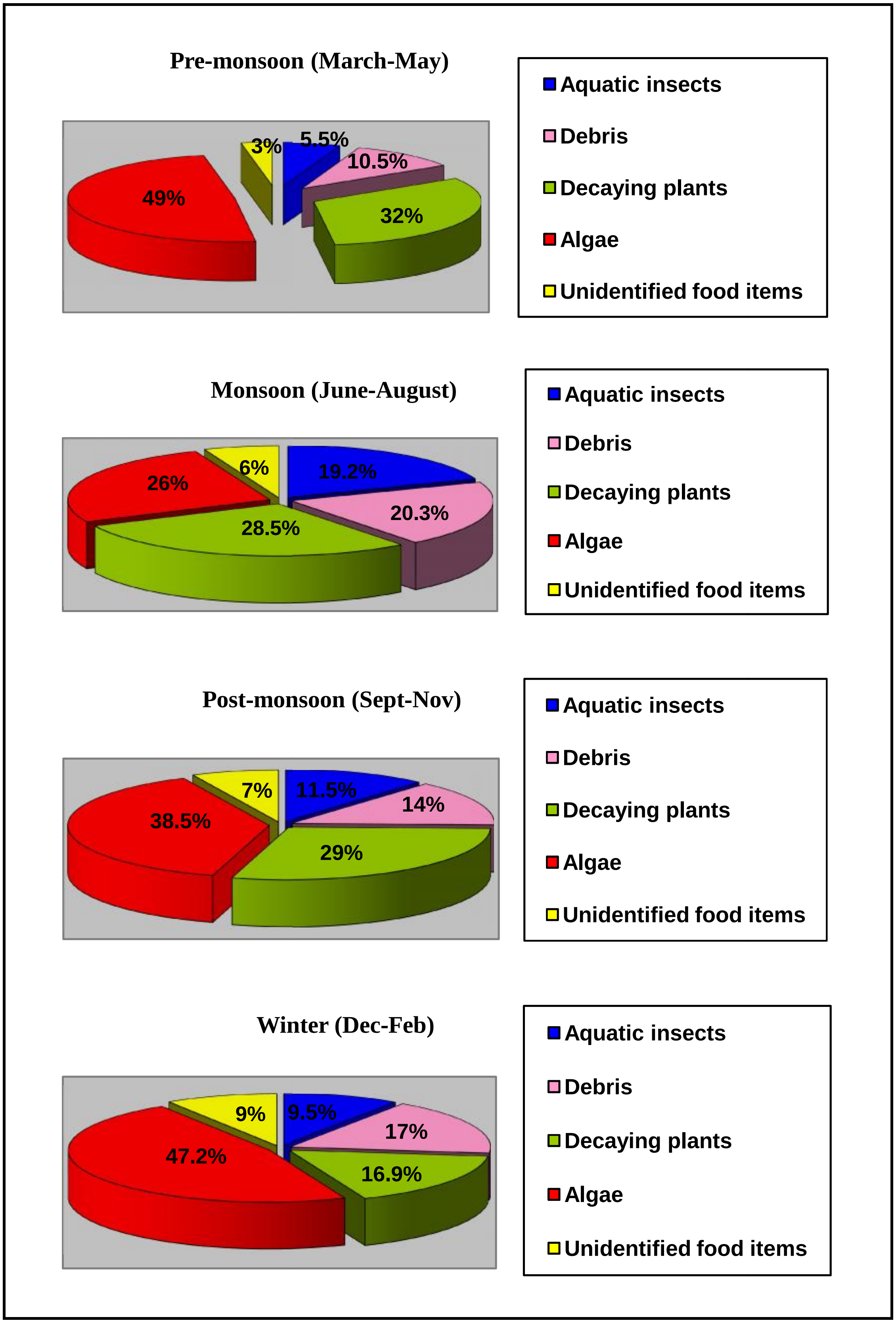

Figure- 10: Victual spectra of *Puntius chola*

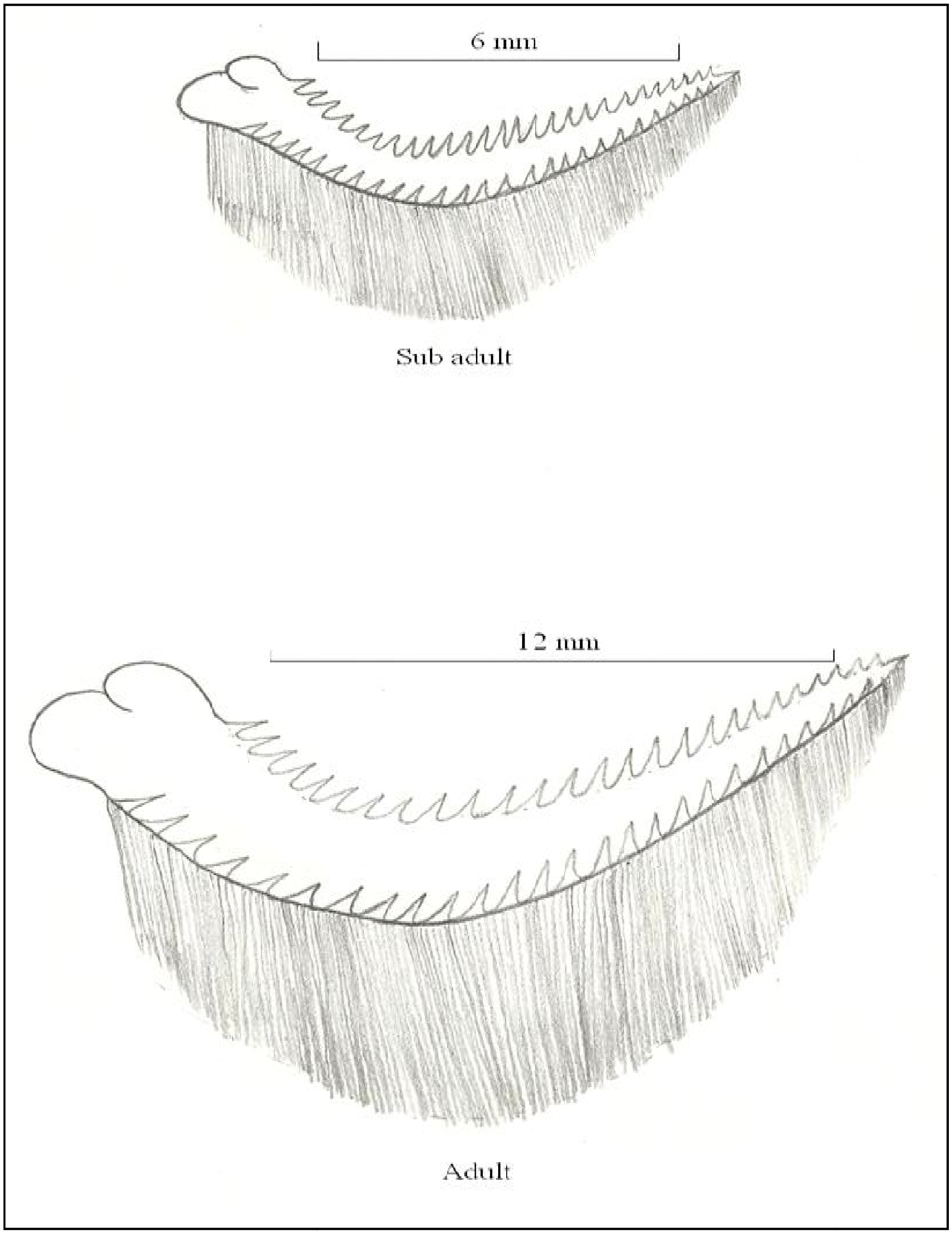

Figure- 11: Schematic diagram showing gill raker traits in *Puntius chola*

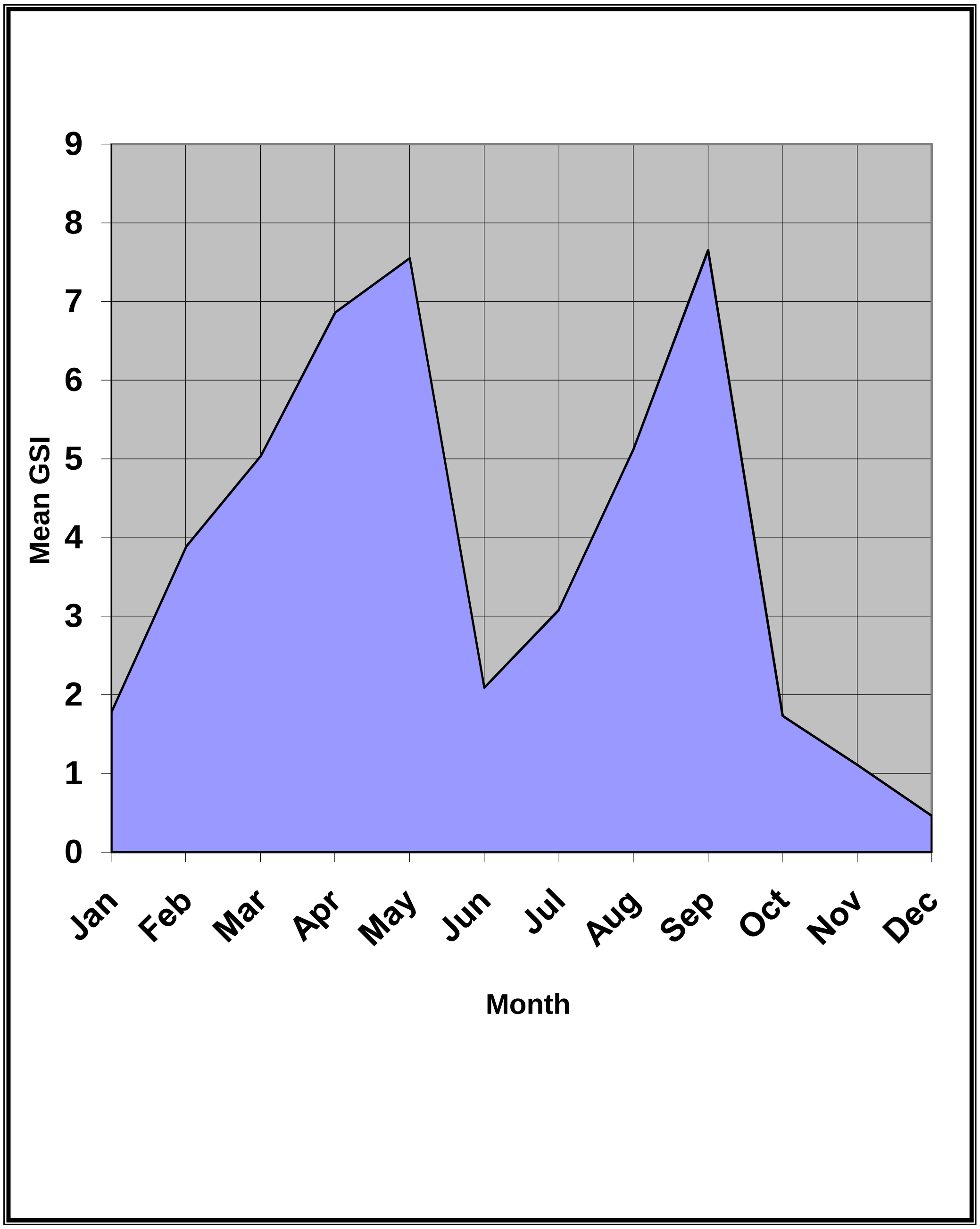

Figure- 13: Gonado-somatic index of *Puntius chola*

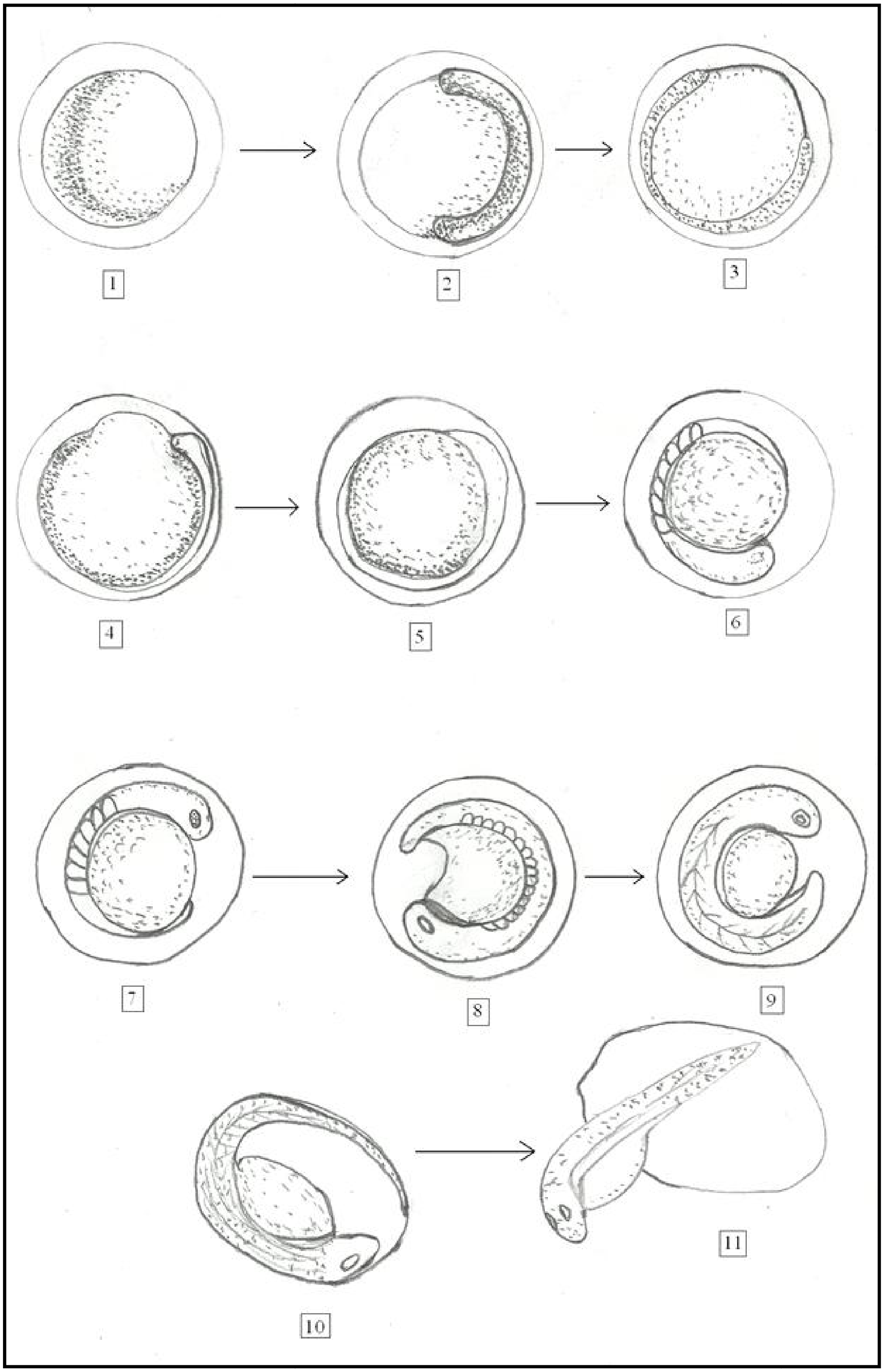

Figure- 16: Schematic diagram showing embryonic developmental stages in *Puntius chola*

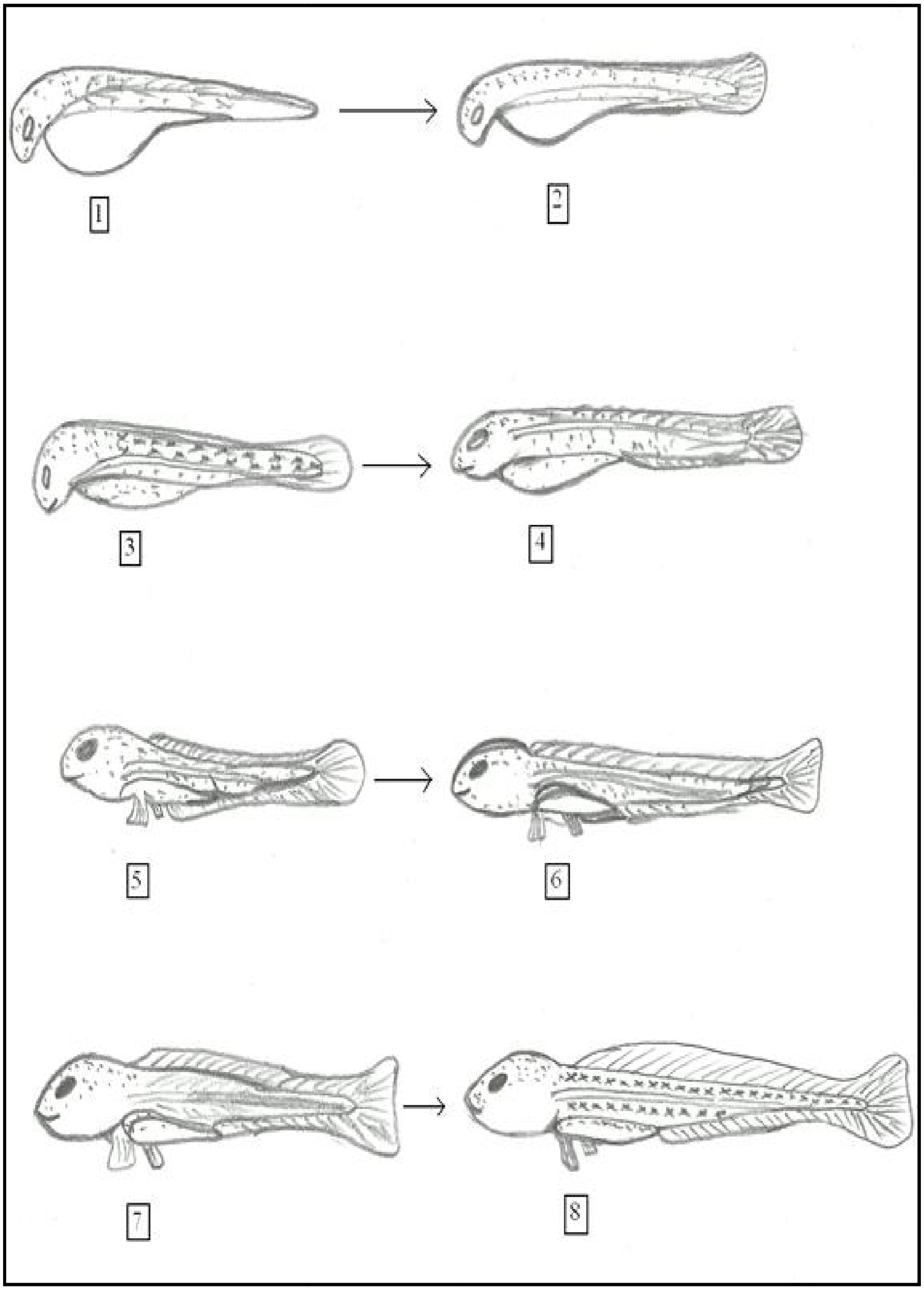

Figure- 17: Schematic diagram showing larval developmental stages in *Puntius chola*