RESULTS

4. RESULTS

4.1. WATER QUALITY

Water is essential for all living organisms like human beings, animals and plants. It is one of the most valuable natural resource. However nowadays, due to human activity it is being polluted. The water quality parameters are very important for the rearing and breeding of both the ornamental and edible fish species. For the present study, it is therefore essential an eco-friendly environment is maintained so as to generate a baseline data of water quality change over a period of time.

4.1.1. Temperature

During the study period, coving from 2013- 2014, the air temperature over the river Kaljani ranged from 22^0C to 36^0C with mean value 30.5^0C (± 3.5); and in captivity at room temperature 20^0C to 34^0C with mean value 28^0C (± 4.53). Monthly variation of air temperature observed at both the sampling sites had an increasing trend.

The Kaljani river water temperature ranged from 26^0C to 31^0C with a mean value of 28.1^0C (± 2.3). Lowest river water temperature was recorded as 26^0C during March to April 2014 and highest 31^0C during August 2014. During this study period, water temperature in captivity ranged from 27^0C to 32^0C which was maintained with the help a Thermostat.

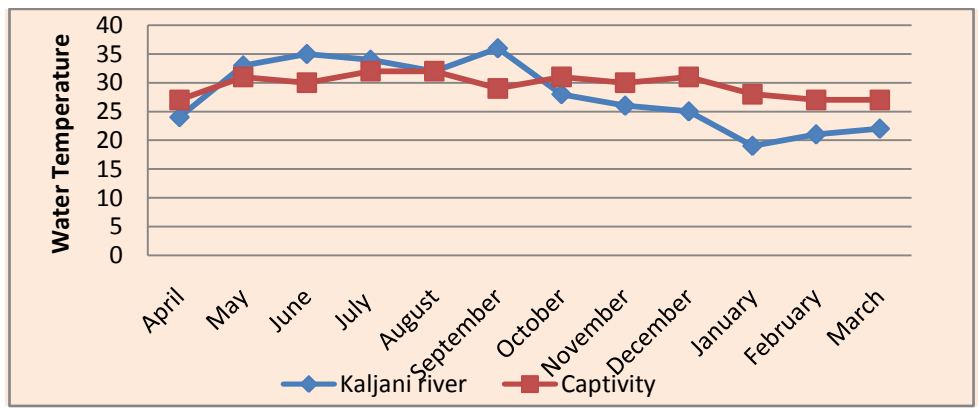


Fig.7a: Monthly variation of water temperature during the study period in Kaljani river and Captivity

4.1.2. Hydrogen ion concentration (pH)

Hydrogen ion concentration (pH) of the Kaljani river water ranged from 7.2 to 8.2 with a mean value of 7.82 (± 0.31). Lowest pH was recorded as 7.2 in April 2014 and highest 8.2 during August 2014. This highest value was found during the rainy season from June to October 2014. The pH value of the captive condition water was however maintained from 7.5 to 8.5 with mean value of 7.88 (± 0.34).

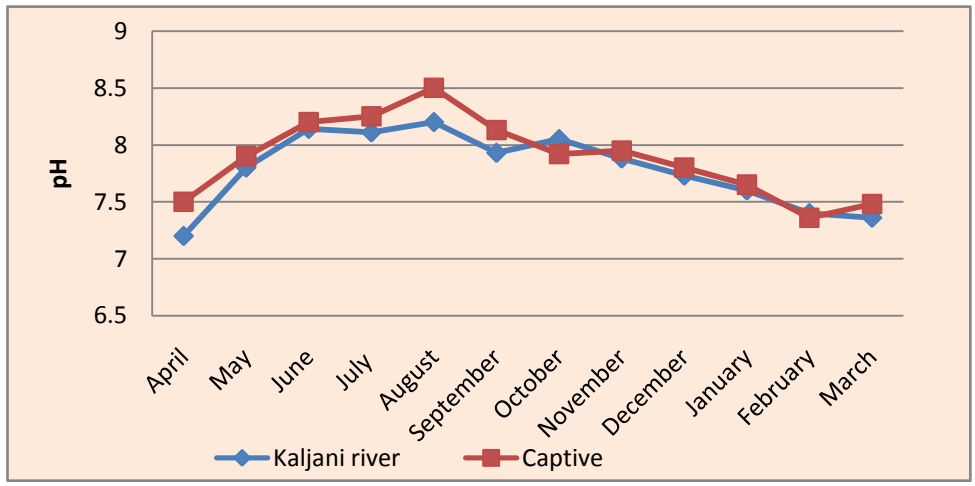


Fig.7b: Monthly variation of pH during the study period in Kaljani river and Captivity

4.1.3. Specific Conductivity

Specific Conductivity of the river water ranged from 110 µS cm^{-1} to 180 µS cm^{-1} with a mean value 151.27 (± 22.30). Lowest value of specific conductivity was recorded as 110 µS cm^{-1} in June 2014 and highest 180 µS cm^{-1} during November 2014. The specific conductivity in the captive condition ranged from 240 µS cm^{-1} to 250 µS cm^{-1} with mean value 246.14 µS cm^{-1} (± 3.18). Lowest value of specific conductivity of breeding tank was recorded as 240 µS cm^{-1} in July 2014 and highest value 250 µS cm^{-1} during April and August 2014.

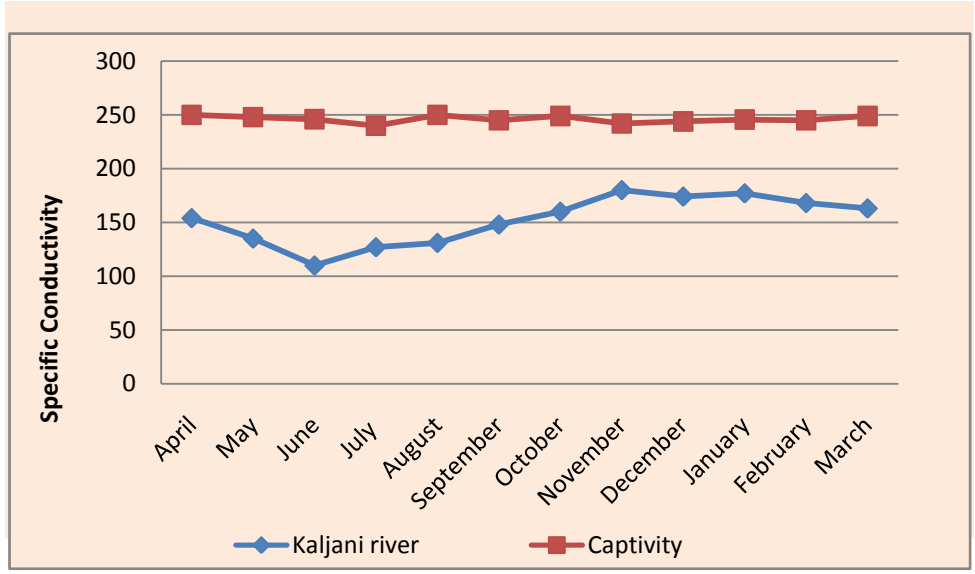


Fig.7c: Monthly variation of Specific Conductivity during the study period in Kaljani river and Captivity

4.1.4. Total Dissolved Solids (TDS)

Total dissolved solids are a measure of dissolved matter (salts, organic matter, minerals and so on) in water. Inorganic constituents comprise most of the total concentration of total dissolved solids. Total dissolved solids are naturally present in water or is the result of mining, oil and gas drilling or some industrial or municipal total dissolved solids. It can be toxic to aquatic life through increase in salinity or changes in the composition of the water, or it may include substances that are toxic to people or aquatic life. Most aquatic ecosystems involving mixed fish fauna can tolerate total dissolved solids levels of 1000 mg L^{-1}. (**Boyd, 1999**). The present study showed that the average value of total dissolved solids of river Kaljani varied from 80 to 120 mg L^{-1} with mean value of 135 mgl^{-1} (± 31.96 mgl^{-1}). Lowest value of total dissolved solids was recorded as 80 mg L^{-1} in September 2014 and highest value of 165 mg L^{-1} during May 2014. Total dissolved solids of breeding tank water were 250 to 260 mg L^{-1} with mean value 255 mg L^{-1} (± 3.97 mgl^{-1}).

4.1.5. Dissolved Oxygen

The amount of dissolved oxygen of the Kaljani river water which ranged from 9.46 mg L^{-1} to 12.4 mg L^{-1} with mean value 10.98 (± 0.914) was quite adequate and characteristic of hill stream. Lowest value of dissolved oxygen was recorded as 9.46 in September 2014 and highest value was recorded as 12.4 during January 2015. During the study period, dissolved oxygen in captivity was maintained at 6.3 mg L^{-1} to 7.6 mg L^{-1} with the help of aeration. This level of dissolved oxygen seemed significant for the survival and activity of *Botia* species.

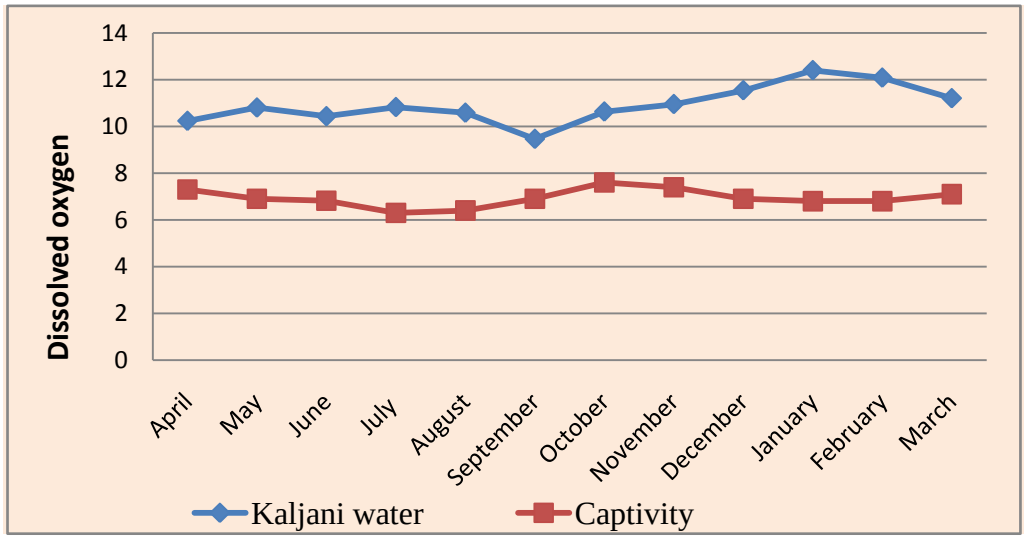


Fig.7d: Monthly variation of Dissolved Oxygen during the study period in Kaljani river and Captivity

4.1.6. Free Carbon Dioxide

The presence of carbonic acid in water may be good or bad depending on the water pH and alkalinity. Carbon dioxide in a water body may be derived from the atmosphere, biotic respiration, inflowing ground water which seep into the pond, decomposition of organic matter due to bacteria and may also be from within the water body itself in combination with other substances namely calcium, magnesium and others (**Abir, 2014**). In the present study, the free carbon dioxide concentration of river water

varied from 2.3 to 4.6 mg L^{-1} with a mean value of 3.76 mg L^{-1} (± 0.801). Free carbon dioxide of breeding tank water varied from 6.0 to 8.0 mg L^{-1} with mean value 6.67 mg L^{-1}(± 0.519 mg L^{-1}).

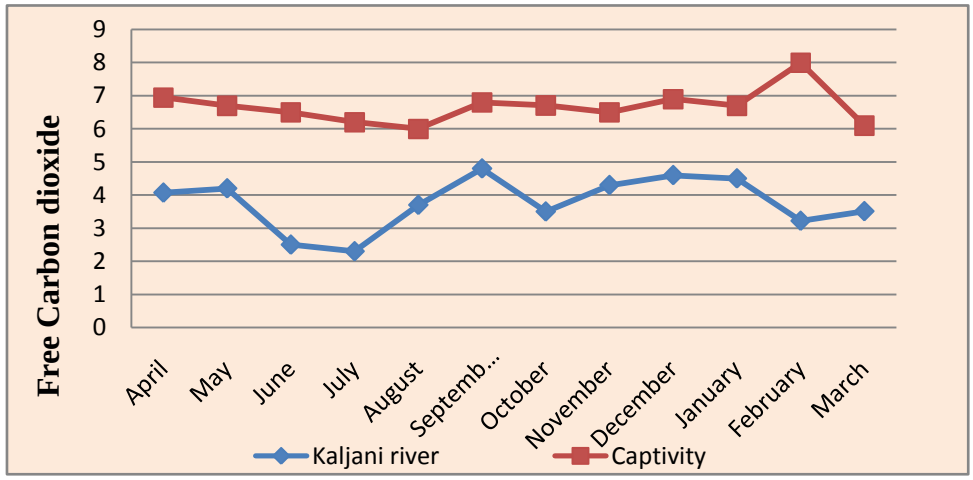


Fig.7e: Monthly variation of Free CO_2 during the study period in Kaljani river and Captivity

4.1.7. Total Alkalinity (TA)

Total alkalinity of the river water ranged from 48.0 mg L^{-1} to 86.2 mg L^{-1} with mean value 69.78 mg L^{-1} (± 12.28). Lowest total alkalinity was recorded as 48.0 mg L^{-1} in March 2015 and highest as 86.2 mg L^{-1} during October 2014. The total alkalinity of the captive condition water ranged from 36.0 mg L^{-1} to 72.0 mg L^{-1} with mean value 54.68 mg L^{-1} (± 14.40). Lowest total alkalinity of breeding tank was recorded as 36.0 mg L^{-1} in January 2015 and highest as 72.0 mg L^{-1} during July 2014.

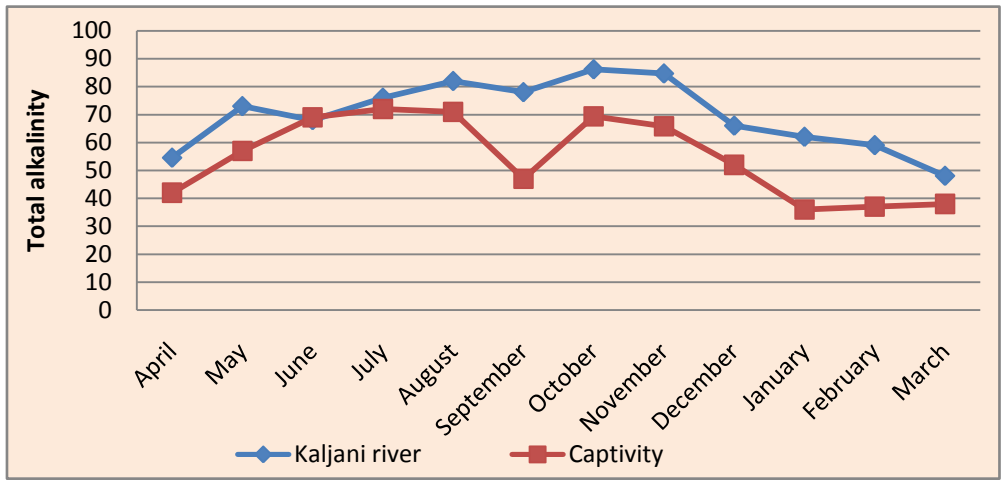


Fig.7f: Monthly variation of Total alkalinity during the study period in Kaljani river and Captivity

4.1.8. Total Hardness

Total Hardness in water is caused by the presence of cations like Ca^{+2} and Mg^{+2}. This property of water helps to precipitate soap by forming a complex with calcium and magnesium present in water. Total hardness of the river water ranged from 18.0 mg L^{-1} to 30.0 mg L^{-1} with mean value 24.0 mg L^{-1}(± 4.03). Lowest total hardness was recorded as 18.0 mg L^{-1} in July 2014 and highest value recorded as 30.0 mg L^{-1} during September 2014. The total hardness of the captive condition of water ranged from 26 mg L^{-1} to 30 mg L^{-1} with mean value 27.29 mg L^{-1}(± 1.29).

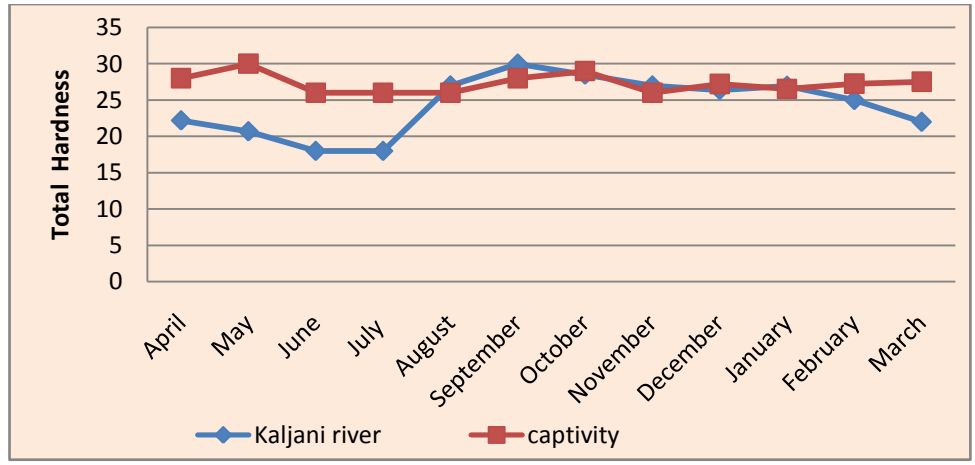


Fig.7g: Monthly variation of Total Hardness during the study period in Kaljani river and Captivity

4.1.9. Ammonium-nitrogen (NH_4-N)

Ammonium-nitrogen concentration of the river water ranged from 0.001 mg L^{-1} in April 2014 to 0.035 mg L^{-1} ; the highest being in June 2014. The average value was 0.0179 mg L^{-1} (± 0.0084). The ammonium-nitrogen concentration in the captive condition ranged from 0.00 mg L^{-1} to 0.007 mg L^{-1} with mean value 0.002 mg L^{-1} (± 0.002). Lowest ammonium-nitrogen of Kaljani river was recorded as 0.001 mg L^{-1} in April 2014, highest of 0.035 mg L^{-1} during June 2014.The low concentrations of ammonium-nitrogen in both the systems revealed no toxic effects.

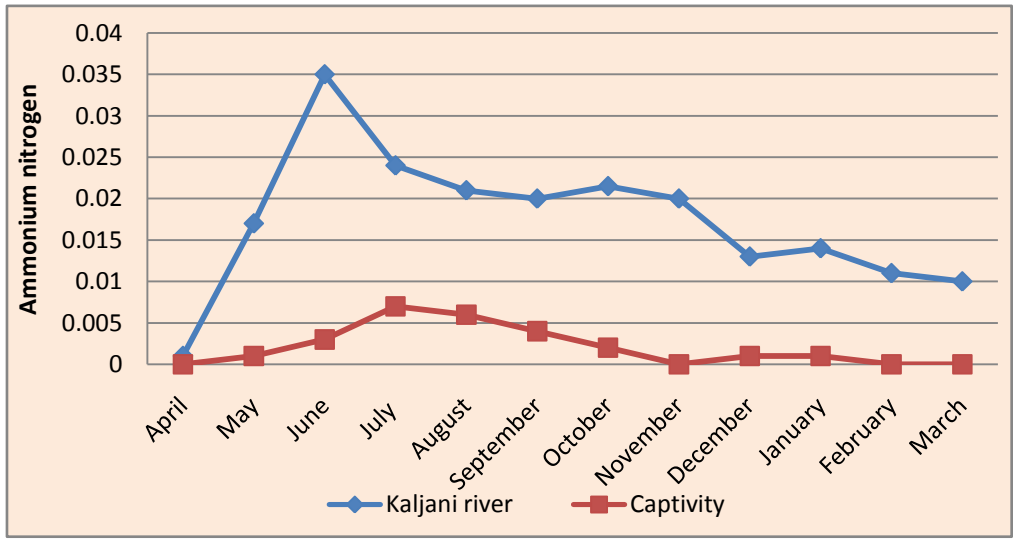


Fig.7h: Monthly variation of Ammonium nitrogen during the study period in Kaljani river and Captivity

4.1.10. Nitrite- nitrogen (NO_2-N)

Nitrite-nitrogen concentration of the river water ranged from 0.001 mg L^{-1} to 0.027 mg L^{-1} with mean value 0.009 mg L^{-1} (± 0.110). Lowest nitrite-nitrogen was recorded as 0.001 mg L^{-1} in April 2014 and highest as 0.027 mg L^{-1} during June 2014. The nitrite -nitrogen concentration of water in the captive condition also revealed a similar low trend 0.001mg L^{-1} to 0.008 mg L^{-1} with mean value 0.003 mg L^{-1} (± 0.003).

Lowest nitrite-nitrogen of breeding tank recorded was 0.001 mg L^{-1} in May 2014 and highest as 0.008 mg L^{-1} during July 2014. Thus toxicity due to Nitrite-nitrogen was negligible.

4.1.11. Nitrate– nitrogen (NO_3-N)

Similar to the low concentration of Ammonium-nitrogen (NH_4-N) and Nitrite-nitrogen(NO_2-N), Nitrate-nitrogen(NO_3-N) concentration of the river water was also low and ranged from 0.149 mg L^{-1} to 0.686 mg L^{-1} with mean value 0.312 mg L^{-1} (± 0.220). Lowest nitrate -nitrogen was recorded as 0.149 mg L^{-1} in June 2014 and highest 0.686 mg L^{-1} during July 2014. The nitrate -nitrogen concentration in the captive condition ranged from 0.085 mg L^{-1} to 0.316 mg L^{-1} with mean value 0.215 mg L^{-1}(± 0.086). Lowest nitrate-nitrogen of breeding tank was recorded as 0.085 mg L^{-1} in September 2014 and highest as 0.316 mg L^{-1} during June 2014.

4.1.12. Phosphate-phosphorous (PO_4-P)

Phosphate-phosphorous (PO_4-P) concentration of the river water ranged from 0.012 mg L^{-1} to 0.197 mg L^{-1} with mean value 0.101 mg L^{-1}(± 0.060). Lowest PO_4-P was recorded as 0.012 mg L^{-1} in August 2014 and highest 0.197 mg L^{-1} during June 2014. The Phosphate-P concentration in the captive condition ranged from 0.110 mg L^{-1} to0.318 mg L^{-1} with mean value 0.172 mg L^{-1} (± 0.078). Lowest Phosphate-P of breeding tank was recorded 0.110 mg L^{-1} in June 2014 and highest 0.318 mg L^{-1} during May 2014.

Tab.5: Summary of water quality parameters in River Kaljani (Natural environment) and Aquaria (Captivity)

Water quality parameters	Kaljani river			Aquaria		
	Min	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**
Air Temperature (^{0}C)	23.0	38.0	30.5 ± 5.79	20.0	34.0	28 ± 4.53
Water Temperature (^{0}C)	19.0	36.0	28.45± 5.8	27.0	32.0	29.54±2.42
pH	7.2	8.2	7.82± 0.31	7.36	8.5	7.88± 0.34
Specific Conductivity (µS cm^{-1})	110.0	180.0	151.27± 22.30	240.0	250.0	246.14± 3.18
Dissolved Oxygen (mg L^{-1})	9.46	12.4	10.98±0.914	6.3	7.6	6.93 ± 0.37
Total Dissolved Solids (mg L^{-1})	80.0	165.0	135± 31.96	250.0	260.0	255.5± 3.97
Free Carbon dioxide (mg L^{-1})	2.3	4.6	3.76± 0.80	6.0	8.0	6.67± 0.51
Total Alkalinity (mg L^{-1})	48.0	86.0	69.78± 12.28	36.0	72.0	54.68±14.40
Total Hardness (mg L^{-1})	18 .0	30.0	24 ± 4.03	26	30.0	27.29 ± 1.29
Ammonium-N (mg L^{-1})	0.001	0.035	0.017±0.008	0.00	0.007	0.002±0.002
Nitrite-N (mg L^{-1})	0.001	0.004	0.009±0.110	0	0.008	0.003±0.003
Nitrate–N (mg L^{-1})	0.149	0.686	0.312±0.22	0.085	0.316	0.215±0.086
Phosphate-P (mg L^{-1})	0.012	0.197	0.101±0.06	0.110	0.318	0.172±0.078

4.2. PROTOCOLS OF FISH BIOLOGY OF *BOTIA* SPECIES

To study the fish biology of *Botia* species in captivity, enough broodstock was procured. Broodstock was maintained in aquarium to promote gonad development. Morphological indicator of matured females was the development of enlarged belly which was lacking in males. During maturation, it was observed that males grew smaller in size than females but matured earlier than females. A common secondary sexual character was the brighter body colour of the male than that of the female fish. The dark bands on the skin of male were deep black during the breeding season, while such colour was absent in female.

4. 2.1. FISH GROWTH PARAMETERS

4.2.1.1. Gonado-Somatic Index (GSI)

Gonado-somatic index (GSI) is the ratio of fish gonad weight to body weight , it is particularly helpful in identifying days or seasons of spawning as the ovaries of gravid females rapidly increase in size just prior to spawning. At maturity stage fish had maximum Gonado-somatic Index value and after spawning the value declined. During the breeding season *Botia* showed maximum Gonado-somatic Index value and after spawning it got reduced. Gonado-somatic Index increased from April to August and declined from September to February. The Index was higher in female than male. In *Botia almorhae* for female the index was 21.36 and for male it was 2.62. The average Gonado-somatic Index of *Botia almorhae* was 11.96±10.29. Gonado-somatic Index of *Botia dario* for female was 13.21 and for male it was 3.4. The average Gonado-somatic Index of *Botia dario* was 8.34±5.4. The Gonado-somatic Index of *Botia lohachata* for female was 24.46 and for male 3.2. The average Gonado-somatic Index of *Botia lohachata* was 13.86±11.50.In *Botia rostrata* Gonado-somatic Index for female was 18.75 and for male 1.82.The average Gonado-somatic Index of *Botia rostrata* worked out

to be 10.29±9.01. Among the *Botia* species, *Botia lohachata* had the highest GSI than other species. Details of Gonado-somatic Index (GSI) of *Botia* species are given below in **Tab. 6**.

Tab.6: Details of Gonado-somatic Index of *Botia* species in Captive condition

Species name	GSI of male	GSI of female	Average of GSI	SD value
B. almorhae	2.62	21.36	11.96	10.29
B. dario	3.4	13.21	8.34	5.4
B. lohachata	3.2	24.46	13.86	11.50
B. rostrata	1.82	18.75	10.29	9.01

4.2.1.2. Condition Factor

Condition factor or Ponderal Index of fish expressed by K Factor, is an index used to monitor feeding intensity and growth rate (**Oni *et al.*, 1983**), and is based on the hypothesis that heavier fish for a given length are in better condition (**Bagenal and Tesch, 1978**). According to **Le Cren (1951),** "K" greater than 1.0 indicates a good general condition of fish. Fish with high values of 'K' are heavy for its length, while with low K are lighter **(Bagenal and Tesch, 1978).** However, in the present investigation it was found that the Relative Condition Factor (K) is interestingly similar in all three fishes studied. Condition Factor were *Botia almorhae* (1.390), *Botia dario* (1.788.), *Botia lohachata* (1.538) and *Botia rostrata* (1.399).

4.2.1.3. Length-Weight Relationship

Length- weight relationship has an important role to play in fish biology, physiology, ecology and fisheries resource management. In biological studies, Length-weight relationship helps in the seasonal variation in fish growth to be followed and the

calculation of Condition Indexes. Length- weight relationship gives us the history and morphological comparisons between different fish species or between different fish by the Least-Square Method from logarithmic data, and the association of degree between Weight-Length variables can be calculated by the determination of Coefficient of Correlation (r).

The Coefficient of Correlation (r) from length-weight relationship of *Botia rostrata* was more significant than other species. The Coefficient of Correlation of *Botia almorhae* was 0.811 (**Fig. 8a**); *Botia dario* 0.802 (**Fig. 8b**); *Botia lohachata* 0.753 (**Fig. 8c**) and *Botia rostrata* 0.936 (**Fig. 8d**). The Coefficient of Correlation (r) showed significance at $p \leq 0.01$. Length –weight relationship of *Botia* species are expressed as follows:

Botia almorhae : LogW =24.49 + 4.027 logL

Botia dario : LogW =23.87 + 4.005 logL

Botia lohachata : LogW =16.28 + 3.006 logL and

Botia rostrata : LogW =17.31 + 3.138 logL

The theoretical value of "b" (regression coefficient) in length-weight relationship is reported to be 3 when the body form of fish remains constant at different length, that is, the growth is isometric (**Allen, 1938**). If this value is less than or more than 3 it indicates that growth is allometric (**Bagenal and Tesch, 1978**).

Tab.7: Details of growth parameters of *Botia* species in Captive condition

Species name	Gonado-somatic Index (GSI)	Coefficient of Correlation (r)	Condi-tion factor (K)	Environ-ment condition for growth	Regression correlation (b)	Growth pattern
B. almorhae	11.96	0.811	1.390	Good	3.006	Allometric (+)
B. dario	8.34	0.802	1.788	Good	4.027	Allometric (+)
B.lohachata	13.86	0.753	1.538	Good	4.005	Allometric (+)
B. rostrata	10.29	0.936	1.399	Good	3.138	Isometric/ allometric (+)

From the length-weight relationship of *Botia* species, it was found that value of b was greater than 3 and therefore could be said to have a positive allometric growth. However, b<3 showed a negative allometric growth, or isometric growth when b=3. Further, when the value of 'b' is less than 3 it indicated that the fish became more slender as they increased in length **(Grover and Juliano, 1976).** During the present investigation, the value of 'b' was greater than 3. This Indicated that growth pattern of fish population was allometric but *Botia lohachata* growth pattern was isometric (b=3.006). *Botia lohachata* growth was slightly higher than isometric growth but present value showed positive allometric growth pattern in captivity. *Botia almorhae* (b=4.027), *Botia dario* (b=4.005) and *Botia rostrata* (b=3.138) indicated positive allometric growth because all values

were greater than 3.0. These results suggest, that all species show positive allometric growth and that the fish grows in proportion to the length in captive condition.

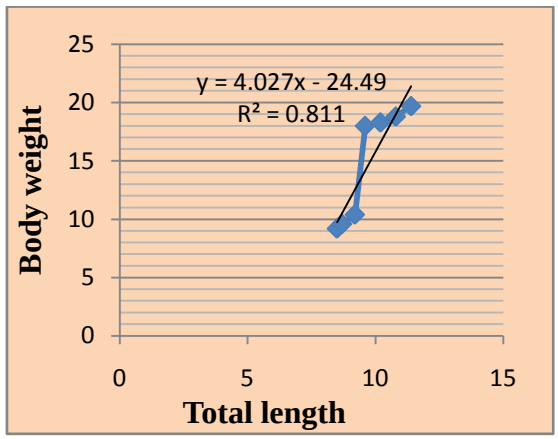


Fig. 8a: Length weight relationship of *Botia almorhae*

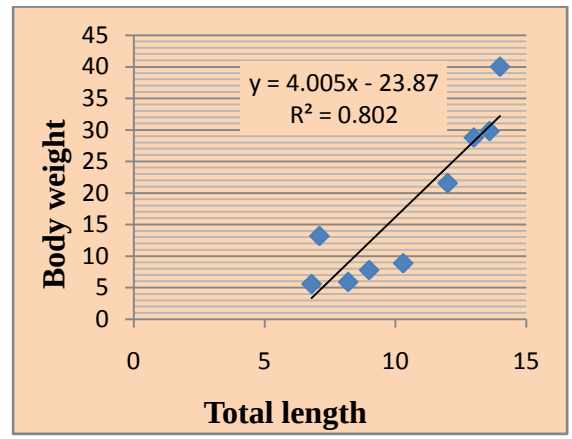


Fig. 8b: Length weight relationship of *Botia dario*

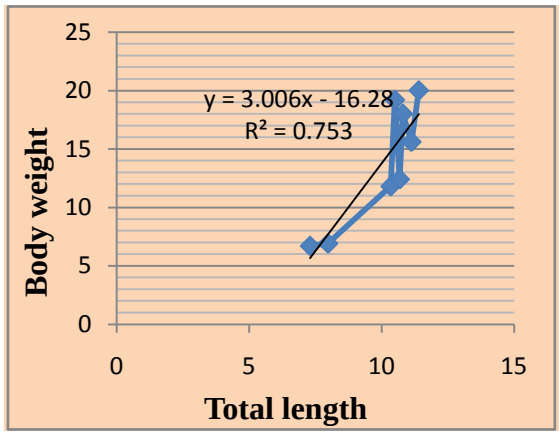


Fig. 8c: Length weight relationship of *Botia lohachata*

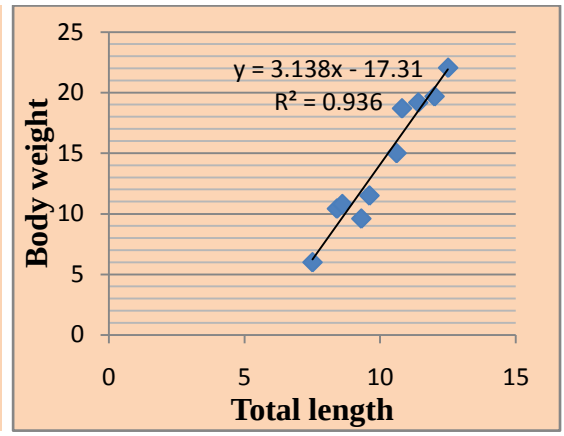


Fig. 8d: Length weight relationship of *Botia rostrata*

4.2.2. Study of fecundity and fertilization rate of the selected fishes

During the breeding period, the ripe male oozed out milt when slight pressure was applied on the vent. Eggs also oozed out with slight pressure on the belly of ripe female. The fecundity and fertilization rate were estimated by random sampling method (**Tab.8**).

4.2.2.1. Fecundity and fertilization rate of *Botia almorhae*

Fecundity of *B. almorhae* showed 12632 to 22456 (18539 ± 3828) numbers. The percentage of fertilization depended on the quality of brood stock. The fertilization rate was found to be 94.44% after one hour of spawning, 91.17% after two hours of spawning; 88.15% after three hours of spawning and 86.36% after four hours of spawning. The average fertilization rate was found to be 90.03%.

4.2.2.2. Fecundity and fertilization rate of *Botia dario*

The fecundity of *Botia dario* ranged from 13880 to 27510 (22573 ± 4949). The fertilization rate was found to be 93.75%, 80.55%, 78.20% and 75.86% after one, two, three and four hours respectively. The average fertilization rate was found to be 82.09 %.

4.2.2.3. Fecundity and fertilization rate of *Botia lohachata*

The fecundity of *Botia lohachata* ranged from 3731 to 23120 (18053 ± 7331). The fertilization rate was found to be 100% after one hour of spawning, 90% after two hours of spawning, 97.26% after three hours of spawning and 96.66% after four hours of spawning. The average fertilization rate was found to be 95.98%.

4.2.2.4. Fecundity and fertilization rate of *Botia rostrata*

The fecundity of *Botia rostrata* ranged from 14103 to 21352 (18698 ± 2772). The fertilization rate was found to be 89.28%, 72.41%, 65.67% and 43.05% after each hour respectively. The average fertilization rate was found to be 67.60%.

Tab.8: Details of Fecundity and fertilization rate of *Botia* species in Captive condition

Sl. No.	Species name	Fecundity			Fertilization rate		
		Lowest	Highest	Average	Lowest	Highest	Average
1	*B. almorhae*	12632	22456	18539	86.36	94.44	90.03
2	*B. dario*	13880	27510	22573	75.86	93.75	82.09
3	*B. lohachata*	3731	23120	18053	96.66	100	95.98
4	*B. rostrata*	14103	21352	18698	43.05	89.28	67.60

4.2.3. Standardization of breeding protocol of *Botia* species

Before hormonal induced breeding, male and female fish were kept in separate glass tanks for at least 2 days. Four different doses of WOVA-FH hormone (0.5 ml/kg as1st dose, 0.25 ml/kg as 2nd dose, 0.025ml/Fish as 3rd dose and 0.0125 ml/fish as 4th dose) were used. The best response to reproduction was obtained from the dosage of WOVA-FH of 0.025 ml/ fish (**Fig.8f**). Higher fertilization, hatching and survival rates were found in fish injected with 0.025 ml/fish in Set-ups: 3, 7, 11 and 15(**Tab.9**). Breeding trails were done twice by applying different doses of hormone in each breeding trial, and the same dose of WOVA-FH hormone was injected to both male and female.

Injected fishes were released in tanks and observed after 4-5 h when they started spawning simultaneously. Spawning was observed in Set-up-3, 7, 11 and 15 but there

was no spawning in others Set-ups. All fishes died in Set-ups-1, 5, 9 and 13 within 2days, whereas, in Set-up-2, 6, 10 and 14 all died within 4 days. In Set-up-4, 8, 12 and 16 all the fishes were most active and fed properly, but spawning was not observed (**Fig.8g**). The present study demonstrated the successful breeding of three species of genus *Botia* in captive condition with small dose of 0.025ml/fish WOVA-FH.

Fig.8e. Cuddling of fishes in a corner after injecting WOVA-FH at 0.5 ml/kg

Fig.8f. Active movement and spawning observed after WOVA-FH (0.025 ml/fish) injection

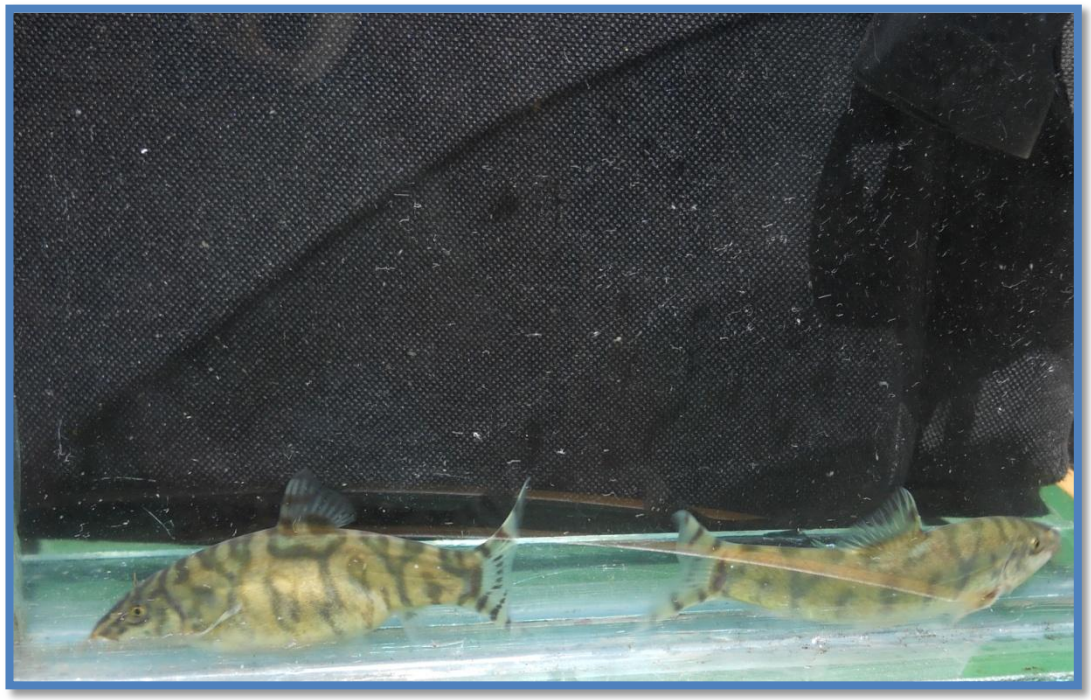


Fig.8g. Fishes most active but spawning not observed after WOVA-FH (0.0125 ml/ fish) injection

The latency period is described as the time interval between injection of hormone on a female fish and the starting of spawning (egg released by the female). The latency period of *Botia almorhae* was between 05.00 to 05.30 hours in fish injected with 0.025ml WOVA-FH per fish; for *Botia dario* it was 5 to 6 hours in fish injected with 0.025ml WOVA-FH per fish; *Botia lohachata* it was 4 to 5 hours in fish injected with 0.025ml WOVA-FH per fish and in *Botia rostrata* it was between 4.30 and 05.00 hours in fish injected with a dosage of 0.025ml WOVA-FH per fish.

Tab. 9: Summary of the different stages of breeding of Genus *Botia* in the different Set-ups.

Time counting after hormone injection	Set-up -1(No 5, 9 and 13) (0.5ml/Kg)	Set-up - 2(No 6,10 and10) (0.25ml /Kg)	Set-up -3(No 7,11 and 15) (0.025ml /fish)	Set-up – 4 (No 8, 12 and 16) (0.0125 ml/fish)
18:00	Transferred to tank	Transferred to tank	Transferred to tank	Transferred to tank
20:00.	Black patch appeared and cuddled in a corner	Some males active	All fishes were active	All fishes were active
21:00	Some males died	Some males active	Fishes were swimming against flow of water	Males were active
22:00	Females swelled up	Females swelled up	Females were being chased by the males at the same time the males were fighting with each other	Females swelled up and males started chasing.
23:00	All fish cuddled in a corner	Some males were chasing the females; no spawning.	Spawning had started; the paired fishes were swimming with the current	Males started chasing the females; no spawning.
24:00	All fish cuddled in a corner	No spawning	Spawning continued	No spawning
03:00	All fish cuddled in a corner	No spawning	Spawning continued	No spawning
04:00	All fish cuddled in a corner	No spawning	Spawning ceased	No spawning
05:00	Not spawning	No spawning	Fishes moved actively	No spawning
After 1 day	Eggs came out on pressing the abdomen	Eggs came out on Pressing the abdomen	Eggs no came out on Pressing the abdomen	Eggs came out on pressing the abdomen
After 2 days	Most of the fish died	Feeding ceased	All fishes took feed and moved actively	All fishes took feed and moved actively
After 4days	All fishes died	Almost all fishes died	All fishes took feed and moved actively	All fishes took feed and moved actively

4.2.4. Behaviour study of *Botia* loaches

Botia species stay together when in shoals or school. They took food in group and swam in a shoal. Naturally, this fish are bottom feeders but in captivity they consume food from the surface of water. They produce a loud cracking sound when feeding. *Botia* species liked to take rest in covered area where sun light did not penetrate. In aquarium or tank they always tried to hide in the sand or beneath the stones. Sometimes, they enjoyed staying under large stones parts and the gap between two stones under the water arranged like a comb. They also preferred to stay in pipe-like structure.

Fig. 9a: *Botia* species stay together in shoals

Fig. 9b: Feed being taken by group of *Botia* sp. from surface water

Fig.9c: Shoal of *Botia* sp. taking shelter in a plastic pipe

Fig. 9d: Group of *Botia* sp. hiding in the gap of two stones arranged like a comb

4.2.4.1. Breeding behaviour

Spawning behaviour was observed during the night or afternoon in absence of light. Male fishes were more actively involved in spawning. At the time of spawning, they made loud cracking sounds repeatedly. Six types of breeding behaviour were observed during the spawning time.

(1) Male hitting the female on the snout region and other body parts.

(2) Male fish was more active than female fish and the male hit the female fish in vent the region more frequently.

(3) Fighting was observed between the males when spawning occurring. Males were attacking each other on the head, tail and fins.

(4) The dominant male chased the female and later the spawning weaker male chased the females.

(5) Most important spawning activity, male and female fish were joined together and swam for about 30 seconds. They were attached together with help of spine that lies below the eye. The spine is a defence organ which causes a painful wound but not venomous. Male fish bent its tail region with female fish and formed "X" shape in tail region. Always male fishes were trying to keep the anal regions close to each other. This activity continued for 3 hours. Male released the sperm and female the egg and fertilization was external.

(6) Cannibalism behaviour was observed after spawning. Some male fishes ate fresh eggs immediately after spawning.

Fig. 10a: Male hitting the female on snout region

Fig. 10b: Male hitting the female on ventral region

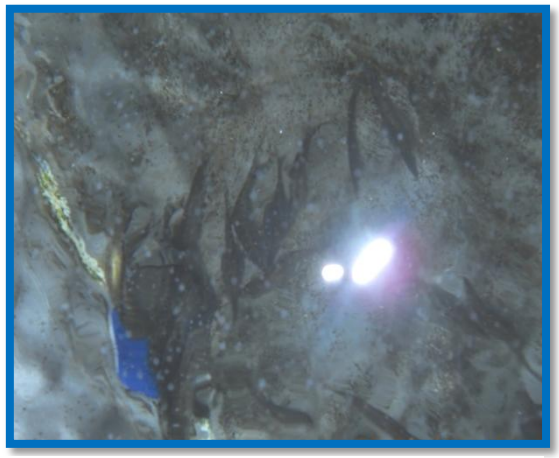


Fig. 10c: Males fighting with each other

Fig. 10d: Dominant male chasing the female and weaker male following them

Fig. 10e: Male and female fish clasping each other and swimming

Fig.10f: Fresh eggs being eaten by *Botia* male

4.2.5. Embryonic development of *Botia* species

The embryonic development of *Botia* species were divided into eight stages- Zygote, Cleavage, Blastula, Gastrula, Segmentation, Pharyngula, Hatching and Early larval period. These observed embryonic development stages are described below.

4.2.5.1. Zygote

The fertilized eggs of *Botia* species were non-adhesive, whitish in colour and optically transparent. Fertilization activated the cytoplasmic movements and the yolkfree cytoplasm began to stretch towards the animal pole gradually segregating the blastodisc from the vegetal cytoplasm. The diameter of the zygote was recorded to be 0.9 to 1mm (**Fig. 11: A1 to A2**).

4.2.5.2. Cleavage

The first cleavage occurred 24 to 28 minutes after fertilization. The two blastomeres rounded in shape just after first cleavage. The blastomeres observed at the animal pole were only half the size of the original cell. After the first cleavage, the blastomeres divided synchronously at an interval of 4 to 10 minutes. Cleavage period was observed to be from 24 min. to 1.15 hour in which the 64 cell stage was completed (**Fig. 11: A3 to A8**).

4.2.5.3. Blastula

The blastula stage with the 128-cell stage ended with the commencement of the gastrula. Blastula stage was observed to be linear from 1.15 to 3.10 hours and it completed 30% of the epiboly stage (**Fig. 11: A9 to A14**).

4.2.5.4. Gastrula

In the gastrula period, extensive cell movement was observed including involution, convergence and extension, producing the three primary germ layers and the embryonic axis. Gastrulation began with cell involution at around 50% epiboly and

completed the bud stage (**Fig. 11: A15-18**). In the bud stage (**Fig. 11: A18**), epiboly ended as the blastoderm completely covered the yolk plug. The tail bud then appeared as a distinct thickening at the caudal end of the embryo near the site of yolk plug closure. Early polster was seen. Gastrula period was observed to range from 3.10 to 6.36 hours. Epiboly continued, in addition the morphogenetic cell movements of involution, convergence and extension occurred producing the primary germ layers and the embryonic axis. As a consequence, within 15 minutes of reaching 90%-epiboly (**Fig. 11: A21**) a thickened marginal region termed the germ ring (**Fig. 11: A22**) appeared, nearly simultaneously all around the blastoderm. Convergence movements then, nearly as rapidly, produced a local accumulation of cells at one position along the germ ring, the so-called embryonic shield (**Fig. 11: A19**). During these events, epiboly temporarily got arrested, but after the shield formation, epiboly continued. The margin of the blastoderm advanced around the yolk cell to cover it completely. The advancement occured at a nearly constant rate, over an additional 15% of the yolk cell each hour, and provided a useful stage index during most of gastrulation (**Fig. 11: A23**). Gastrulation began with cell involution at around 50% epiboly (**Fig. 11: A18**) and completed at the bud stage. In the bud stage (**Fig. 11: A23**) epiboly ended as the blastoderm completely covered the yolk plug. The tail bud then appeared as a distinct thickening at the caudal end of the embryo near the site of yolk plug closure.

4.2.5.5. Segmentation

The segmentation period was characterized by the sequential formation of the somites, and lasted just prior to hatching. During this period, the embryo elongated along the animal pole axis, the tail bud developed longer and rudiments of the primary organs became visible. Somites, formed in bilateral pairs as the developing embryo, extended

posteriorly. Segmentation period was observed to be from 6.46 to 14.30 hours (**Fig. 11:A24** to **A34**).

4.2.5.6. Pharyngula

During this period the embryo were bilaterally organized, with a well-developed notochord and a newly completed set of somites that extended to the end of a long post-anal tail. Body axis straightened from its early curvature. The yolk sac, circulation, pigmentation, and fins development continued. The nervous system was hollow. The head straightened out and lifted to the dorsal side. The brain was prominently sculptured. The blood flow was visible. Pigment formation began in cells of the pigmented retinal epithelium. The embryo continued to exhibit spontaneous side-to-side contractions involving the trunk and tail and the rate of contractions increased in bursts till the embryo hatched out of the chorion (**Fig. 11: A35-42**). The C-shaped embryo elongated and gradually differentiated into a head and tail. The body formed into a C-shape (**Fig. 11: A42**). The yolk was attached between the tail and head. Myotomes development was observed. The embryo started occasional movement. At the twitching stage the tail got completely detached from the yolk. The yolk sac was restricted to the head region. The number of myotomes increased and the embryo became active and exhibited continuous twitching movement.

4.2.5.7. Hatching

Just after hatching from the chorion the larva at 14.30 to 14.45 hours measured 2.5 mm (**Fig. 11: A44**). Head was slightly bent on the yolk, the eyes were large, yolk sac was present on the anterior-ventral side of the body and the heart and optic vesicle were seen. They were responsive to stimulus and settled in the substrate. During this stage (**Fig. 11: A44 to A45**) the embryo continued to grow at about the same rate as earlier.

Morphogenesis of many of the organ rudiments were now rather complete and slowed down considerably, with some notable exceptions including the gut and its associated organs. However, these endodermal structures are difficult to visualize in the living embryo because of their deep position, and thus are not considered here. Pectoral fin development continued to be a useful feature for staging, especially during the early part of the hatching period. At the onset of the period, the paired fin rudiments were like elongated buds, each already containing centrally located mesenchymal condensation that formed the girdle cartilages.

4.2.5.8. Larval development

The mouth appeared to be opened and slit-like. After 22 h of hatching larvae started swimming and feeding. At first larvae, *Paramecium* sp. then *Artemia* were fed after 3days. The larvae consumed small sized zooplanktons (**Fig. 11:A46- A48**). There was complete resorption of the yolk sac and minute pectoral fins were observed. The eyes were well set in the optic sockets.

Tab. 10: Summary of the embryonic development of *Botia* species

Species name	Hormone dose	Latency period	Incubation period
Botia almorhae	0.025ml WOVA-FH/Fish	5 to 5.30 hours	14.40 hours
Botia dario	0.025ml WOVA-FH/Fish	5 to 6 hours	14.40 hours
Botia lohachata	0.025ml WOVA-FH/Fish	4 to 5 hours	14.30 hours
Botia rostrata	0.025ml WOVA-FH/Fish	4.30 to 5 hours	14.45 hours

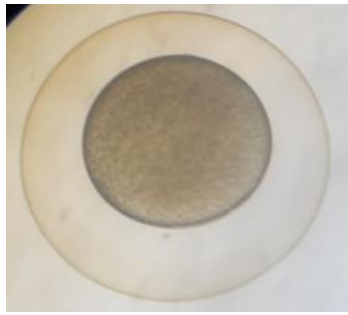
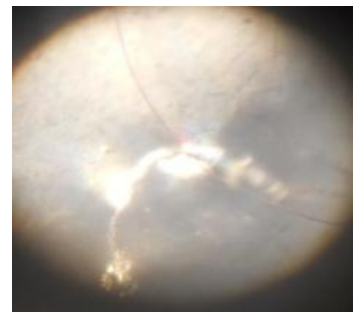
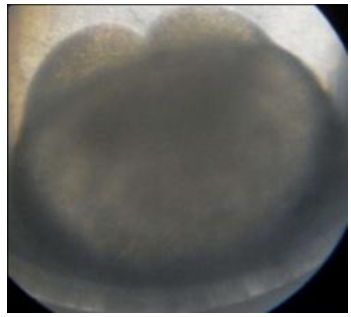
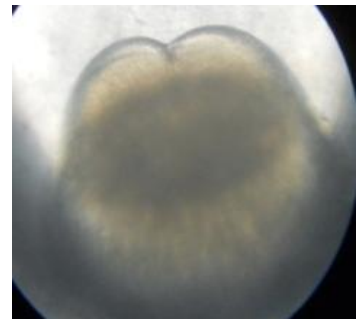
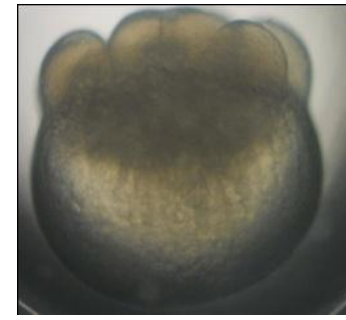
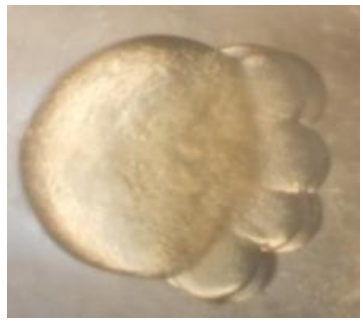
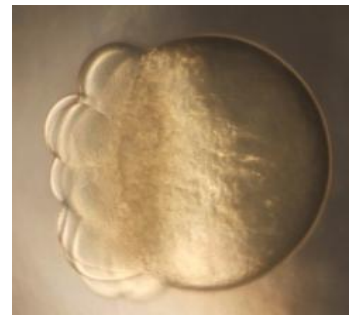



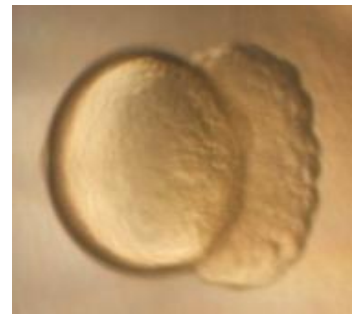
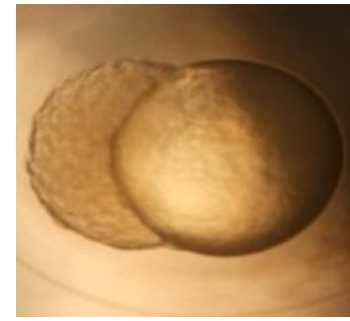

Fig. 11: A1 = Single cell A2 = Fertilization process A3 =Fertilized eggs

A4= 2-cell stage A5=4-cells stage A6= 8-cells stage


A7= 16 -cells stage A8= 32-cells stage A9 =64-cells stage

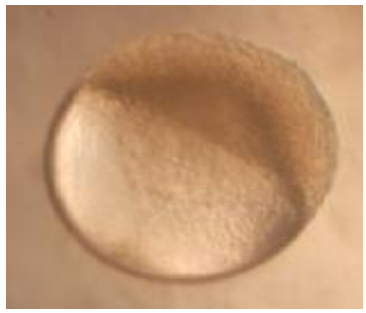




A10=128-cells stage A11= 256-cells stage A12= 512-cells stage

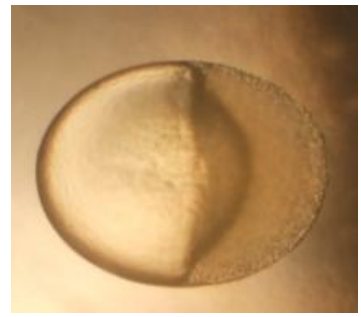
A13= 1,K cells stage

A14= Oblong stage

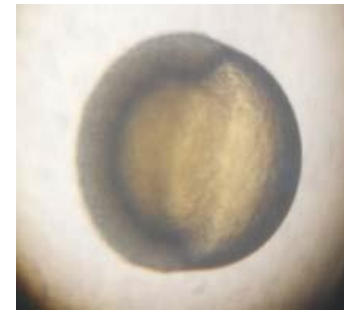
A15= Sphere stage



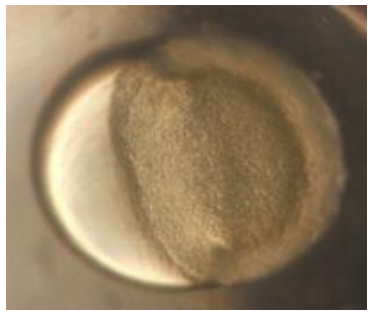
A16 = Dome stage



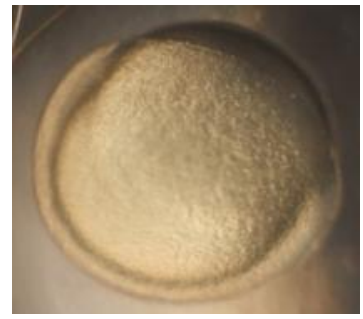
A17 = 30% epiboly



A18 =50% epiboly

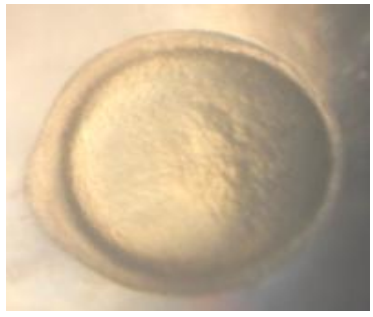


A19= embryonic shield stage



A20=75% epiboly

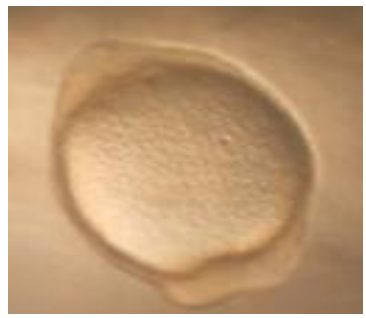
A21= 90% epiboly



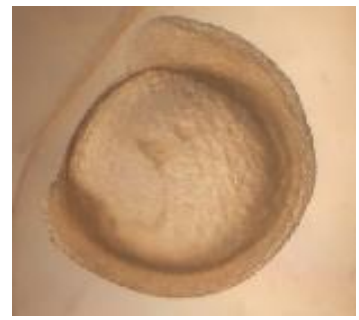
A22= Germ ring stage

A23= Bud stage

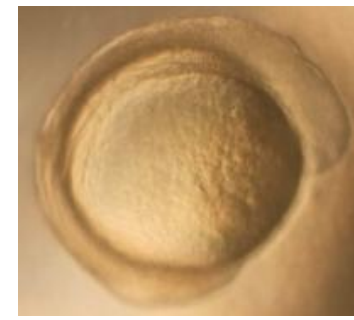
A24 = 1, somite stage



A25=2,somites stage



A26= 3, somites stage



A27= 4, somites stage

A28 = 7, somites stage

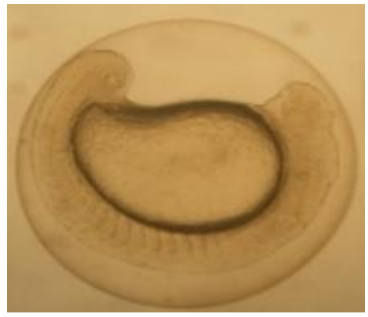
A29 = 8, somites stage

A30=12, somites stage

A31=14, somites stage

A32=16, somites stage

A33=18, somites stage

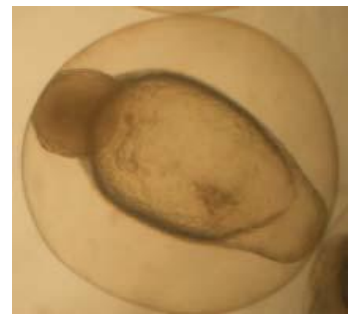


A34=20, somites stage

A35= Pharyngula stage

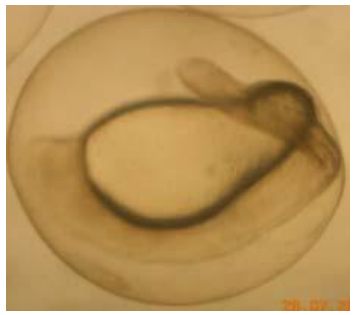
A36 = Pharyngula stage

A37= Pharyngula stage

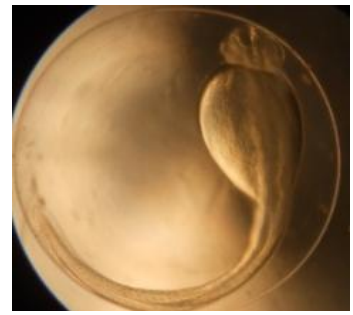


A38= Pharyngula stage

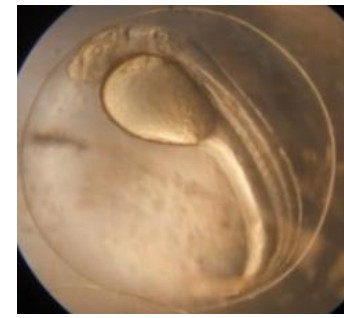
A39= late pharyngula stage



A40= Twitching stage



A41=C-shaped embryo

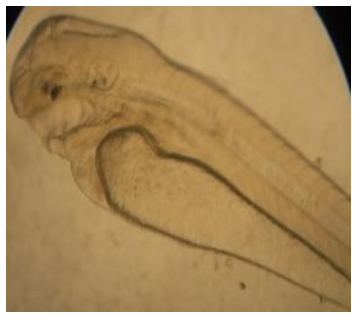


A42=C-shaped embryo

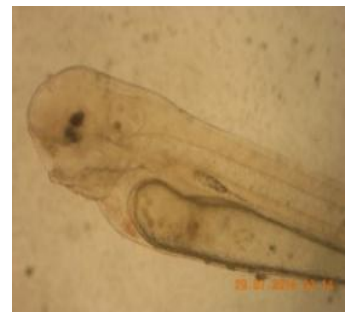
A43=Before hatching

A44= Hatching stage

A45=Newly hatched larva

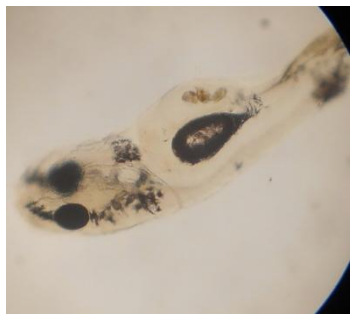


A46=larva take Paramecium

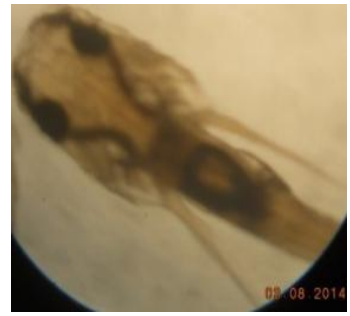


A47= larva take Artemia

A48= larva take zooplankton



A49= *Daphnia* inside the stomach of larva



A50= 6 day old larva

A51=7,day old larva

4.2.6. Supplementary feed for larval rearing of *Botia* species

Four glass aquariums containing 50 litres of water were used for feeding in experimental trials. Juveniles from the same parents were stocked in different experimental tanks with same stocking density (20 fishes per tank). Absolute Growth Rate in Tank-A was 0.037, Tank-B: 0.081, Tank-C: 0.086 and Tank-D: 0.117. In the present study, good growth was observed in Tank-D where only minced snail or bivalve flesh fed. The other experimental tanks, namely Tank-A, was fed only commercial fish feed, Tank-B: live zooplanktons and Tank-C: boiled minced meat. The growth rates were similar in Tank B and Tank C. Lowest growth rate was observed in Tank-A.

4.3. Histological study of the gonads of *Botia* species

4.3.1 Histology of the gonad of *Botia* species

Gonad maturation was a complicated process in *Botia* species and took one year time. Ovary developed slowly during its primary growth phase and then rapidly with the incorporation of yolk protein during its secondary growth phase, and finally attained maturation with the germinal vesicle breakdown. On the basis of size, morphology and cytoplasmic inclusion the growing oocytes were categorised into three stages and testes

were divided into two stages. Gonad development of *Botia almorhae, Botia dario* and *Botia lohachata* were similar. In *Botia rostrata,* gonad maturation took more than one year in captive culture. The size of gonad was too small, because *Botia rostrata* size was small than other *Botia* species. The distinctive features of each stages are given below.

4.3.1.1 Pre-spawning stage

Immature ovary of *Botia* species were small, elongated, thread like and translucent without visible oocytes. Immature stage of ovary of *Botia* species was found from February to March **(Fig.12a)**. The distinctive features of immature stage were presence of Chromatin nuclear oocytes (CNO) and Perinuclear oocytes (PNO). Chromatin nuclear oocytes are characterized by a nucleus containing a single nucleolus, surrounded by chromatin threads. In Perinucleolar oocytes, nucleolus was found in the periphery of nuclear membrane. Ovary occupied only a small portion of the abdominal cavity.

The pre-spawning phase or developing phase of ovary was found to be during March to May. Matured ovary was larger than immature ovary, pale yellow in colour and oocytes were visible to naked eye. Characteristics of early maturity stages of ovary were presence of Perinucleolar oocytes (PNO) and Cortical alveoli (CA) or Yolk vesicle Oocytes **(Fig.12b and 12c)**. Perinucleolar oocytes were round in shape and large in size than Chromatin and contained more than 15 peripheral nucleoli. Cortical alveoli possessed a large round nucleus which contained dispersed chromatin and numerous small nucleoli. Distinct zona granulosa (ZG) and zona radiata (ZR) layers appeared around the oocytes of CA. Yolk deposition stage was found to be slightly large in size and cytoplasm of oocytes was basophilic.

The testes were white in colour, thin, slender, thread-like, translucent and showed gradual increase in their volume and weight. They extended to about two third the length of abdominal cavity. No spermiation occurred after applying slight pressure on the abdomen. Developing stage of testis of *Botia* species were found from April to May. Histological features of developing testis were the presence of spermatids (ST), spermatocyts (SC) and spermatogonia (SG) (**Fig.12d**).

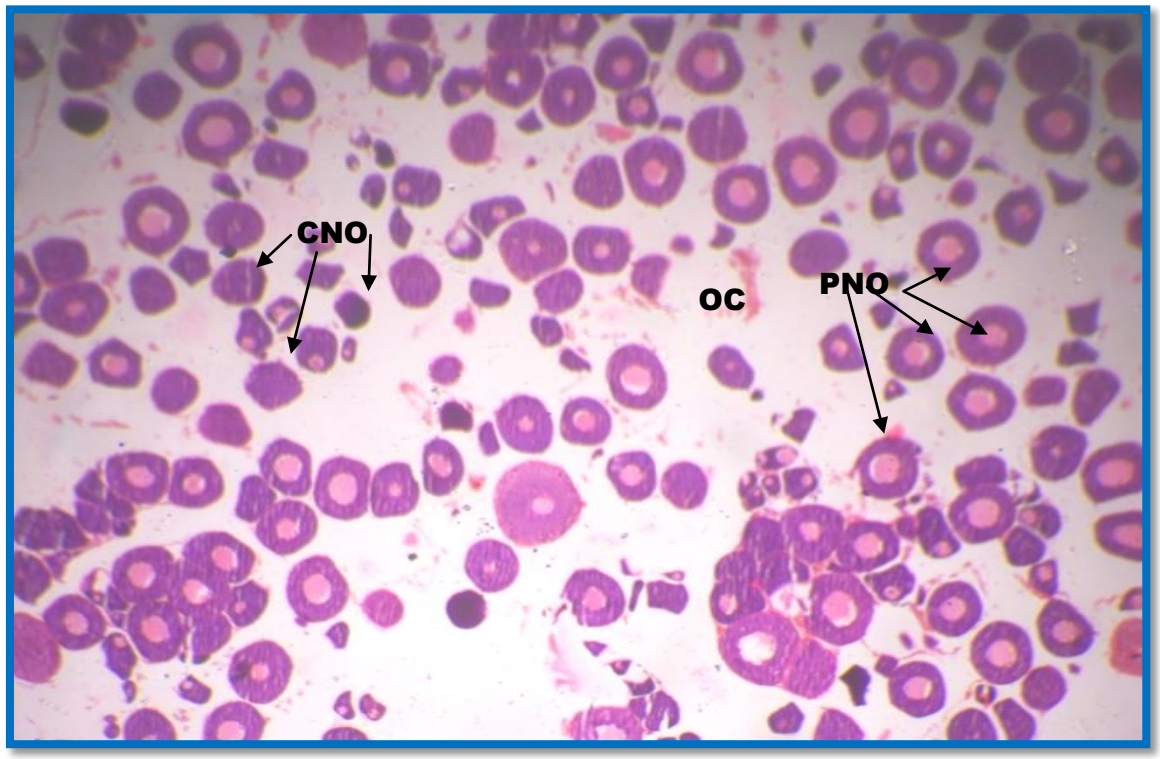


Fig.12a: Transverse section of primary growth phase of ovary of *Botia* species showing Chromatin nuclear oocytes (CNO) and Perinuclear oocytes (PNO)

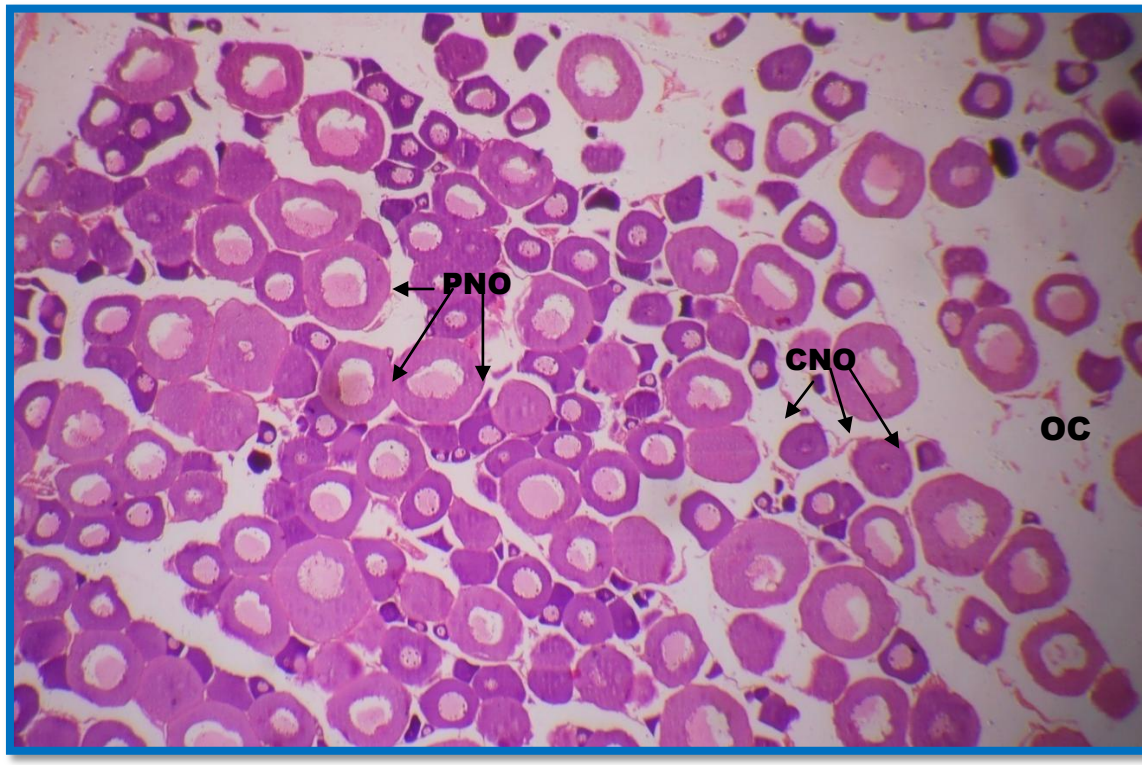


Fig.12b: Transverse section of primary growth phase of ovary of *Botia* species showing Chromatin nuclear oocytes (CNO), Perinuclear oocytes (PNO) and peripheral nucleus (PN)

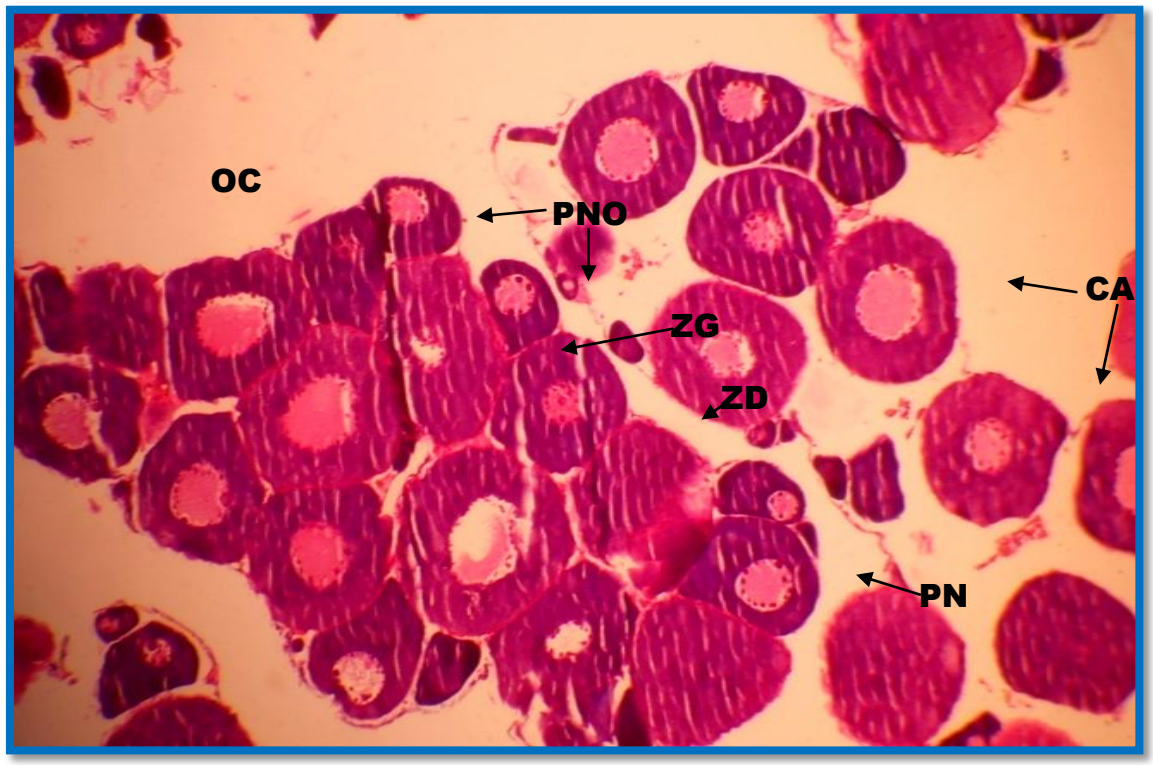


Fig.12c: Transverse section of pre-spawning phase of ovary of *Botia* species showing Perinuclear oocytes (PNO), peripheral nucleus (PN) and Cortical alveoli with zona granulosa (ZG) and zona radiate (ZR)

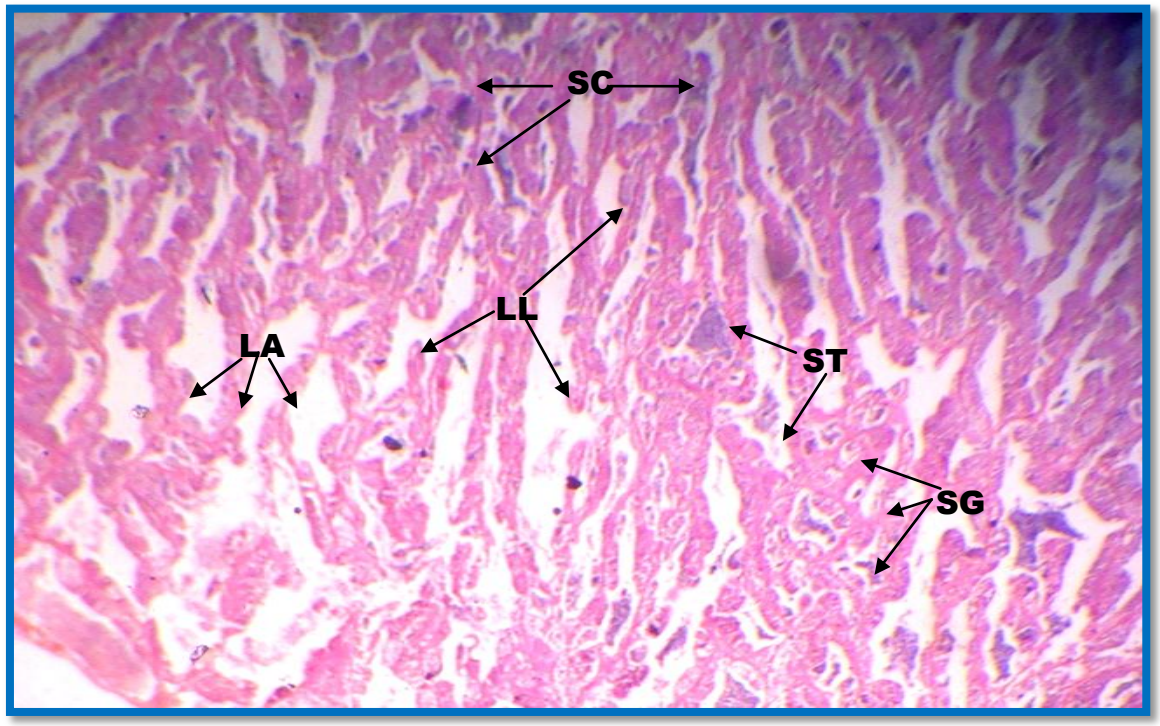


Fig.12d: Transverse section of developing stage of testis of *Botia* species showing Spermatogonia(SG), Spermatids (ST), Spermatocyts (SC), Lobule lumen (LL) and Lobule anastomose (LA)

4.3.1.2 Spawning stage

Spawning phase of ovary of *Botia almorhae, Botia lohachata* and *Botia rostrata* were found to be during June to August and *Botia dario* was found to be during May to July. Matured ovary was enlarged in size and yellow in colour. The oocytes were visible to the naked eye. Spawning phase of ovary was characterized by the occurrence of Cortical alveoli (CA) and vitellogenic oocytes (YGO) but post-ovulatory follicles were not present (**Fig.12e and 12f)**. The yolk globules increased in number, lipid droplets enlarged and was scattered between the yolk granules. At the running stage of ovary, germinal vesicle collapsed and migration of germinal vesicle occurred in Yolk globule oocytes and migration nucleus (MN) started to move to the animal pole.

The testes were milky white, long and flat, narrower behind, ribbon-like and increased in size. Spawning phase of testis of *Botia* species was found during May to September. Spawning stage was characterized by the onset of spermiation on applying

slight pressure to the abdomen. Testes extended to the entire length of the visceral cavity. Histological features of matured testis were the presence of spermatozoa (SP), spermatids (ST), spermatocyts (SC) and spermatogonia (SG) (**Fig.12g**).

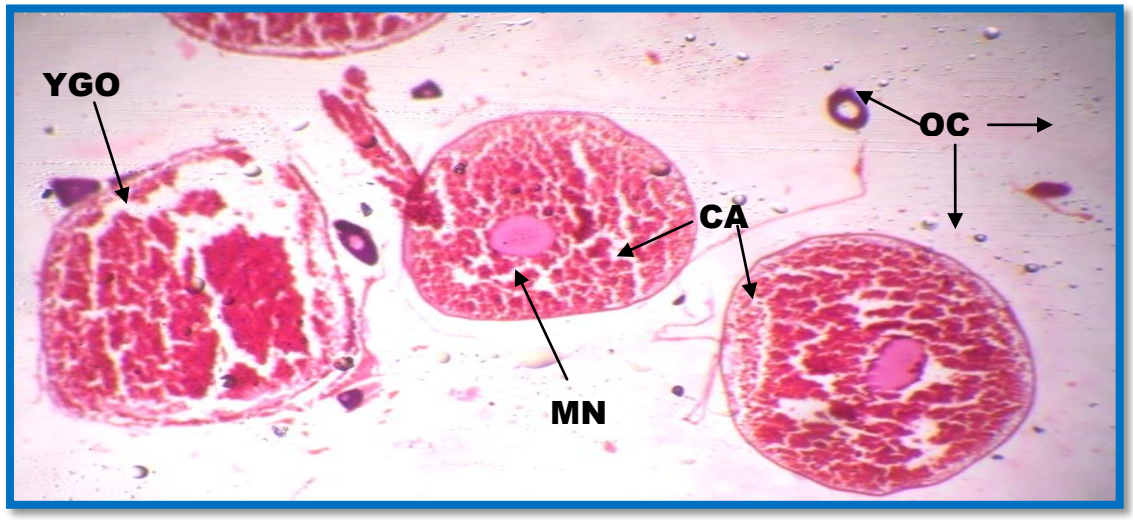


Fig.12e: Transverse section of spawning phase of ovary of *Botia* species showing Cortical alveoli (CA), Yolk globule oocyte (YGO) and Migratory nucleus (MN)

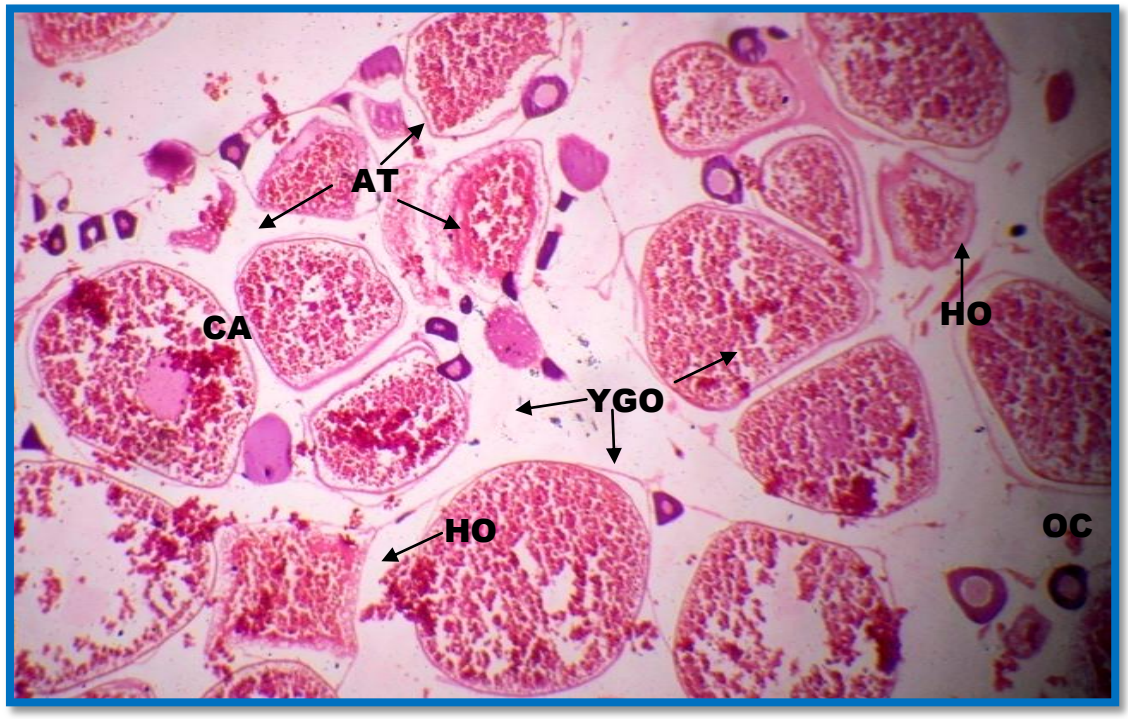


Fig.12f: Transverse section of running phase of ovary of *Botia* species showing Cortical alveoli (CA), Yolk globule oocyte (YGO), Hyaline oocytes (HO)and Atresia (AT)

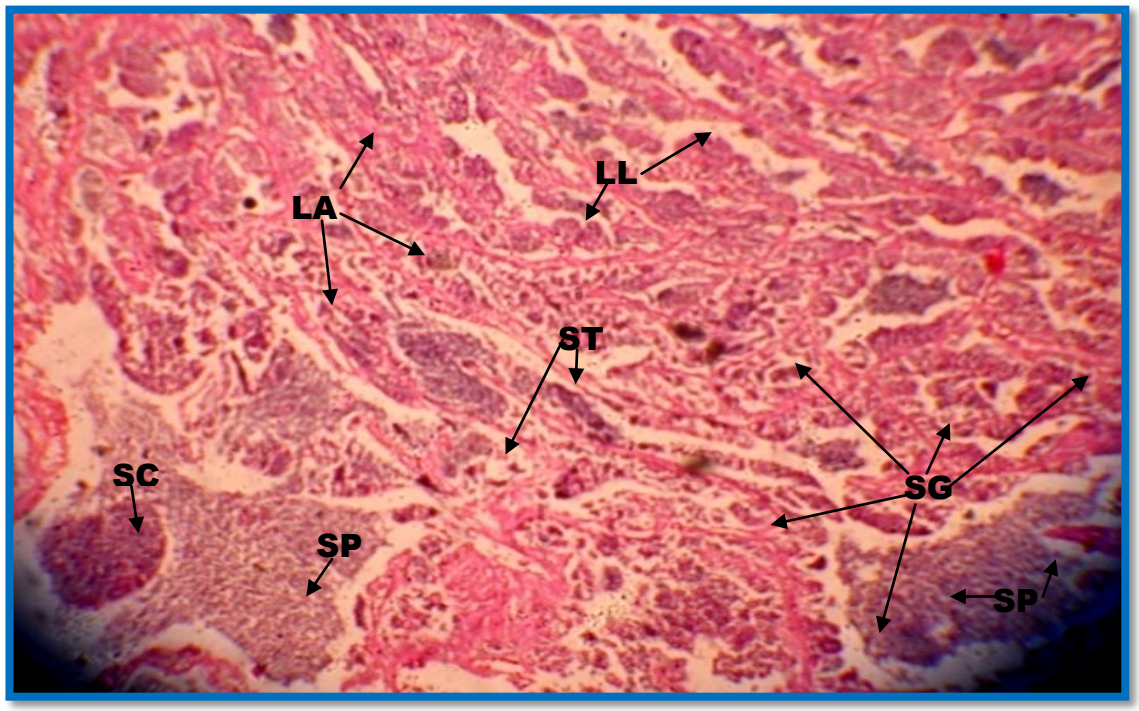


Fig.12g: Transverse section of spawning phase of testis of *Botia* species showing Spermatozoa (SP), Spermatogonia(SG), Spermatids (ST), Spermatocyts (SC), Lobule lumen (LL) and Lobule anastomose (LA)

4.3.1.3 Post-spawning stage

Post ovulated ovary of *Botia* species were elongated, thread like and transparent. Post-spawning phase of ovary of *Botia almorhae*, *Botia lohachata* and *Botia rostrata* were found to be during September to October. Post ovulated ovary of *Botia dario* was found to be during August to October. The ovaries were flat whitish with wrinkled membranes and residual eggs were occasionally visible through the ovarian walls. The weight of the ovary also decreased. Follicular cells of the atretic follicles were hypertrophid and the average oocyte diameter sharply declined. Characteristic features of post ovulated ovary were convoluted ovigerous folds containing large number of ruptured atretic follicles (AT), perinucleolar oocytes (PNO), post ovulated follicle (POF) and hyaline oocytes (HO) (**Fig.12h**). The unovulated mature oocytes (UOMO) became highly vacuolated, collapsed and shrunken inward, during the post-spawning period.

The testes were white in colour, thin, slender, and gradually decreased in their volume and weight. No spermiation occurred after applying slight pressure on the abdomen. Post spawning stage of testis of *Botia* species were found from October to February **(Fig.12i)**. Histological features of post spawning stage of testis were the presence of Spermatogonia (SG) and Residual spermatozoa (RSP).

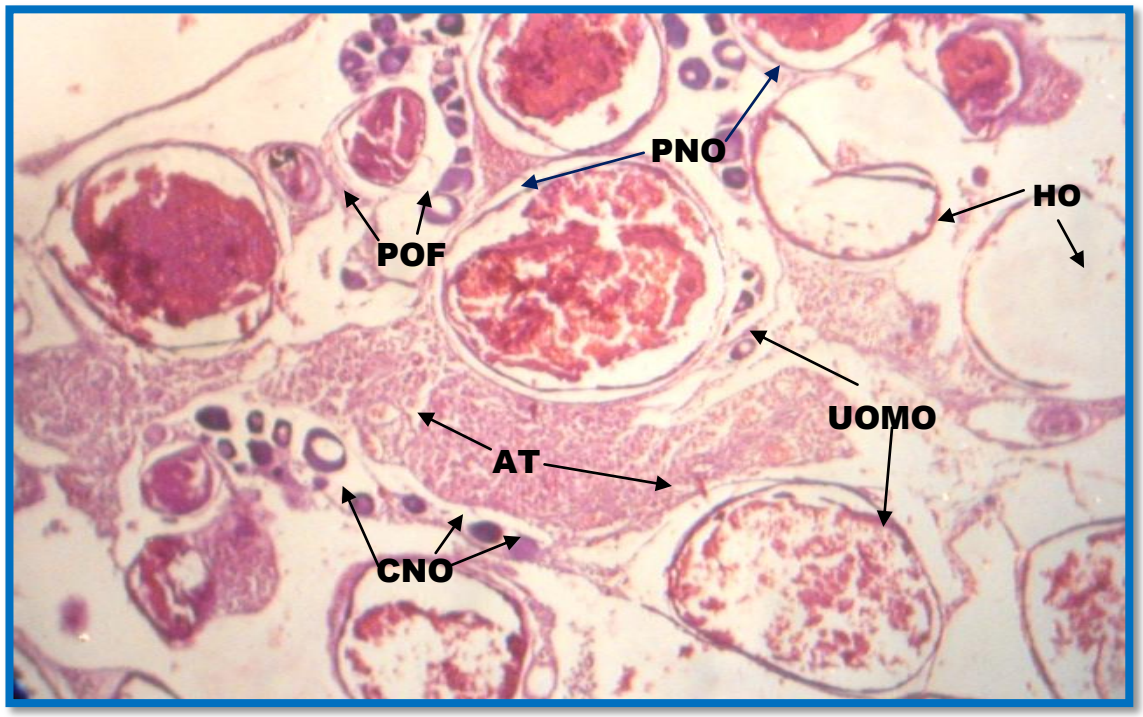


Fig.12h: Transverse section of Post-ovulated ovary (Spent stage) of *Botia* species showing Unovulated matured oocyte (UOMO), Hyaline oocytes (HO), Atresia (AT), Chromatin nuclear oocytes (CNO), Perinuclear oocytes (PNO) and Post ovulatory follicle (POF)

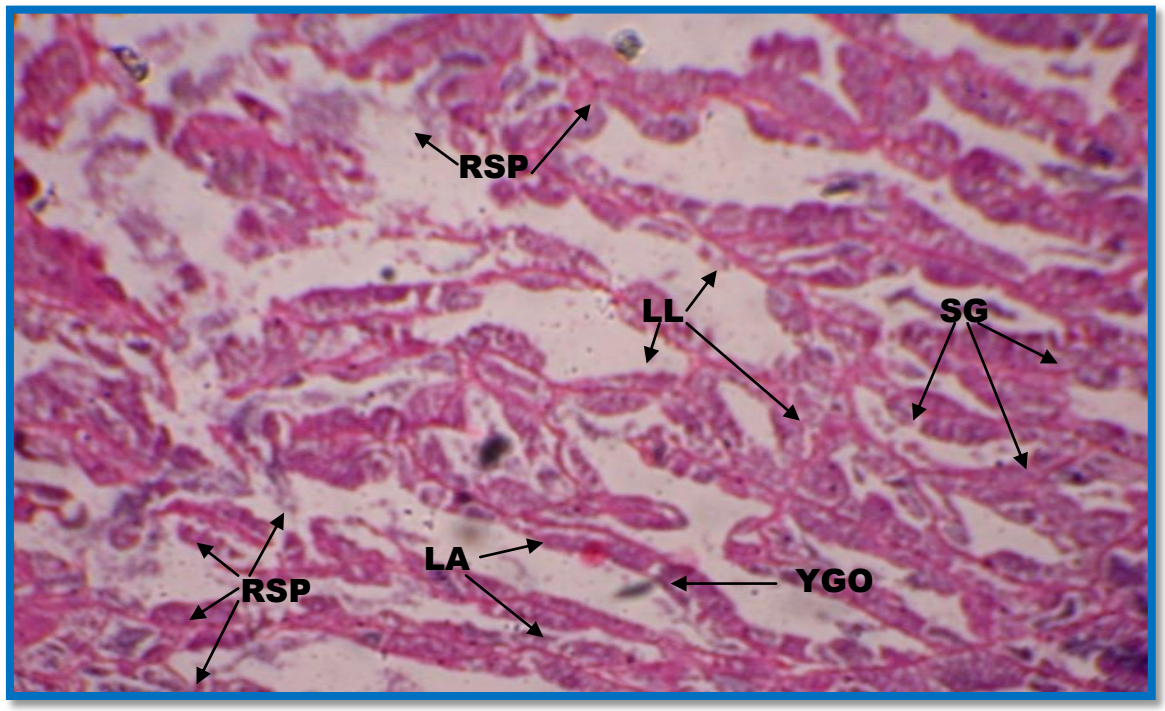


Fig.12i: Transverse section of post-spawning phase of testis of *Botia* species showing Spermatogonia(SG), Residual spermatozoa(RSP), Lobule lumen (LL) and Lobule anastomose (LA)

4.4. Molecular characterization of DNA barcoding and evolutionary relationship among *Botia* species

4.4.1. DNA sequence (FASTA sequence)

FASTA provides sequence similarity searching of query sequence against nucleotide and protein databases using FASTA programmes. The sequences in FASTA formatted files are preceded by a line starting with a ">" symbol. FASTA files containing multiple sequences are just same, with one sequence listed right after another. This format is accepted for many multiple sequence alignment programs. Ten FASTA sequences of COI of *Botia* species were obtained from the present study. FASTA sequences of Botia species are given below.

>Botia rostrata BR1

CCTATATCTCGTATGTGGTGCCTGAGCCGGAATAGTGGGCACGGCCATCAGC
CTTTTAATTCGGGCTGAACTCAGCCAACCTGGGTCCCTTCTAGGTGATGATCA
AATTTATAATGTTATCGTCACTGCACATGCCTTTGTTATGATTTTCTTTATAGT
AATACCAATCCTTATTGGGGGATTCGGGAACTGGCTTCTTCCACTTATGATTG
GAGCCCCTGATATAGCATTCCCTCGAATAAATAATATAAGCTTTTGACTTCTA
CCCCCATCTTTTCTTCTTCTCCTAGCATCCTCTGGAGTTGAAGCCGGAGCCGG
AACTGGGTGAACAGTATATCCTCCACTTGCTGGCAACTTAGCCCACGCAGGA
GCATCCGTAGACTTAACTATTTTCTCATTACATTTAGCAGGAGTTTCATCCAT
TTTAGGAGCAATTAATTTTATTACCACATCCATTAATATGAAACCCCCAGCAA
TTTCTCAATACCAAACACCATTATTTGTATGAGCCGTACTTGTAACGGCAGTT
CTACTGCTTTTATCCCTACCCGTACTGGCCGCCGGAATTACAATGCTGTTAAC
AGACCGTAATTTAAACACAACATTCTTCGACCCCGCTGGAGGAGGAGACCCA
ATCCTTTATCAACATTTATTC

>Botia rostrata BR2

CCTTTATCTCGTATGTGGTGCCTGAGCCGGAATAGTTGGCACGGCCCTCAGCC
TTTTAATTCGGGCTGAACTCAGCCAACCTGGGTCCCTTCTAGGTGATGATCAA
ATTTATAATGTTATCGTCACTGCACATGCCTTTGTTATGATTTTCTTTATAGTA
ATACCAATCCTTATTGGGGGATTCGGGAACTGGCTTCTTCCACTTATGATTGG
AGCCCCTGATATAGCATTCCCTCGAATAAATAATATAAGCTTTTGACTTCTAC
CCCCATCTTTTCTTCTTCTCCTAGCATCCTCTGGAGTTGAAGCCGGAGCCGGA
ACTGGGTGAACAGTATATCCTCCACTTGCTGGCAACTTAGCCCACGCAGGAG
CATCCGTAGACTTAACTATTTTCTCATTACATTTAGCAGGAGTTTCATCCATTT
TAGGAGCAATTAATTTTATTACCACATCCATTAATATGAAACCCCCAGCAATT
TCTCAATACCAAACACCATTATTTGTATGAGCCGTACTTGTAACGGCAGTTCT
ACTGCTTTTATCCCTACCCGTACTGGCCGCCGGAATTACAATGCTGTTAACAG
ACCGTAATTTAAACACAACATTCTTCGACCCCGCTGGAGGAGGAGACCCAAT
CCTTTATCAACATTTATTC

>Botia rostrata BR3

CCTATATCTCGTATGTGGTGCCTGAGCCGGAATAGTTGGCACGGCCCTCAGC
CTTTTAATTCGGGCTGAACTCAGCCAACCTGGGTCCCTTCTAGGTGATGATCA
AATTTATAATGTTATCGTCACTGCACATGCCTTTGTTATGATTTTCTTTATAGT
AATACCAATCCTTATTGGGGGATTCGGGAACTGGCTTCTTCCACTTATGATTG
GAGCCCCTGATATAGCATTCCCTCGAATAAATAATATAAGCTTTTGACTTCTA
CCCCCATCTTTTCTTCTTCTCCTAGCATCCTCTGGAGTTGAAGCCGGAGCCGG
AACTGGGTGAACAGTATATCCTCCACTTGCTGGCAACTTAGCCCACGCAGGA
GCATCCGTAGACTTAACTATTTTCTCATTACATTTAGCAGGAGTTTCATCCAT
TTTAGGAGCAATTAATTTTATTACCACATCCATTAATATGAAACCCCCAGCAA
TTTCTCAATACCAAACACCATTATTTGTATGAGCCGTACTTGTAACGGCAGTT
CTACTGCTTTTATCCCTACCCGTACTGGCCGCCGGAATTACAATGCTGTTAAC
AGACCGTAATTTAAACACAACATTCTTCGACCCCGCTGGAGGAGGAGACCCA
ATCCTTTATCAAC

>Botia rostrata BR4

CCTATATCTCGTATTTGGTGCCTGAGCCGGAATAGTTGGCACGGCCCTCAGCC
TTTTAATTCGGGCTGAACTCAGCCAACCTGGGTCCCTTCTAGGTGATGATCAA
ATTTATAATGTTATCGTCACTGCACATGCCTTTGTTATGATTTTCTTTATAGTA
ATACCAATCCTTATTGGGGGATTCGGGAACTGGCTTCTTCCACTTATGATTGG
AGCCCCTGATATAGCATTCCCTCGAATAAATAATATAAGCTTTTGACTTCTAC
CCCCATCTTTTCTTCTTCTCCTAGCATCCTCTGGAGTTCAAGCCGGAGCCGGA
ACTGGGTGAACAGTATATCCTCCACTTGCTGGCAACTTAGCCCACGCAGGAG
CATCCGTAGACTTAACTATTTTCTCATTACATTTAGCAGGAGTTTCATCCATTT
TAGGAGCAATTAATTTTATTACCACATCCATTAATATGAAACCCCCAGCAATT
TCTCAATACCAAACACCATTATTTGTATGAGCCGTACTTGTAACGGCAGTTCT
ACTGCTTTTATCCCTACCCGTACTGGCCGCCGGAATTACAATGCTGTTAACAG
ACCGTAATTTAAACACAACATTCTTCGACCCCGCTGGAGGAGGAGACCCAAT
CCTTTATCAACATTTATTC

>Botia lohachata BL1

CCTTTATCTAGTATGTGGTGCCTGAGCCGGAATAGGTGGCACGGCCCTCAGC
CTTTTAATTCGGGCTGAACTTAGCCAACCTGGGTCCCTTCTAGGTGACGATCA
AATTTACAATGTTATCGTCACTGCACATGCCTTTGTTATGATTTTCTTTATAGT
AATACCAATCCTTATTGGAGGATTCGGAAACTGGCTTCTTCCACTTATGATTG
GAGCCCCTGACATAGCATTCCCCCGAATGAATAATATAAGTTTTTGACTTCTG
CCCCCATCTTTTCTTCTTCTCTTAGCATCCTCTGGAGTTGAAGCCGGAGCCGG
AACTGGTTGAACAGTTTATCCCCCACTTGCTGGTAATTTGGCCCACGCAGGA
GCATCCGTAGACTTAACTATTTTCTCACTACATTTAGCAGGAGTCTCATCCAT
TTTAGGGGCAATTAATTTTATTACCACATCCATTAATATGAAACCTCCAGCAA
TTTCTCAATACCAAACACCATTATTTGTATGAGCCGTACTTGTAACGGCAGTT
CTACTGCTTTTATCCTTACCAGTACTAGCCGCCGGAATTACAATGCTGTTAAC
AGATCGTAATTTAAACACAACATTCTTCGACCCCGCTGGAGGAGGAGACCCA
ATCCTTTATCAACATTTATTC

>Botia almorhae BL3

CCTATATCTAGTATGTGGTGCGTGAGCCGGAATAGTTGGGACGGCCCTCAGC
CTTTTAATTCGGGCTGAACTTAGCCAACCTGGGTCCCTTCTAGGTGACGATCA
AATTTACAATGTTATCGTCACTGCACATGCCTTTGTTATGATTTTCTTTATAGT
AATACCAATCCTTATTGGGGGATTCGGAAACTGGCTTCTTCCACTTATGATTG
GAGCCCCTGACATAGCATTCCCCCGAATGAATAATATAAGTTTTTGACTTCTG
CCCCCATCTTTTCTTCTTCTCTTAGCATCCTCTGGAGTTGAAGCCGGAGCCGG
AACTGGTTGAACAGTTTATCCCCCACTTGCTGGTAATTTGGCCCACGCAGGA
GCATCCGTAGACTTAACTATTTTCTCACTACATTTAGCAGGAGTCTCATCCAT
TTTAGGGGCAATTAATTTTATTACCACATCCATTAATATGAAACCTCCAGCAA
TTTCTCAATACCAAACACCATTATTTGTATGAGCCGTACTTGTAACGGCAGTT
CTACTGCTTTTATCCCTACCAGTACTAGCCGCCGGAATTACAATGCTGTTAAC
AGATCGTAATTTAAACACAACATTCTTCGACCCCGCTGGAGGAGGAGACCCA
ATCCTTTATCAACATTTATTC

>Botia modesta BM1

CCTATATCTAGTATGAATTGGGTGAGCCGGAATAGTTGGGACTGCCCTCAGC
CTTTTAATTCGAGCTGAACTAAGCCAACCCGGATCACTTCTGGGCGATGATC
AAATCTACAATGTTATCGTTACTGCACATGCTTTCGTAATAATTTTCTTTATA
GTAATACCAGTCCTTATTGGAGGGTTTGGAAATTGACTCCTCCCGCTAATAAT
TGGAGCCCCAGACATAGCATTTCCGCGAATAAATAATATGAGTTTTTGACTC
CTACCCCCCTCTTTCCTACTACTCCTGGCCTCTTCCGGAGTTGAAGCAGGCGT
CGGAACAGGGTGAACAGTGTACCCGCCACTTGCGGGAAACTTAGCCCATGCA
GGCGCATCCGTAGACTTAGCTATCTTTTCTCTACACTTAGCAGGTGTATCCTC
CATCCTAGGCGCTATTAATTTTATCACCACCTCTATTAATATAAAACCCCCAG
CCATCACCCAATATCAAACTCCCCTATTTGTATGAGCTGTACTTGTAACAGCC
GTTCTTTTACTGCTATCCCTACCTGTTTTAGCCGCTGGAATTACAATGCTGTTA
ACAGACCGTAACTTAAATACAACATTCTTTGACCCCGCAGGAGGAGGAGACC
CAATTCTTTACCAACACCTATGC

>Botia macracanthus BMC1

CCTATATCTAGTATTTGGTGCCTGAGCCGGAATAGTTGGCACTGCCCTCAGTC
TCTTAATTCGAGCTGAACTTAGCCAACCTGGTTCACTTCTAGGTGACGATCAA
ATCTATAACGTTATCGTAACTGCACATGCCTTTGTTATAATTTTCTTTATAGTA
ATACCAATCCTCATCGGAGGATTCGGAAATTGACTTCTTCCATTAATAATTGG
AGCCCCCGATATAGCATTCCCCCGAATAAACAATATAAGCTTCTGACTCCTG
CCCCCATCATTCCTTCTACTTTTAGCCTCCTCTGGAGTTGAAGCAGGAGCTGG
AACGGGATGAACTGTTTATCCGCCACTCGCGGGTAACTTAGCCCACGCAGGG
CCATCCGTAGACCTAACTATCTTCTCACTACATTTGGCGGGTGTTTCATCAAT
TTTAGGGGCAATTAATTTTATCACCACCTGCATTAATATGAAACCTCCAGCCA
TTTCTCAATATCAAACACCTTTATTTGTATGAGCCGTCCTTGTAACGGCTGTC
CTGTTATTATTATCTTTACCAGTATTAGCTGCTGGAATTACAATACTTTTAAC
AGACCGTAATCTTAACACAACATTCTTTGACCCCGCAGGAGGAGGTGACCCA
ATCCTTTATCAACATTTATTT

>Botia dario BD1

ACTAGATTTAGTATATGATGCCTGAGCCGGCATCGGTGGGACAGCCCACAGC
CTTATATTACGAGCTGTACTCAGCCAACCTGGGTCCCTCCTAGGTGATGATCA
AATTTACAACGTTATCGTCACTGCCCATGCTTTCGTTATAATTTTCTTTATAGT
AATACCAATCCTTATTGGGGGATTCGGGAACTGACTCCTTCCACTTATAATTG
GAGCCCCTGACATAGCATTCCCTCGAATAAATAATATAAGCTTTTGACTTCTC
CCACCATCTTTTCTTCTCCTTTTAGCATCCTCTGGTGTCGAAGCTGGGGCCGG
AACTGGTTGAACAGTATACCCACCACTTGCTGGCAACTTAGCCCACGCAGGA
GCATCCGTAGACTTAACTATTTTTTCACTACACTTAGCAGGAGTTTCATCTAT
TTTAGGAGCAATCAATTTTATTACCACATCCATCAACATGAAACCCCCAGCTA
TTTCTCAATACCAAACACCATTATTCGTGTGAGCTGGACTTGTAACAGCAGTC
CTATTACTTTTATCCCTACCAGTGCTAGCTGCCGGAATTACAATACTGTTAAC
AGACCGTAATTTAAATACAACATTCTTTGACCCCGCCGGAGGAGGTGACCCA
ATCTTTTACCAACACTTATTC

>Botia dario BD2

ACTAGATTTAGTATATAGTGCGTGAGCCGGCATATGTGGCACAGCCCTCAGC
CTTTTAATTCGAGCTGAACTCAGCCAACCCGGGTCCCTTCTAGGTGATGATCA
AATTTACAACGTTATCGTCACTGCACATGCTTTCGTTATAATTTTCTTTATAGT
AATACCAATCCTTATTGGGGGATTCGGGAACTGACTCCTTCCACTTATAATTG
GAGCCCCTGACATAGCATTCCCTCGAATAAATAATATAAGCTTTTGACTTCTC
CCACCATCTTTTCTTCTCCTTTTAGCATCCTCTGGTGTCGAAGCTGGGGCCGG
AACTGGTTGAACAGTATACCCACCACTTGCTGGCAACTTAGCCCACGCAGGA
GCATCCGTAGACTTAACTATTTTTTCACTACACTTAGCAGGAGTTTCATCTAT
TTTAGGGGCAATCAATTTTATTACCACATCCATCAACATGAAACCCCCAGCTA
TTTCTCAATACCAAACACCATTATTCGTGTGAGCTGTACTTGTAACAGCAGTC
CTATTACTTTTATCCCTACCAGTGCTAGCTGCCGGAATTACAATACTGTTAAC
AGACCGTAATTTAAATACAACATTCTTTGACCCCGCCGGAGGAGGTGACCCA
ATTCTTTACCAACACTTATTC

4.4.2. DNA sequence variation analysis

Mitochondrial DNA 655bp Cytochrome Oxidase Subunit I (COI) gene were successfully amplified from individuals of *Botia dario, Botia rostrata, Botia almorhae, Botia lohachata, Botia macracanthus* and *Botia modesta* and sequences were submitted to Genbank databases (**Tab.11**). Simplicity and un-ambiguity were observed among all the sequences, and no insertions, deletions or stop codons were observed in any of the sequences. Some sequences were also derived from NCBI. Out of 655 positions in the COI gene sequences analyzed in 10 specimens, 196 positions were variable, and 172 were parsimoniously informative. The average base composition [Thymine/Uracil (T/U); Cytosine (C); Adenine (A) and Guanine (G)] over all the four codon positions is 31.5, 25.7, 25.8 and 17.0, respectively. The transition/transversion rate ratios are k1 =4.836 (purines) and k2 = 6.772 (pyrimidines). The overall transition/transversion bias is R = 3.084(**Tab.12**).

Tab. 11: The mitochondrial COI sequences of genus *Botia* with the accession number

Sl. No.	Species name	Genbank Accession Number	Authors
1	*Botia almorhae*	KF738184	NCBI
2	*Botia almorhae*	KF738185	NCBI
3	*Botia almorhae*	KF738183	NCBI
4	*Botia almorhae*	KT781504	PRESENT STUDY
5	*Botia lohachata*	KT781505	PRESENT STUDY
6	*Botia lohachata*	KF742423	NCBI
7	*Botia kubotai*	KF738178	NCBI
8	*Botia kubotai*	KF738179	NCBI
9	*Botia kubotai*	KF738180	NCBI
10	*Botia kubotai*	KF738181	NCBI
11	*Botia rostrata*	KT781497	PRESENT STUDY
12	*Botia rostrata*	KT781498	PRESENT STUDY
13	*Botia rostrata*	KT781499	PRESENT STUDY
14	*Botia rostrata*	KF738189	NCBI
15	*Botia rostrata*	KF738190	NCBI
16	*Botia rostrata*	KF738191	NCBI
17	*Botia rostrata*	KT781500	PRESENT STUDY
18	*Botia rostrata*	KF738192	NCBI
19	*Botia striata*	KF738186	NCBI
20	*Botia striata*	KF738187	NCBI
21	*Botia striata*	KF738188	NCBI
22	*Botia dario*	KT781502	PRESENT STUDY
23	*Botia dario*	KT781503	PRESENT STUDY
24	*Botia dario*	JX105475	NCBI
25	*Botia dario*	KF511556	NCBI
26	*Botia dario*	JX105468	NCBI
27	*Botia dario*	JX105477	NCBI
28	*Botia dario*	JX105478	NCBI
29	*Botia macracanthus*	KT781506	PRESENT STUDY
30	*Chromobotia macracanthus*	KF738204	NCBI
31	*Chromobotia macracanthus*	KF738207	NCBI
32	*Chromobotia macracanthus*	KF738205	NCBI
33	*Chromobotia macracanthus*	KF738206	NCBI
34	*Botia modesta*	KT781501	PRESENT STUDY
35	*Botia modesta*	JQ346170	NCBI
36	*Glyptothoraxbrevipinnis*	EU637829	NCBI

Table 12: Molecular characterization information content of the mtDNA COI region of analyzed *Botia*

No. of bases analyzed	Nucleotide composition				Invariable Sites	Polymorphic informative Sites	Parsimony informative Sites	Estimated tv/ts bias (R)
	%A	%G	%T	%C				
655	25.8	17	31.5	25.7	196	164	172	3.084

Table 13: Evolutionary divergence between Genus *Botia*

	B. dario	***B. lohachata***	***B. almorhae***	***B. macracanthus***	***B. modesta***	***B. rostrata***	***B. kubotai***	***B. striata***
B.dario		0.015	0.015	0.017	0.018	0.013	0.015	0.014
B.lohachata	0.112		0.002	0.017	0.020	0.009	0.009	0.014
B.almorhae	0.107	0.004		0.017	0.020	0.008	0.009	0.014
B.macracanthus	0.169	0.137	0.140		0.020	0.018	0.018	0.018
B. modesta	0.186	0.200	0.199	0.198		0.019	0.019	0.019
B. rostrata	0.094	0.045	0.040	0.151	0.193		0.008	0.012
B. kubotai	0.112	0.047	0.049	0.158	0.192	0.035		0.013
B. striata	0.109	0.093	0.097	0.155	0.199	0.074	0.088	

4.4.3. Evolutionary distances

Intra-species pair wise distances of *Botia* genus is highlighted in Table 3. The COI sequence pair of *Botia* evolutionary distances ranged from 0.004 to 0.200. The interspecies Kimura's 2- parameter pair-wise distance was highest (0.200) between *B. modesta* and *B. lohachata* and lowest (0.004) for *B. almorhae* and *B. lohachata* (**Tab.13**). Best fit models for COI dataset was Hasegawa-Kishino-Yano (HKY+ I) model for different population of *Botia* and closely related species such as *B. lohachata* and *B. almorhae*. 500 bootstrap re-sampling strategy was used to assess the reliability of a

phylogenetic tree. All the populations of *Botia* clearly separated from each other in the phylogenetic tree (**Fig.13**).

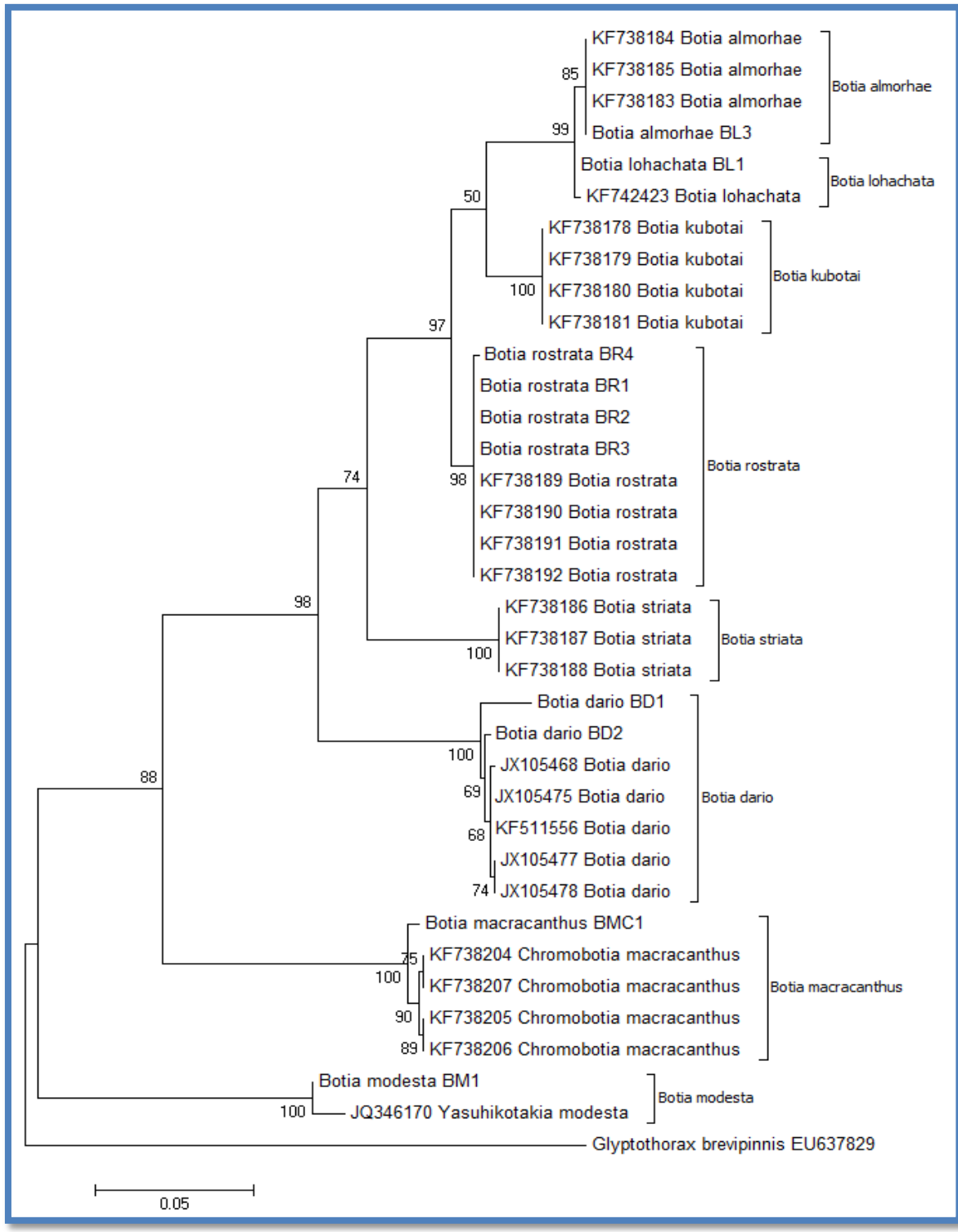


Fig. 13: Molecular Phylogenetic Analysis by Maximum Likelihood Method

4.4.4. Phylogenetic analysis

The nucleotide sequences of COI gene were aligned in order to determine the phylogenetic relationship among 6 species of *Botia*. The topology of ML and NJ tree were estimated. The phylogenetic tree showed that *B. almorhae* and *B. lohachata* formed a monophyletic group (supported by 100% bootstrap value) and then constituted one clade with *Botia kubotai*. Other Asian species, *Botia rostrata, Botia striata, Botia dario, Botia modesta and Botia macrocanthus* also contributed to this clade but were distant to native *Botia* species.

The evolutionary history was inferred by using the Maximum Likelihood Method based on the Hasegawa- Kishino-Yano model (**Hasegawa *et al.*, 1985**). The tree with the highest log likelihood (-2520.3362) is shown in Figure 8. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour- Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior loglikelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 0.0010% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 36 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions contained gaps and missing data were eliminated. There were a total of 592 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 **(Tamura *et al.*, 2011).**

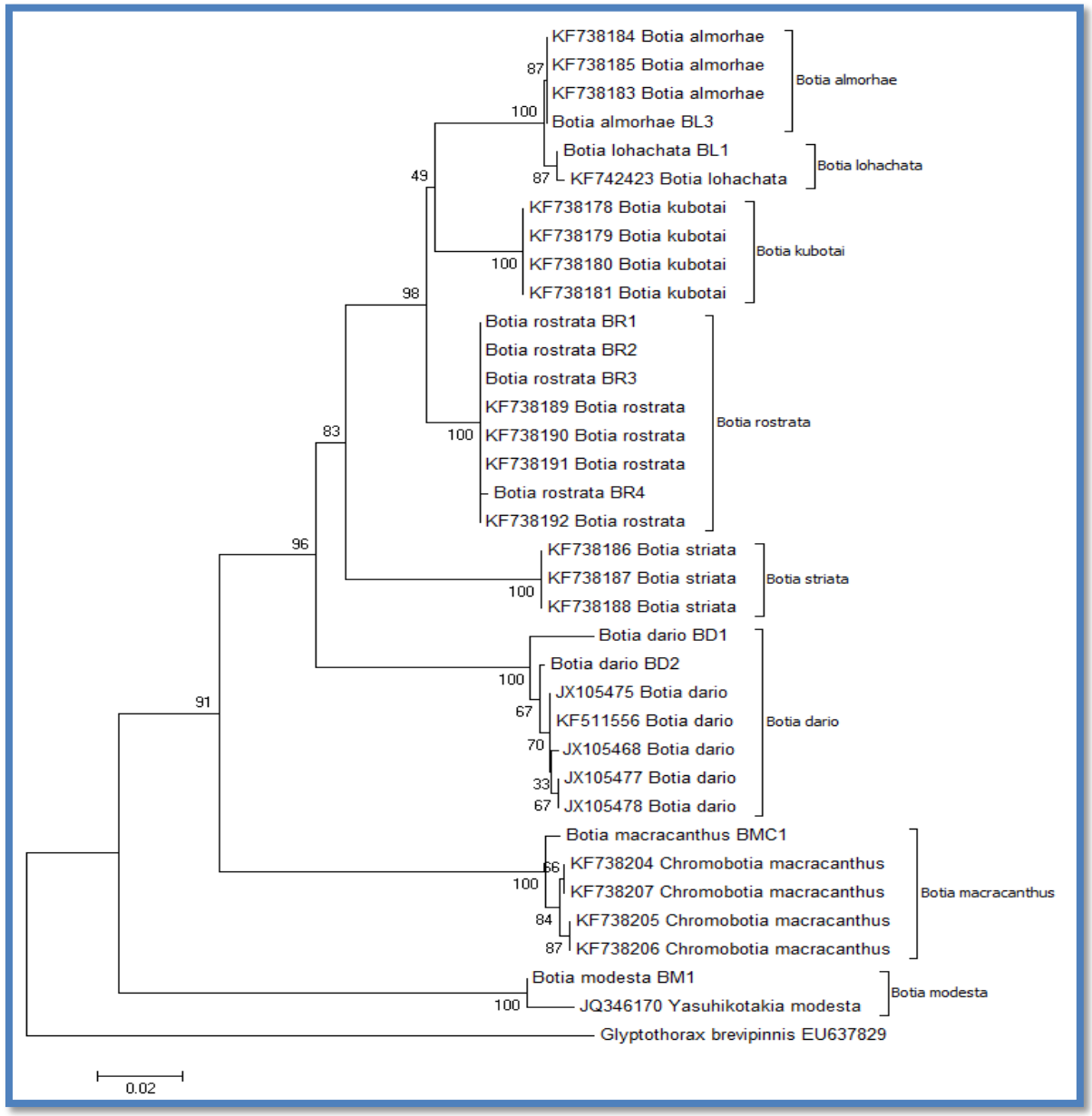


Fig. 14: Evolutionary relationships of taxa by Neighbour-Joining method

The evolutionary history was inferred using the Neighbour- Joining method. The optimal tree with the sum of branch length = 0.60289640 is shown in **Fig.14**. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method

and are in the units of the number of base substitutions per site. The analysis involved 36 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were a total of 592 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

4.4.5. DNA Barcoding of four *Botia* loaches

The Barcode ID number was generated from the Barcode of Life Data Systems Version 3. The Barcode ID number of four *Botia* species were **SDP657007-17** (*Botia almorhae*), **SDP657005-17** (*Botia dario*), **SDP657002-17** (*Botia lohachata*) and **SDP657006-17** (*Botia rostrata*).

Fig.14a: Barcode view of *Botia almorhae* in BOLD system

Fig.14b: Barcode view of *Botia dario* in BOLD system

Fig.14c: Barcode view of *Botia lohachata* in BOLD system

Fig.14d: Barcode view of *Botia rostrata* in BOLD system

4.5. Statistical analysis

In *Botia* species the mathematical relationship between body weight and gonad weight, body length and gonad length, body weight and fecundity and Gonado-somatic Index of male and female, Coefficient of Correlation (r) was done using MS- Excel.

4.5.1. Correlation study among growth parameters of *Botia almorhae*

The Coefficient of Correlation of *Botia almorhae* among gonad weight and body weight was 0.819, gonad length and body length was 0.611, fecundity and body weight was 0.848 and female and male Gonado-somatic Index was 0.87. The Coefficient of Correlation (r) among all relationships showed significance at $p \leq 0.01$.Correlation between the different parameters of *Botia almorhae,* Gonado-somatic Index of male and female were more significant than other parameters. The regression equation Y= 0.418X

+ 3.824 for body weight and gonad weight (**Fig. 15a**), Y= 0.364X – 0.735 for body length and gonad length (**Fig. 15b**), Y= 764.1X – 5812 for body weight and fecundity (**Fig. 15c**), Y= 9.155X + 2.734 for Gonado-somatic Index of male and female (**Fig. 15d**) showed a linear relationship by using the linear regression equation Y = a+ bx.

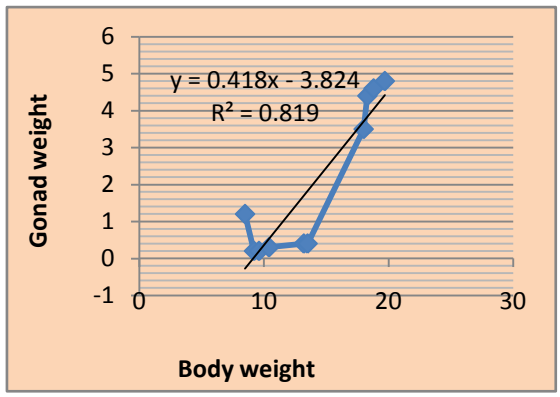


Fig. 15a: Correlation among gonad weight and body weight of *B. almorhae*

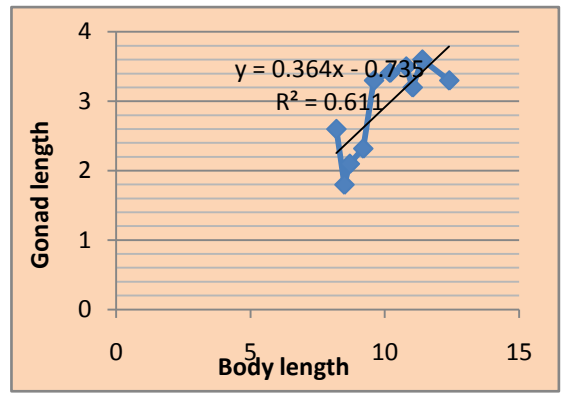


Fig. 15b: Correlation among gonad length and body length *B. almorhae*

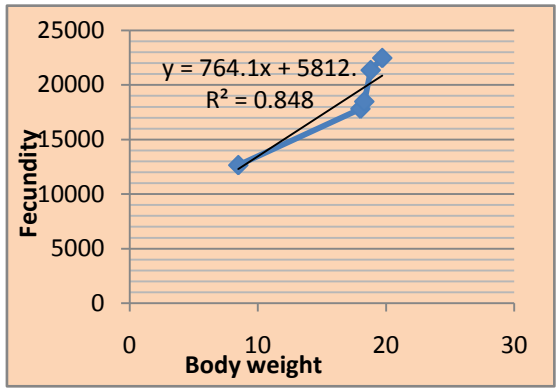


Fig. 15c: Correlation among fecundity and body weight of *B. almorhae*

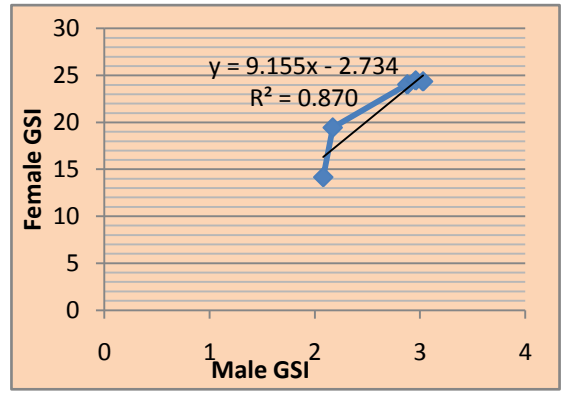


Fig. 15d: Correlation among female and male GSI of *B. almorhae*

4.5.2. Correlation study among growth parameters of *Botia dario*

The Coefficient of Correlation of *Botia dario* among gonad weight and body weight was 0.948, gonad length and body length was 0.410, fecundity and body weight was 0.676 and female and male Gonado-somatic Index was 0.99. The Coefficient of Correlation (r) among all relationships showed significance at $p \leq 0.01$.Correlation between the different parameters of *Botia dario*, Gonado-somatic Index of male and female was more significant than other parameters. The regression equation Y= 0.178X + 1.209 for body weight and gonad weight (**Fig. 16a**), Y= 0.208X – 1.377 for body length and gonad length (**Fig. 16b**), Y= 523.5X – 7806 for body weight and fecundity (**Fig. 16c**), Y= 3.613X + 0.655 for Gonado-somatic Index of male and female (**Fig. 16d**) showed a linear relationship by using the linear regression equation Y = a+ bx.

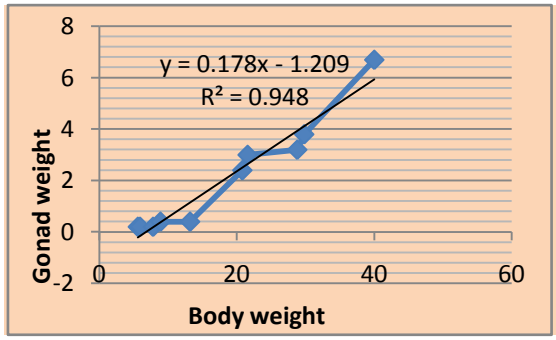


Fig. 16a: Correlation among gonad and body weight of *B. dario*

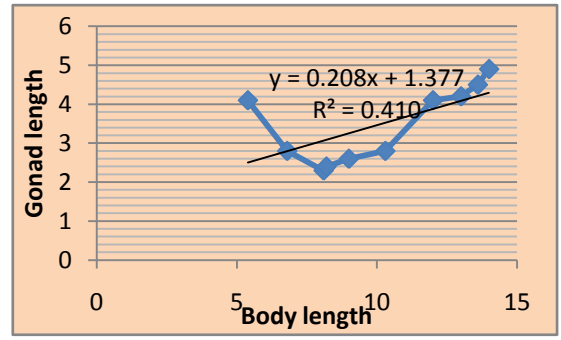


Fig. 16b: Correlation among gonad and body length of *B. dario*

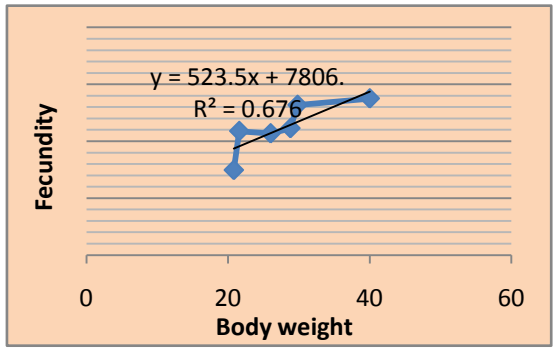


Fig. 16c: Correlation among fecundity and body weight of *B. dario*

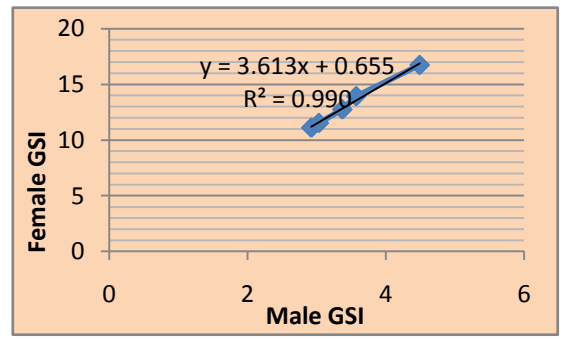


Fig. 16d: Correlation among female and male GSI of *B. dario*

4.5.3. Correlation study among growth parameters of *Botia lohachata*

The Coefficient of Correlation of *Botia lohachata* among gonad weight and body weight was 0.70, gonad length and body length was 0.865, fecundity and body weight was 0.832 and female and male Gonado-somatic Index was 0.949. The Coefficient of Correlation (r) among all relationship showed significance at $p \leq 0.01$.Correlation between the different parameters of *Botia lohachata*, Gonado-somatic Index of male and female was more significant than other parameter. The regression equation Y= 0.194X + 0.727 for body weight and gonad weight (**Fig. 17a**), Y= 0.386X – 0.754 for body length and gonad length (**Fig. 17b**), Y= 1237X – 1610 for body weight and fecundity (**Fig.17c**), Y= 3.434X + 15.48 for Gonado-somatic Index of male and female (**Fig.17d**) showed a linear relationship by using the linear regression equation Y = a+ bx.

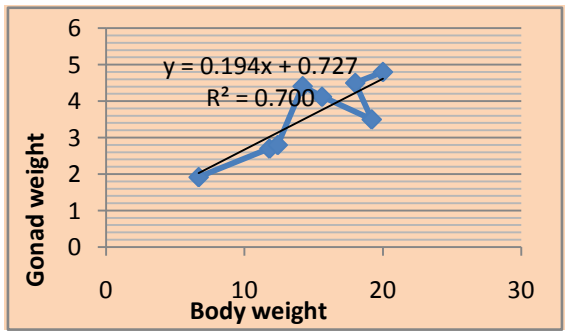


Fig. 17a: Correlation among gonad and body weight of *B. lohachata*

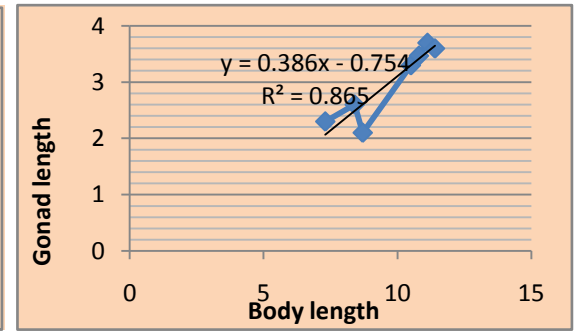


Fig. 17b: Correlation among gonad and body length of *B. lohachata*

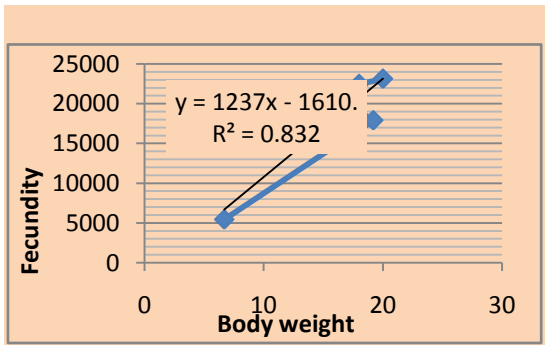


Fig. 17c: Correlation among fecundity and body weight of *B. lohachata*

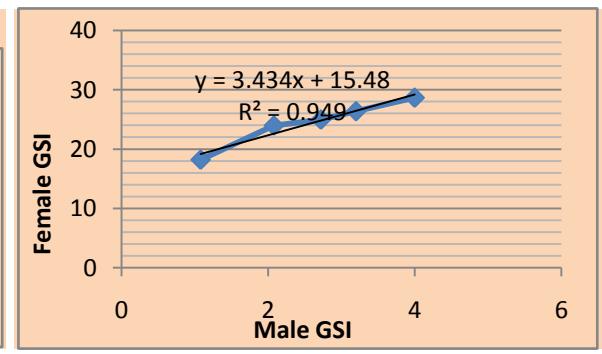


Fig. 17d: Correlation among female and male GSI of *B. lohachata*

4.5.4. Correlation study among growth parameters of *Botia rostrata*

The Coefficient of Correlation of *Botia rostrata* among gonad weight and body weight was 0.889, gonad length and body length was 0.949, fecundity and body weight was 0.956 and female and male Gonado-somatic Index was 0.817. The Coefficient of Correlation (r) among all relationship were showed significance at $p \leq 0.01$.Correlation between the different parameters of *Botia rostrata*, fecundity and body weight was more significant than other parameter. The regression equation Y= 0.284X + 2.032 for body weight and gonad weight (**Fig. 18a**), Y= 0.359X – 0.855 for body length and gonad length (**Fig. 18b**), Y= 1067X – 1498 for body weight and fecundity (**Fig. 18c**), Y= 10.04X + 0.420 for Gonado-somatic Index of male and female (**Fig. 18d**) showed a linear relationship by using the linear regression equation Y = a+ bx.

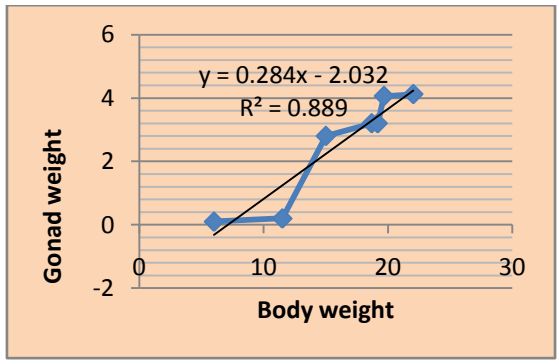


Fig.18a: Correlation among gonad and body weight of *B. rostrata*

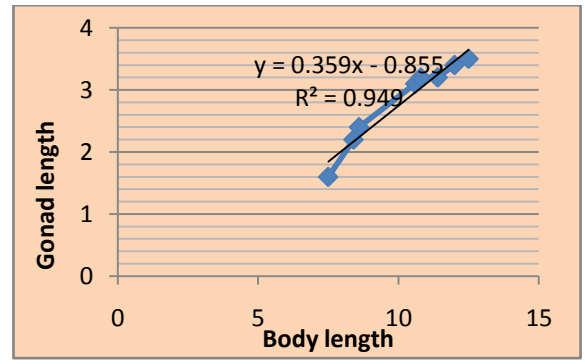


Fig. 18b: Correlation among gonad and body length of *B. rostrata*

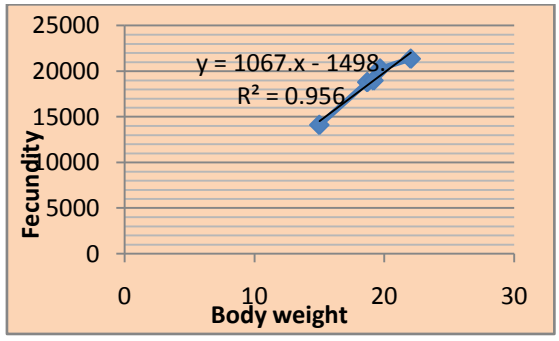


Fig. 18c: Correlation among fecundity and body weight of *B. rostrata*

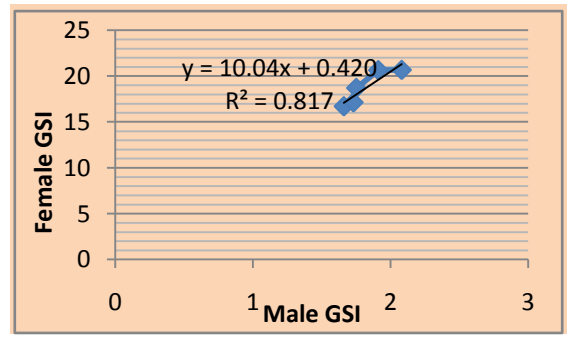


Fig. 18d: Correlation among female and male GSI of *B. rostrata*

4.6. ICHTHYOFAUNA DIVERSITY OF RIVER KALJANI

River Kaljani has the richest fish diversity among all other rivers of Cooch Behar district, originates from Gabaur Bachhra forest lying in the Bhutan ghat hills of Eastern Himalaya and outfalls into Shiltorsa in Cooch Behar district, West Bengal, India. Ornamental fish are dominant over the food fish in the Kaljani river.

About 138 fish species belonging to 31 families were recorded in the present study (**Tab.14**). As seen from **Fig. 19**, the most dominant fish families contributing to the study were Cyprinidae: 50 species and Sisoridae: 14 species. The less dominant family than Cyprinidae was Bagridae contributing 11 species and Cobitidae: 8 species. The families Belontiidae, Channidae, and Schilbeidae contributed 6 species. Mastacembelidae represented 4 species and Balitoridae, Badidae and Siluridae represented 3 species. Ambassidae, Amblycipitidae,Clupeidae and Notopteridae contributed 2 species.Other families Anabantidae, Anguillidae, Aplocheilidae. Belonidae, Chacidae, Clariidae, Engraulididae, Gobiidae, Heteropneustidae, Mugilidae, Nandidae, Ophichthidae, Pangasiidae, Synbranchidae, Syngnathidae and Tetradontidae all contributed 1 species each. Among the 138 species, 53 species had food value, 60 species ornamental value and 25 species both ornamental and food value **(Tab.14).** Therefore, in the present study, an attempt had been made to explore the available indigenous ornamental fish fauna including *Botia* species of River Kaljani, northern part of West Bengal. It was observed, ornamental fishes were dominant over the food fishes. All the three types of feeding habit of fishes like carnivorous, omnivorous and herbivorous were available in this region. About 97 species of fishes were carnivorous, 28 species were omnivorous and 13 species were herbivorous (**Tab.14**). According to IUCN (International Union for Conservation of Nature) and CAMP (Conservation Assessment

and Management Plan), the conservation status of the fishes listed are as, 1 species as Critically Endangered, 13 species as Endangered, 41 species as Vulnerable, 35 species as at Lower Risk Near Threatened, 41 species as Lower Risk Least Concerned, 4 species as Data Deficient and 3 species as Not Evaluated.

The evaluation of conservation status of the fishes and the results of the present study revealed that 25.36% of the fishes belonged to lower risk near threatened (LRnt); 29.71% vulnerable (VU); 29.71% lower risk least concern (LRlc); 2.17% not evaluated (NE); 9.42% endangered (EN); 0.72% critically endangered (CEN) and 2.89% data deficient (DD) category (**Fig-20)**. Month wise availability of fish species were high in the months of November (2012) to April (2013) and September (2013). Chhat Bhelakopa (Site -4) had the richest diversity than the other sites. *Pangasius pangasius* is a critically endangered species, found in this region. *Tenualosa toli* was also found at Chhat Bhelakopa (Site-4) only during monsoon. The above data showed that 11 species such as *Puntius ticto, Puntius sophore, Puntius conchonius, Puntius chola, Brilius barila, Barilius bendelisis, Cirrhinus mrigala, Mystus tengra, Channa punctatus, Mystus vittatus* and *Channa marulius* were abundant in the system and were collected from all locations throughout the year. The highest demandable ornamental species present are *Pseudambassis ranga, Chanda nama, Colisa lalia, Botia dario, Ctenops nobilis, Danio devario, Botia almorhae, Badis badis, Botia lohachata, Botia rostrata, Botia histrionic, Oreichthys casuatis, Oreichthys crenuchoides, Osteobrama cotio, Danio devario, Hara hara* and *Microphis deocata*. The present study showed that 20 endemic species were found in this region. According to **CAMP (1998),** India has 191 endemic species. Eastern Himalayan rivers represented many endemic species like *Badis badis, Badis bengalensis, Badis assamensis, Ctenop nobilis, Chaca chaca, Conta conta, Olyra longicaudata* and so on. The Eastern Himalayas is an area of considerable endemicity in

its freshwater ichthyofauna. Much of this endemicity stems from the presence of numerous hill stream species with highly restricted distributions for example, many members of the Balitoridae and Sisoridae. All known species of the genus *Aborichthys* are endemic to Brahmaputra drainage in northern Bengal, Meghalaya and Arunachal Pradesh.

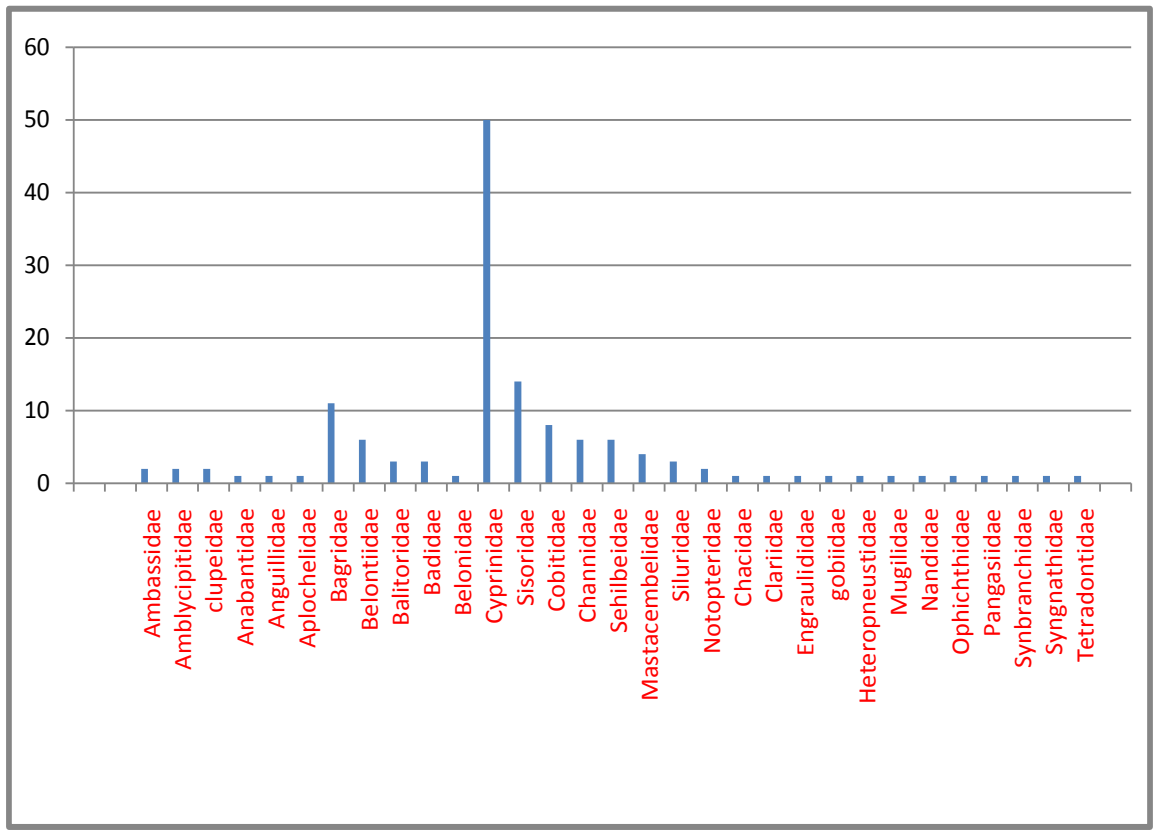


Fig. 19: Bar diagram showing the family wise distribution of fishes in the river Kaljani

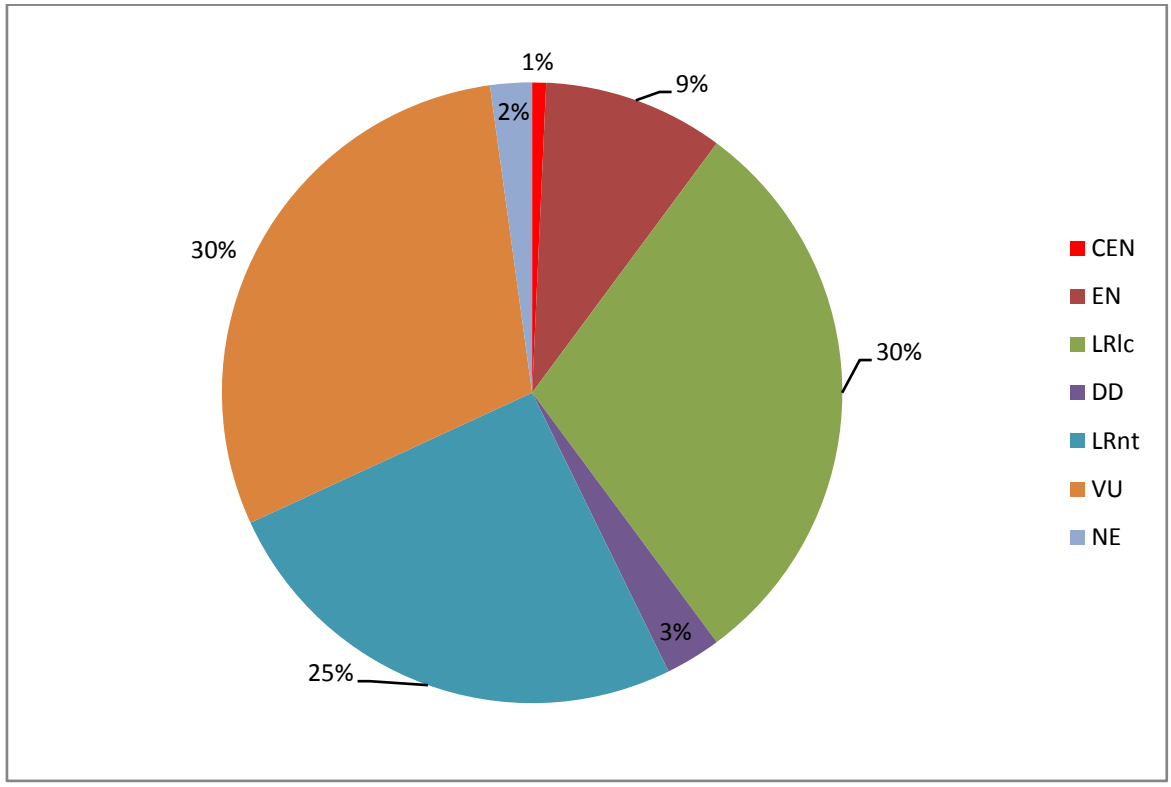


Fig. 20: Sector diagram showing the percentage of conservation status of fishes in river Kaljani recorded during the period 2012-14

Tab. 14: Ichthyofauna Diversity of River Kaljani in Cooch Behar District

Sl. No.	Scientific name	Family	Conser-vation status	Econ-omic value	Food habit
1	*Anabas testudineus* (Bloch)	Anabantidae	VU	Fd	C
2	*Pseudambassis ranga* (Hamilton-Buchanan)	Ambassidae	LRnt	Or	C
3	*Chanda nama* (Hamilton-Buchanan)	Ambassidae	LRnt	Or	C
4	*Amblyceps mangois* (Hamilton-Buchanan)	Amblycipitidae	EN	Or	C
5	*Amblyceps tuberculatum* (Linthoingambi and Vishwanath)	Amblycipitidae	LRlc	Or	C
6	*Anguilla bengalensis*(Gray)	Anguillidae	EN	Fd	O

7	*Aplocheilus panchax* (Hamilton)	Aplocheilidae	LRlc		O
8	*Mystus bleekeri* (Day)	Bagridae	VU	Fd/Or	C
9	*Mystus carcio* (Hamilton)	Bagridae	LRlc	Fd/Or	C
10	*Mystus cavasius* (Hamilton	Bagridae	LRnt	Fd/Or	O
11	*Mystus tengara* (Hamilton)	Bagridae	LRlc	Fd/Or	C
12	*Mystus gulio* (Hamilton)	Bagridae	LRlc	Fd/Or	C
13	*Mystus vittatus* (Bloch)	Bagridae	VU	Fd/Or	C
14	*Sperata aor* (Hamilton)	Bagridae	VU	Fd	C
15	*Sperata seenghala* (Sykes)	Bagridae	VU	Fd	C
16	*Batasio affinis* (Blyth)	Bagridae	LRnt	Fd/Or	C
17	*Rita rita* (Hamilton -Buchanan)	Bagridae	VU	Fd/Or	C
18	*Balitora brucei* (Gray)	Balitoridae	VU	Or	O
19	*Schistura fasciata*(Lokeshwar and Vishwanath)	Balitoridae	NE	Or	C
20	*Schistura tirapensis*(Kottelat)	Balitoridae	LRlc	Or	C
21	*Xenentodon cancila* (Hamilton)	Belonidae	LRlc	Or	C
22	*Badis assamensis* (Ahl)	Badidae	DD	Or	C
23	*Badis badis* (Hamilton)	Badidae	LRlc	Or	C
24	*Badis bengalensis* (Hamilton)	Badidae	LRlc	Or	C
25	*Ctenops nobilis* (McClelland)	Belontiidae	LRnt	Or	O
26	Colisa fasciatus (Schneider)	Belontiidae	LRnt	Or	C
27	*Colisa labiosus* (Day)	Belontiidae	LRlc	Or	C
28	*Colisa lalia* (Hamilton - Buchanan)	Belontiidae	LRlc	Or	C
29	*Colisa sota*(Hamilton-Buchanan)	Belontiidae	LRlc	Or	C
30	*Colisa chuna*(Hamilton)	Belontiidae	LRnt	Or	C
31	*Chaca chaca*(Hamilton-Buchanan)	Chacidae	EN	Or	C
32	*Channa striata* (Bloch)	Channidae	LRlc	Fd	C
33	*Channa bleheri* (Vierke)	Channidae	LRnt	Fd/Or	C
34	*Channa gachua* (Hamilton)	Channidae	LRlc	Fd/Or	C
35	*Channa marulius* (Hamilton)	Channidae	LRnt	Fd	C

36	*Channa punctatus* (Bloach)	Channidae	LRlc	Fd	C
37	*Channa barca*(Hamilton)	Channidae	DD	Fd/Or	C
38	*Clarius batrachus* (Linnaeaus)	Clariidae	VU	Fd	C
39	*Gudusia chapra*(Hamilton-Buchanan)	Clupeidae	EN	Fd	O
40	*Tenualosa toil* (Valenciennes)	Clupeidae	VU	Fd	C
41	*Botia dario* (Hamilton)	Cobitidae	VU	Fd/Or	C
42	*Botia rostrata* (Gunther)	Cobitidae	VU	Or	C
43	*Botia lohachata* (Chaudhuri)	Cobitidae	EN	Or	C
44	*Botia almorhae* (Grey)	Cobitidae	EN	Or	C
45	*Pangio pangio*(Hamilton)	Cobitidae	VU	Or	C
46	*Cantophrys gongota* (Hamilton)	Cobitidae	EN	Or	C
47	*Lepidocephalichthys berdmorei* (Blyth)	Cobitidae	LRlc	Or	C
48	*Lepidocephalichthys manipurensis* (Arunkumar)	Cobitidae	LRlc	Or	C
49	*Oreichthys casuatis*(Hamilton-Buchanan	Cyprinidae	LRlc	Or	C
50	*Oreichthys crenuchoides* (Schäfer)	Cyprinidae	DD	Or	C
51	*Chagunius chagunius*(Hamilton)	Cyprinidae	EN	Fd/Or	O
52	*Osteobrama belangeri* (Valencienes	Cyprinidae	LRnt	Fd	C
53	*Osteobrama cotio*(Hamilton	Cyprinidae	LRnt	Fd	C
54	*Tor putitora* (Hamilton)	Cyprinidae	EN	Fd	O
55	*Tor tor* (Hamilton)	Cyprinidae	EN	Fd	O
56	*Amblypharyngodon mola* (Hamilton-Buchanan)	Cyprinidae	LRlc	Fd/Or	H
57	*Cirrhinus reba* (Hamilton)	Cyprinidae	VU	Fd	O
58	*Crossocheilus burmanicus* (Hora)	Cyprinidae	VU	Fd	O
59	*Garra kempi* (Hora)	Cyprinidae	LRlc	Fd	H
60	*Garra gotyla* (Gray)	Cyprinidae	VU	Fd	H

61	*Garra lamta* (Hamilton)	Cyprinidae	LRlc	Fd	H
62	*Barilius barila* (Hamilton)	Cyprinidae	VU	Fd	O
63	*Barilius tileo* (Hamilton)	Cyprinidae	VU	Fd	O
64	*Barilius vagra* (Hamilton)	Cyprinidae	VU	Fd	O
65	*Barilius dogarsinghi* (Hora)	Cyprinidae	EN	Fd	O
66	*Barilius ngawa* (Vishwanath and Manojkumar)	Cyprinidae	LRlc	Fd	O
67	*Barilius bendelisis* (Hamilton)	Cyprinidae	VU	Fd	O
68	*Barilius barna* (Hamilton)	Cyprinidae	VU	Fd	O
69	*Aspidoparia morar* (Hamilton)	Cyprinidae	VU	Fd/Or	C
70	*Devario devario*(Hamilton)	Cyprinidae	LRnt	Or	C
71	*Devario assamensis* (Barman)	Cyprinidae	VU	Or	C
72	*Rasbora daniconius* (Hamilton)	Cyprinidae	LRlc	Or	C
73	*Rasbora rasbora* (Hamilton)	Cyprinidae	LRlc	Or	C
74	*Raiamas bola* (Hamilton)	Cyprinidae	VU	Fd/Or	C
75	*Salmophasia bacaila* (Hamilton)	Cyprinidae	LRnt	Fd/Or	C
76	*Psilorhynchus sucatio* (Hamilton)	Cyprinidae	LRlc	Or	O
77	*Psilorhynchus balitora* (Hamilton)	Cyprinidae	VU	Or	O
78	*Psilorhynchus homaloptera* (Hora and Mukherji)	Cyprinidae	VU	Fd	O
79	*Psilorhynchus brucei* (Gray)	Cyprinidae	LRnt	Or	O
80	*Schizothorax labialus* (McClelland and Griffith)	Cyprinidae	LRnt	Fd/Or	C
81	*Labeo rohita* (Hamilton - Buchanan)	Cyprinidae	LRnt	Fd	H
82	*Labeo calbasu* (Hamilton)	Cyprinidae	LRlc	Fd	H
83	*Labeo gonius* (Hamilton)	Cyprinidae	VU	Fd	H
84	*Labeo dyocheilus* (McClelland)	Cyprinidae	VU	Fd	H
85	*Labeo bata* (Hamilton)	Cyprinidae	LRnt	Fd	H
86	*Labeo boga* (Hamilton)	Cyprinidae	LRnt	Fd	H
87	*Labeo pangusia* (Hamilton)	Cyprinidae	LRnt	Fd	H

88	*Catla catla* (Hamilton-Buchanan)	Cyprinidae	VU	Fd	H
89	*Cirrhinus mrigala* (Hamilton-Buchanan)	Cyprinidae	LRnt	Fd	O
90	*Puntius chola* (Hamilton-Buchanan)	Cyprinidae	LRlc	Or	C
91	*Puntius conchonius* (Hamilton)	Cyprinidae	LRlc	Or	C
92	*Puntius phutunio* (Hamilton)	Cyprinidae	LRlc	Or	C
93	*Puntius sarana* (Hamilton)	Cyprinidae	VU	Fd	C
94	*Puntius sophore* (Hamilton)	Cyprinidae	LRnt	Or	C
95	*Puntius stolickanus* (Day)	Cyprinidae	LRlc	Or	C
96	*Puntius terio* (Hamilton)	Cyprinidae	LRnt	Or	C
97	*Puntius ticto* (Hamilton)	Cyprinidae	LRnt	Or	C
98	*Esomus danricus*(Hamilton-Buchanan)	Cyprinidae	LRlc	Or	O
99	*Setipinna phasa* (Hamilton-Buchanan)	Engraulididae	LRnt	Fd	C
100	*Glossogobius giuris* (Hamilton-Buchanan)	Gobiidae	LRnt	Fd	C
101	*Heteropneustes fossilis* (Bloch)	Heteropneustidae	VU	Fd	O
102	*Rhinomugil corsula* (Hamilton)	Mugilidae	VU	Fd/Or	H
103	*Macrognathus aral* (Bloch and Schneider)	Mastacembelidae	LRnt	Fd/Or	C
104	*Macrognathus morehensis* (Arunkumar and Tombi)	Mastacembelidae	LRlc	Fd/Or	C
105	*Macrognathus pancalus* (Hamilton)	Mastacembelidae	LRnt	Fd/Or	C
106	*Mastacembelus armatus* (Lacepede)	Mastacembelidae	LRlc	Fd/Or	C
107	*Nandus nandus* (Hamilton-Buchanan)	Nandidae	LRnt	Or	C
108	*Notopterus notopterus* (Pallas)	Notopteridae	EN	Fd	O

109	*Notopterus chitala* (Hamilton-Buchanan)	Notopteridae	EN	Fd	C
110	*Olyra longicaudata* (McClelland)	Bargridae	LRnt	Or	C
111	*Pisodonophis chilkensis* (Chaudhuri)	Ophichthidae	LRnt	Fd	C
112	*Pangasius pangasius* (Hamilton-Buchanan)	Pangasiidae	CNE	Fd	C
113	*Bagarius bagarius* (Hamilton)	Sisoridae	VU	Fd	C
114	*Gagata cenia* (Hamilton)	Sisoridae	LRnt	Fd/Or	C
115	*Gagata dolichonema* (He)	Sisoridae	LRlc	Fd/Or	C
116	*Hara hara* (Hamilton)	Sisoridae	LRlc	Or	C
117	*Hara Jerdoni* (Day)	Sisoridae	LRlc	Or	C
118	*Hara horai* (Misra)	Sisoridae	NE	Or	C
119	*Conta conta* (Hamilton-Buchanan)	Sisoridae	NE	Or	C
120	*Conta pectinata* (Ng)	Sisoridae	LRlc	Or	C
121	*Sisor barakensis* (Vishwanath and Darshan)	Sisoridae	VU	Or	C
122	*Sisor rhabdophorus* (Hamilton)	Sisoridae	LRlc	Or	C
123	*Sisor chennuah* (Ng and Lahkar)	Sisoridae	DD	Or	C
124	*Glyptothorax indicus* (Talwar)	Sisoridae	LRlc	Or	C
125	*Glyptothorax cavia* (Hamilton)	Sisoridae	LRlc	Or	C
126	*Glyptothorax telchitta* (Hamilton)	Sisoridae	LRlc	Or	C
127	*Ompok pabda* (Hamilton)	Siluridae	VU	Fd	C
128	*Ompok pabo* (Hamilton)	Siluridae	EN	Fd	C
129	*Wallago attu* (Schneider)	Siluridae	VU	Fd	C
130	*Neotropius atherinoides* (Bloach)	Schilbeidae	LRlc	Fd	C
131	*Ailia coila* (Hamilton)	Schilbeidae	VU	Fd	C
132	*Clupisoma garua* (Hamilton)	Schilbeidae	VU	Fd	C
133	*Clupisoma Montana* (Hora)	Schilbeidae	VU	Fd	C
134	*Eutropiichthys murius* (Hamilton)	Schilbeidae	LRnt	Fd	C
135	*Eutropiichthys vacha* (Hamilton)	Schilbeidae	VU	Fd	C

136	*Amphipnous cuchia* (Hamilton-Buchanan)	Synbranchidae	VU	Fd	C
137	*Microphis deocata* (Hamilton-Buchanan)	Syngnathidae	LRnt	Or	O
138	*Tetradon cutcutia* (Hamilton-Buchanan)	Tetradontidae	LRnt	Or	O

Note: Feeding habit: O= Omnivorous, C= Carnivorous, H=Herbivorous, **Economic importance:** Fd=Food fish, Or= Ornamental fish. **Conservation status:** According to IUCN (2010) and CAMP (1998), DD= Data deficient, NE= Not evaluated, VU= Vulnerable, EN= Endangered, CNE= Critically endangered, LRnt=Lower risk near threatened, LRlc=lower risk least concern.