DISCUSSION

5. DISCUSSION

5.1. WATER QUALITY

5.1.1 Temperature

Temperature controls fish growth and has profound effects on the chemistry, physical and biochemical reactions of aquatic organisms. No other factor has much influence as temperature as far as solubility of gas and salts in water is concerned (**Welch, 1952**). In the present study, the mean air temperature of the River Kaljani had 30.5^{0}C and in captivity at room temperature was 28^{0}C. This result was supported by similar finding of **Acharjee (2013**), where that highest air temperature recorded in hill stream river Relli was 32.3^{0}C and 32.4^{0}C for river Teesta.

Kaljani river water temperature ranged from 19^{0} C to 36^{0}C with a mean value of 28.45^{0}C in the present study. Whereas, in captivity it was 27^{0}C to 32^{0}C with a mean value of 29.54^{0}C. These findings were supported by **Dubey (1978),** who reported 8.5^{0}C to 26^{0}C from rivers of Bhutan Himalaya and 29.6^{0}C from Trishuli river in Nepal (**Thapa *et al.*, 2010**). **Barat and Jha (2002) and Bhadra *et al.*, (2003)** reported water temperature to range from 17.5^{0}C to 34 ^{0}C in the rivers Mahananda and Torsa; **Saha (2014)** 16.9^{0}C to 29.5^{0}C in Shutunga river at Cooch Behar district and 26.5^{0}C to 26.9^{0}C in Lotchka river in Darjeeling **Roy (2014)**. The optimum temperature required for growth of carp was reported to be 27^{0}C to 32^{0}C (**ICAR, 2006**). In the present study, the range of temperature was appropriate for the good growth of fishes. Good growth of fish was observed in summer season than winter season in the present study. For this reason in captivity, the water temperature was maintained from 27^0C to 32^0C in the winter season for broodstock development. This report was supported by **Weldermeriam (2013)** who reported that standard temperature for sustaining aquatic life varies between 28^0C to 30^0C. From the present investigation it was observed that water temperature increased when air temperature was high. This means that in summer season the water temperature increases and corroborates with the findings of **Wetzel (2001).**

5.1.2. pH (Hydrogen Ion Concentration)

pH measures the basic or acidic nature of water. Most natural waters are generally alkaline due to presence of sufficient quantity of carbonates. Average Hydrogen ion concentration (pH) of the Kaljani river water was 7.82. The highest value found during the rainy season from July to August 2014 was due to rainfall and low decomposition. (**Pandit *et al.*,2001).** They reported that pH is dependent on the amount of CO_2 and inversely proportional to the activity of photosynthesis**.** The mean value of pH in the captive condition water was 7.88. The pH value in captivity was constant due to strong buffering capacity of water and was found in both natural water system and captive water to be very approximate to the normal limit of 6.5 to 8.5 as suggested by the **BIS (1991**) and 7 to 8.5 by **WHO (1992).**

From the present investigation, it was also observed that Kaljani river water and laboratory water (Captive condition) were alkaline in nature. The higher value of pH (8.2) during rainy season may be due to the leaching of dolomite because, Kaljani river is connected with small streams arising from Buxa Reserve Forest. In captive condition, water pH was also high indicating that ground water also probably contained calcium carbonate. The present investigation agreed with the findings of **Renold *et al.* (1998),** who found 6.5 to 8.7 in the surface water of upper Khumbu region of Nepal; **Mondal *et al.*, (2011a** and **2011b**) and **Mozumder *et al.*, (2015)** observed alkaline pH in Torsa river, Kaljani river and Mahananda river water. In the present study, the pH showed positive correlation with hardness (r = 0.776, P<0.01) and negatively correlation with free CO_2 (r = -0.292,) and total alkalinity (r = -0.180) in Kaljani river, respectively. Similar relations have been observed by **Acharjee (2013)** in river Teesta**.**

5.1.3. Specific Conductivity

The range (110 μS cm^{-1} to 180 μS cm$^{-1)}$, mean value (151.27 ± 22.30) of specific conductivity of River Kaljani was lower than the range (240 μS cm^{-1} to 250 μS cm^{-1}), mean value (246.14± 3.18) of the captive condition water. The optimum level (110 to 250 μS cm^{-1}) of Specific Conductivity water bodies studied was found within the prescribed range (300 μS cm^{-1}) of **(BIS, 1991).** The Specific Conductivity, as observed was higher in captivity than river water. This could be due to higher value of dissolved solids, than ions in the water **(Bhatt *et al.*, 1999)**. The specific conductivity between captive and Kaljani river water had negatively correlation (r = -0.100, P<0.01). In the present study, specific conductivity was found to be higher during winter season which may be due to low flow rate of water and increased in dissolved solids. Low values of specific conductivity during rainy season may be due to high water level for rainfall. Similar observations were also recorded by **Thapa *et al.*, (2010)** from Nepal**; Mandal *et al.*, (2012); Acharjee (2013) Saha (2014)** and **Mozumder *et al.*, (2015)** from different rivers of northern region of West Bengal.

5.1.4. Total Dissolved Solids (TDS)

In natural waters, dissolved solids are composed mainly of carbonates, bicarbonates, chlorides, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium, iron and manganese. The present study showed that the average value of total dissolved solids in river Kaljani varied from 80 to 120 mg L^{-1} with mean value of 135 (± 31.96) mg L^{-1}. Total dissolved solids of breeding tank water were 250 to 260 mg L^{-1} with mean value 255 mg L^{-1} (± 3.97). The present study showed that the TDS of the sampling water ranged from 80 mg L^{-1} to 260 mg L^{-1} in the both the system of culture which was as per the **BSI (1991)** standard of 500 mg L^{-1}. Further, the TDS level was higher than the earlier workers like **Mondal *et al.*, (2011a** and **2011b**) and **Mozumder *et al.*, (2015)** who also worked on Torsa river, Kaljani river and Mahananda river water. Present investigation also corroborated with the observations of other workers like **Kumar *et al.*, (2014)** where TDS was 126.33 to 170.33 mg L^{-1} in Kali river; **Patela and Vaghanib (2015)** on Par River, Gujarat TDS (227 to 250.6 mg L^{-}) and **Baitule *et al*., (2015)** (240 to 430 mg L^{-}) in Nag River, Nagpur city.

5.1.5. Dissolved Oxygen

Dissolved oxygen (DO) plays a vital role for aquatic life, and oxygen content depends on many factors such as temperature, photosynthesis, presence of algae, decomposition activities, pollution and the level of aeration. In the present investigation, the average concentration of dissolved oxygen found in the Kaljani river water was 10.98 (± 0.914) which was quite adequate and characteristic of hill streams. 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. From the present study, dissolved oxygen was higher than the standard limit of DO (5.0 mg L^{-1})**.** According to **Swingle (1967)** in dissolved oxygen content ranging between 1.0 - 5.0 mg l^{-1} fishes can survive, but their reproduction is poor and growth is slow. **Mondal and Barat (2004)** also reported that dissolved oxygen concentration less than 5.0 mg l^{-1}, is not considered conducive for fish growth. The maximum DO (12.4 mg l^{-1}) found during winter in the present study, may be due to low temperature. The decreased water temperature during winter season has a greater capacity to hold DO than warm water and probably led to a lower rate of respiration thereby allowing maximum DO in winter **(Welch, 1952).** In the present study, DO showed negative correlation with water temperature (r = -0.765, P<0.01). Present investigation also corroborated with the observations of other works like **McColl (1972)** and **Acharjee (2013)** in their studies on New Zealand Lakes, Teesta and Relli respectively.

5.1.6. Free Carbon Dioxide

Carbon dioxide present in surface water in the form of carbonic acid is called Free Carbon dioxide. Carbon dioxide dissolveds in water more readily and the dissolution depends on temperature, pressure and mineral content of the water. The variation of CO_2 was due to the absorption by aquatic plants for photosynthesis and the activity of other living organisms. In the present study, the mean of Free Carbon dioxide concentration of river water was 3.76 mg L^{-1} (± 0.801) and breeding tank water was 6.67 mg L^{-1}(± 0.519). Lowest value of Free Carbon dioxide (2.3 mgL^{-1}) was found in July was probably due to high rain fall and highest value recorded in December may be due to high decomposition load. In the present study Free CO_2 showed negative correlation with pH (r = -0.292, P<0.01) in Kaljani river. **Dhanze *et al.* (1998)** observed negative correlation of Free CO2 with alkalinity; and **Acharjee (2013)** also observed negative correlation between Free CO_2 and pH in their study on Teesta and Relli.

5.1.7. Total Alkalinity

Alkalinity measures the buffering capacity of water and is caused by calcium carbonate and bicarbonate and also to some extent to phosphates and organic matter. Kaljani river water and aquarium water were medium productive as, total alkalinity ranged from 40 to 90 mg L^{-1}. **Jhingran (1991)** considered total alkalinity of 40 to 90 mg L^{-1} as medium productive. Kaljani river water showed bicarbonate alkalinity mostly whereas, Captive water showed carbonate alkalinity some timed as pH values were greater than 8.2 (**Trivedy and Goel, 1984). Barat and Jha, (2002**), observed bicarbonate alkalinity of river Mahananda and **Acharjee (2013)** observed it is river Teesta and Relli. In the present study, total alkalinity was positively correlation with pH ($r = 0.834$, $P<0.01$) in Kaljani river. **Dobriyal and Singh (1988), Trivedi (1988)** reported similar positive significant correlation of total alkalinity with pH.

5.1.8. Total Hardness

Total hardness of water is an important component to determine the suitability of water for domestic and industrial uses. Hardness is caused by multivalent metallic cations and certain anions present in the water. The principla hardness-causing cations are the divalent calcium, magnesium, strontium, ferrous iron and mangnous ions. Average value of total hardness of the river water was 24.0 mg L^{-1}(± 4.03) and the captive water was 27.29 mg L^{-1}(± 1.29). The results of total hardness indicated that water of both condition was soft (**Swingle, 1967).** According to **Boyd (1982),** normal fish culture needs at least 20 mg L^{-1} total hardness. The values of total hardness were soft in the present study and it agreed with the observations **Barat and Jha, (2002); Roy and Barat, (2011); Mandal *et al.*, (2011); Mandal *et al.*, (2012)** and **Acharjee, (2013).** The total hardness of water was positively correlation with pH ($r = 0.021$) and this result was supported by **Acharjee (2013)** in river Teesta and Relli.

5.1.9. Ammonium-N

The results of the ammonium-nitrogen (NH_4-N) revealed that in both the systems there was no toxic effect due to ammonia and suggested that the low concentrations may be due to the fact, that aquatic autotrophs rapidly utilize ammonium ions preferring these to nitrates; as such NH_4-N does not reach harmful concentrations. Sewage has large quantities of nitrogen matter, thus its disposal tends to increase the ammonia content of the water (**Trivedy and Goel, 1984**). Distribution of NH_4-N as found in the present study corroborates with the distribution of NH_4-N in unpolluted rivers as mentioned by **Wetzel (2001). Jha and Barat (2003)** recorded, NH_4-N concentration to vary from 0.006 to 0.072 mg L^{-1} in Mirik lake, **Thapa *et al.* (2010)** observed to haveNH_4-N ranged from nil to 0.013 mg L^{-1} in the Trishuli River of Nepal. The NH_4-N of water was negatively correlated with dissolved oxygen (r = -0.302) in the Kaljani river. **Jana and Barat (1984)** and **Acharjee (2013)** observed similar relations between NH_4-N and dissolved oxygen.

5.1.10. Nitrite-N.

Nitrite-N is a very unstable ion and gets converted into either ammonia or nitrate depending upon the conditions prevailing in the water (**Trivedy and Goel, 1984**). The range (0.001 mg L^{-1} to 0.027 mg L^{-1}), mean value (0.009 mg L^{-1} ± 0.110) of NO_2-N of river Kaljani was found slightly higher than the range (nil to 0.008 mg L^{-1}), mean value (0.003 mg L^{-1} ± 0.003) of the Captive water NO_2-N. In the present study toxicity due to Nitrite-nitrogen (NO_2-N) was negligible. **Barat and Jha, (2002)** reported NO_2-N level 0.002 to 0.030 mg L^{-1} in Mahananda river and again, **Jha and Barat (2003)** reported similar low values of NO_2-N ranged from 0.002 to 0.032 mg L^{-1}in Mirik lake. Range of NO_2-N in the two rivers (Torsha and Teesta) of northern part of West Bengal were 0.001 to 0.008 mg L^{-1} and 0.006 to 0.014 mg L^{-1} respectively (**Bhadra *et al.*, 2003; Acharjee, 2013).**

5.1.11. Nitrate–N

The nitrate-N is one of the most oxidisable forms of nitrogen and is essential for plant nutrient and also associated with sewage and sullage discharge (**Barat and Jha, 2002**). Mean nitrate-nitrogen concentration of the river water was 0.312 mg L^{-1} (± 0.220) and the Captive 0.215 mg L^{-1}(± 0.086). According to **Lester (1969)** Kaljani river water and captive water was very clean <0.5. The quantities of NO_3-N as found in the present investigation were slightly higher than the distribution of NO_3-N as mentioned by **Wetzel (2001)** in unpolluted rivers. In the present study nitrate- nitrogen was greater than ammonium-nitrogen and nitrite- nitrogen. So, water quality was good because nitrate-nitrogen is a good form of nitrogen as it can be returned to the nitrogen cycle quickly. Similar type of observation was done by **Bhadra *et al.*, (2003)** who reported NO_3-N level in river Torsha 0.090 to 2.200 mg L^{-1}. **Acharjee (2013)** reported NO_3-N level in Teesta to range from 0.032 to 0.062 mg L^{-1}. NO_2-N studied in the water of Lukha River, Meghalaya to vary from 2.14 to 12.81 mg L^{-1} (**Lamare and Singh, 2016).**

5.1.12. Phosphate-P

Phosphorus is an essential nutrient for the growth of organisms and helps in the primary productivity of a water body. The presence of phosphate in large quantities in fresh waters indicates pollution through sewage and industrial wastes. Phosphate-phosphorous (PO_4-P) concentration of the river water ranged (0.012 mg L^{-1} to 0.197 mg L^{-1}) with mean value 0.101 mg L^{-1}(± 0.060) and ranged (0.110 mg L^{-1} to 0.318 mg L^{-1})with mean value 0.172 mg L^{-1} (± 0.078) of captive water. PO_4-P concentration in the Kaljani river and Captive condition were normal level. According to **CPAB (2008),** the maximum permissible limit of phosphate is 5.0 mg l^{-1} for inland surface water. Similar observation had been made by different workers in different rivers of North Bengal like **Barat and Jha, (2002); Roy and Barat, (2011); Achajee,(2013); Saha, (2014)** and **Mozumder *et al.*, (2015).**

5.2. FISH BIOLOGY

5.2.1. Fish growth parameters

5.2. 1.1. Gonado-Somatic Index (GSI)

Gonado Somatic Index (GSI) is a reliable indicator of gonadal maturity; as the weight of the gonad increases with maturity when it spawns; there is a reduction in the weight of the gonad on account of the release of gametes (**Vlaming *et al.*, 1982**). Gonado-somatic index is particularly helpful in identifying days or seasons of spawning as the ovaries of gravid females rapidly increase in size just prior to spawning. The present study revealed that Gonado-somatic index increased from April and declined from September. The Index was higher in female than male of *Botia* species. The maximum value of GSI was observed during the spawning time of *Botia* species and this observation has been supported by **Bouain and Sian (1983)** who reported GSI was indicative of fish spawning in temperate and tropical regions. The average Gonado-somatic Index of *Botia* species were, *Botia almorhae* (11.96±10.29), *Botia dario* (8.34±5.4), *Botia lohachata* (13.86±11.50) and *Botia rostrata* (10.29±9.01). Among the *Botia* species *Botia lohachata* had the highest GSI and *Botia rostrata* had the lowest. GSI reduced after spawning of *Botia* species and this study was supported by **Vlaming *et al.* (1982).**

The Coefficient of Correlation between Gonado-somatic Index of male and female of *Botia* species were *Botia almorhae* (0.87), *Botia dario* (0.99), *Botia lohachata* (0.949) and *Botia rostrata* (0.817). The Coefficient of Correlation (r) among all relationship showed significance at $p \leq 0.01$ and positively correlated. Similar observations were made by **Dey *et al.*, (2015d** and **2015c)** on *Botia dario* and *Botia lohachata*.

From the present investigation, it was found that GSI increased with the increase of gonad size of *Botia* species. GSI values of both males and females followed more or less the same trend. The peak GSI value was found only in the breeding season and so it confirms that *Botia* species breeds only once a year. *Botia almorhae, Botia lohachata* and *Botia rostrata* spawned from June to August and *Botia dario* from May to July. Similar types of observations were made on ornamental fish by many authors like **Joshi and Pathani (2009**) on *Botia almorhae*; **Oliveira *et al.*, (2015)** on *Hemiramphus brasiliensis* from Brazil; **Dey *et al.*, (2015d, 2015c and 2015d**) on *Botia dario, Botia lohachata* and *Botia rostrata*; **Pal and Mahapatra (2016)** on *Amblypharyngodon mola* and **Dey *et al.*, (2016)** on *Barilius barila.*

5.2. 1.2. Condition Factor

Condition factor or K- factor or Ponderal index of fish is an Index used to monitor feeding intensity and growth rate (**Oni *et al.*, 1983**). From the present investigation, Condition Factor was for *Botia almorhae* (1.390), *Botia dario* (1.788.), *Botia lohachata* (1.538) and *Botia rostrata* (1.399). According to **Le Cren (1951)**, “K” > 1 indicates good general condition of fish. Therefore, in Captivity Condition Factor of *Botia* species was good. Among the four *Botia* loaches, *Botia dario* had the highest Condition Factor because, *Botia dario* was healthy or robust than the others fish. Fish with high value of ‘K’ are heavy for its length, while with low K are lighter **(Bagenal and Tesch, 1978)**. Similar type of work on Condition Factor was made by **Arockiaraj *et al.*, (2004**) on *Mystus montanus* (4 to 9); **Bindu (2006)** on *Etroplus suratensis* (2.29 to

3.2) and *Horabagrus brachysoma* (1.13 to 1.38) respectively; **Rahman *et al.*, (2016)** on *Mystus vittatus* (0.95 to 1.32) and **Lal *et al.*, (2016)** on *Mystus armatus* (0.22 to 2.84).

5.2. 1.3. Length-Weight Relationship

Length- Weight relationship gives us history and morphological comparisons between different fish species or between different fish by the Least-Square Method from logarithmic data. The association of degree between weight-length variables can be calculated by the determination of Coefficient of Correlation (r). The Coefficient of Correlation of *Botia almorhae* was 0.811; *Botia dario* 0.802; *Botia lohachata* 0.753 and *Botia rostrata* 0.936. The Coefficient of Correlation (r) of *Botia rostrata* was more significant than other species. The Coefficient of Correlation (r) showed significance at $p \leq 0.01$. 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**). When 'b' value was greater than 3, then the growth was a positive allometric growth. However, $b<3$ showed a negative allometric growth, or isometric growth when equal to 3.0.

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 higher than 3.0. These results suggested, that all species showed positive allometric growth and that the fish grew in proportion to the length in captive condition. **Beverton and Holt (1957)** stated, that serious departures from isometric growth (n= 3.0) are rare. In the present study, 'b' value of *Botia lohachata* was almost equal to 3. According to **Tesch (1971)** and **Wootton (1990)** 'b' value depends on many factors like habitat, degree of stomach fullness, gonadal maturity; sex and so on. Similar observations were made by authors like **Islam *et al.*, (2012)** on *Sillaginopsis panijus* Bangladesh; **Jan *et al.*, (2014)** on *Schizothorax plagiostomus* from Kashmir; **Rejitha and Pillai (2015)** on six coral reef fishes and **Dey *et al.*, (2016)** on *Barilius barila*.

5.2.2. Fecundity and fertilization rate

Absolute fecundity refers to the number of eggs produced per female per year (**Wootton, 1979**), and can also be defined as the number of mature Oocytes present in the ovary immediately before spawning (**Bagenal, 1963**). Fecundity is an important tool to understand the reproductive capacity of a fish species and in regulating the rate of reproduction to changing environmental condition. Population size of fish was also dependent on fertilization rate. The percentage of fertilization depended on the quality of brood stock.

Average fecundity of *Botia* species was *Botia almorhae* (18539 ± 3828), *Botia dario* (22573 ± 4949), *Botia lohachata* (18053 ± 7331) and *Botia rostrata* (18698 ± 2772). The average fertilization rate had *Botia almorhae* (90.03%), *Botia dario* (82.09 %.), *Botia lohachata* (95.98%) and *Botia rostrata* (67.60%).

In the present study it was observed, that among the four *Botia* loaches *Botia dario* had high fecundity and *Botia rostrata* the lowest fecundity **Dey *et al.*, (2015b, 2015c** and **2015d)**. It was also found that individual fecundity increased with body weight and length **Dey and Barat (2015)**. This finding was supported by workers like **Nikolsky(1969); Guar and Pathani (1996); Islam *et al.*, (2012)** and **Dey *et al.*, (2016).**

The Coefficient of Correlation of *Botia* species was between gonad weight and body weight, gonad length and body length and fecundity and body weight; *Botia almorhae* (0.819, 0.611 and 0.848); *Botia dario* (0.948, 0.410 and 0.676); *Botia lohachata* (0.70, 0.865 and 0.832) and *Botia rostrata* (0.889, 0.949 and 0.956) respectively.

All the Coefficient of Correlation (r) among all relationships of *Botia* species showed significance at $p \leq 0.01$. All correlation of *Botia* species showed that fecundity increased with the gonad weight, gonad length, body weight and body length. These findings were supported by **Simpson (1951); Bagenal (1957); Sarkar *et al*.,(2004)** and **Mahapatra *et.al*, (2004).**

The fertilized eggs were transparent and unfertilized ones were opaque and white. Fertilization rate of *Botia* loaches in captivity was high. Highest fertilization was observed in *Botia lohachata*.Similar type of captive breeding and fertilization rate were reported by **Kimmel *et al.(*1995)** on *Danio rerio*, **Udit *et al*. (2014)** on *Puntius sarana*, **Dey *et al*. (2014)** on *Devario aequipinnatus* and **Dey *et al*., (2016)** on *Barilius barila.*

5.2. 3. Captive breeding standardization

The loaches of *Botia* species are good candidate species for ornamental fish. They are near to the door of extinction due to indiscriminate fishing. Loaches do not breed spontaneously in captivity and as such breeding technique was developed with the help of synthetic hormone in captivity. Artificial breeding of wild spawners under controlled conditions is often implemented as a conservation method for endangered freshwater fish species, to produce fry for the enhancement or restoration stocking (**Philippart, 1995; Poncin and Philippart, 2002).**

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, with the best response to reproduction obtained from the dosage of WOVA-FH of 0.025 ml/ fish. Among the four different doses 3rd dose had appropriate for the captive breeding of *Botia* loaches. The higher fertilization, hatching and survival rates were found in fish injected with 0.025 ml/fish. Same dose of WOVA-FH (0.025 ml/fish) hormone was injected to both male and female.

Spawning was observed after 4-5 hours in Set-up-3, 7, 11 and 15 which fishes were injected 0.025 ml/ fish of WOVA-FH. The present study demonstrated the successful breeding of four species of genus *Botia* in captive condition with little dose 0.025ml/fish WOVA-FH (**Dey and Barat, 2015a).**

The present study revealed that flowing water was essential for induced spawning of *Botia species*. The spawning behaviour of *Botia species* was similar to the Indian Major Carps like flowing water systems. *Botia* species easily matured and bred successfully under captive condition **Dey and Barat (2015)** on *Botia almorhae*; **Dey *et al.*, (2015d, 2015c and 2015b)** on *Botia dario, Botia lohachata* and *Botia rostrata.*

The latency period is described as the time interval between injection of hormone on a female fish and starting the spawning (egg release by the female) of fish. In the present investigation, latency period of *Botia almorhae* was between 05.00 to 05.30 hours; for *Botia dario* it was 5 to 6 hours; *Botia lohachata* was 4 to 5 hours and in *Botia rostrata* was between 4.30 and 05.00 hours in fish injected with a dosage of 0.025ml WOVA-FH per fish respectively. It was observed that latency period of *Botia* species was short than other species. *Botia dario* was highest latency period than other three species. Similar types of finding was reported by **Udit *et al*., (2014**) on *Puntius sarana;* (**Purkayastha *et al*., 2012**) on *Ompok pabda* and **Dey *et al*.,(2016)** on *Barilius barila*.

According to **Kiyzhanovsky (1949),** spawning type of *Botia* species was Pelagophils (fishes which spawn freely in column of water and the eggs float). *Botia* loaches spawn once in a year (**Dey and Barat, 2015b; Dey** ***et al.*****, 2015d, 2015c and 2015b**). Similar type of captive breeding of ornamental fishes was reported by many authors *Danio rerio* **(Kimmel** ***et al.*****,1995);** *Macrognathus aculeatus* (**Das and Kalita, 2003**); *Etroplus suratensis* **(Bindu, 2006);** *Puntius gelius* (**Sarma,2008**); *Devario aequipinnatus (***Dey** ***et al*****., 2014**) and *Sahyadria denisonii* (**Sajeevan** ***and*** **Anna Mercy, 2016**).

5.2.4. Breeding behaviour study of fish

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 a loud cracking sound repeatedly. Six types of breeding behaviour were observed during spawning time like a) male hitting the female on snout, b) male hitting the female fish in the vent region more frequently, c) fighting between the males, d) male chasing the female, e) male and female fish were embraced together and swimming and f) Cannibalism behaviour. In the present investigation, no jumping activity on the surface, no nest building activity and did not showed any types of parental care of *Botia* species. Both male and female exhibited swimming movements in pairs, circling and pushing the female on the abdomen during courtship and similar type of study was done by **Guthric and Mutz (1993)** on *Brachydanio rerio*; **Kharbuli** ***et.al,*** **(2004)** on *Danio aequipinnatus;* **Angami (2012)** on *Danio dangila* and *Puntius chola*

The male initiates the motivation for courtship and in the process nudges the female with the snout and pushes the female upwards and then bends down and brings its genital pore in proximity with the female’s genital pore enticing and interlocking the female with the pelvic and anal fin. This study was supported by **Angami (2012).** Similar type of breeding behaviour was observed in other fishes by many workers like, **Hutchings *et al*., (1999)** on *Gadus morhua*; **Anna Mercy *et al*., (2003)** on *Pristolepis marginata;* **Chandran *et al*., (2013)** studied on breeding behaviour of *Pseudosphromenus cupanus;* **Paray *et al*., (2013)** on breeding behavior of the *Channa striatus* and **Behera *et al*., (2016)** observed pairing and chasing courtship behaviour of climbing perch, *Anabas testudineus.*

5.2.5. Embryonic development

The colour of fertilized eggs was whitish and transparent initially and then changed to creamy as the embryonic development proceeded. The embryonic development of *Botia* species were divided into eight stages-Zygote, Cleavage, Blastula, Gastrula, Segmentation, Pharyngula, Hatching and Early larval period **(Dey and Barat, 2015; Dey *et al*., 2015b; 2015c and 2015d).**

In the present study, egg incubation period ranged between *Botia almorhae* (15.30 and 16.00 hours), *Botia dario* (14.30 to 14.40 hours), *Botia lohachata* (14.00 to 14.30 hours) and *Botia rostrata* (15 to 15.30 hours). The incubation period was also lower than other species **(Dey and Barat, 2015**; **Dey *et al*., (2015b, 2015c and 2015d).**

The first cleavage occurred at 28 minutes (*Botia almorhae*), 25 minutes (*Botia dario*), 24 minutes (*Botia lohachata)* and 26 minutes (*Botia rostrata*) after the eggs were fertilized of *Botia* species. First cleavage formed in *Botia* species within 28 minutes and this development took less time than other ornamental fish. similar type of observation was made by **Kimmel *et al. (*1995)** after 40 minutes in *Danio rerio;* **Udit *et al*. (2014)** reported first cleavage occurred after 30 minutes in *Puntius sarana*, **Dey *et al.* (2014)**

after 45 min. in *Devario aequipinnatus* and Dey *et al.*, (2016) after 34 minutes in *Barilius barila*.

5.2.6. Supplementary feed for larval rearing of fish

According to **Nikolsky (1963),** juveniles of *Botia* species are "euryphagic" because they consume different types of food. At first larvae, *Paramecium* sp. were fed and then *Artemia* after 3days, the larvae consumed small sized zooplanktons of *Botia* species in captivity **Dey *et al.*, (2015b, 2015c and 2015d)** and **Dey and Barat (2015).**

In the present study, good growth was observed in Tank-D (only minced snail or bivalve flesh fed) than other experimental tanks where 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. Similar type of observation was reported on ornamental fish culture by **Karim and Hossain (1972)** on *Mastacembelus pancalus;* **Serajuddin and Mustafa (1994)** on *Mastacembelus armatus*; **Das and Kalita (2003)** on *Macrognathus aculeatus*; **Williot *et al.*, (2009)** on *Acipenser sturio;* **Rahman and Awal (2016)** on *Channa striatus*; **Ghosh *et al.*, (2016)** on Clownfish, *Amphiprion Clarkii*.

5.3. HISTOLOGICAL STUDY OF THE FISH GONADS

Immature ovary of *Botia* species are small, elongated, thread like and translucent without visible oocytes. Immature stage of ovary of *Botia* species was seen from February to March.

The pre-spawning phase or developing phase of ovary was found to be during March to May. Developing stage of testis of *Botia* species were found from April to May. 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.

The testes were milky whitish, long and flat, narrower behind, ribbon-like and increased in size. Spawning phase of testis of *Botia* species were found during May to September.

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 Post spawning stage of testis of *Botia* species were found from October to February. Histological features of post spawning stage of testis were presence of spermatogonia (SG). Spermatogonia(SG) and Residual spermatozoa(RSP). This finding was similar to (**Sanwal and Khanna 1972**).

In the present investigation it was found that gonad developmental stages were similar Indian major carp or minor carps.Present study was similar with other works done by **Roy and Manda (2015)** on *Labeo bata*; **Chakraborty and Choudhury (2015)** on *Notopterous notopterous;* **El-Nasr (2016)** on *Gerres filamentosus* from Hurghada Red Sea, Egypt; **Mahmud *et al.*, (2016)** on *Channa striata* from Bangladesh; **Silva *et al.*, (2016)** on *Cynoscion leiarchus;* and **Sales *et al.*, (2016)** on *Hypostomus francisci* from Brazil.

5.4. DNA BARCODING OF FISH

The blast search analyses of sequences were carried out for strengthening of the sequenced data. The phenotypical identification of the present studied species of *Botia* showed 100% similarity with same species sequence in Genbank. **Hebert *et al*., (2004)** proposed a concept; a short nucleotide sequence of mitochondrial genome will act as a DNA barcode of species identification of eukaryotic in particular animals. The technology has proven to be a rapid tool for precise identification of biological specimens. DNA barcoding works under the principle that interspecies variations are greater than the intraspecies variations allowing one to distinguish the species using nucleotide sequences. Six hundred fifty (650) nucleotide bases of 5 Cytochrome C oxidase sub – unit I gene (COI) have been accepted as universal barcode to delineate animal life in this planet. Identification of juveniles and immature stages of loach are very difficult using traditional taxonomic approach. Therefore, molecular phylogenies help resolve the taxonomic confusion of species.

In the present investigation, evolutionary distances among *Botia* genus 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*. 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* .

The nucleotide sequences of COI gene were aligned in order to determine the phylogenetic relationship among 6 species of *Botia*. 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 *B. kubotai*. Other Asian species, *B. rostrata, B. striata, B. dario B. modesta and B. macrocanthus* also contributed to this clade but are distant to native *Botia* species**.**

The Barcode ID number of four *Botia* species was **SDP657007-17** (*Botia almorhae*), **SDP657005-17** (*Botia dario*), **SDP657002-17** (*Botia lohachata*) and **SDP657006-17** (*Botia rostrata*). The present study thus highlighted the validity of DNA barcoding to differentiate the loaches at the species level and helped to understand the loaches in different reaches of rivers of Terai region of West Bengal.

DNA barcoding of fishes in different parts of the globe has gained momentum and is well established in Australia **(Ward *et al.*, 2005).** In Indian waters, similar types of findings were reported on barcoding by **Lakra *et al.*, (2009, 2011); Chandra *et al.*, (2012); Ambili *et al.*, (2014); Persis *et al.*, (2009); Ajmal *et al.*, (2010); Ajmal et al., (2011); Akbar *et al.*,(2010); Prasanna *et al.*,*(*2011) and Kannan *et al.*, (2014).**

5.5. ICHTHYOFAUNA DIVERSITY OF RIVER KALJANI

Kaljani river originating from Eastern Himalaya has the richest fish diversity among all other rivers of Cooch Behar district. The present study revealed, 138 fish species belonging to 31 families in the river **(Dey *et al.*, 2015a)**.

The most dominant fish families contributing to the study were Cyprinidae (50 species) and Sisoridae(14 species). The less dominant family than Cyprinidae were Bagridae (11 species) and Cobitidae (8 species). The families Belontiidae, Channidae, and Schilbeidae contributed to 6 species. Mastacembelidae represented 4 species and Balitoridae, Badidae and Siluridae represented 3 species each. Ambassidae, Amblycipitidae,Clupeidae and Notopteridae contributed 2 species whereas, 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, 55 species had food value, 58 species ornamental value and 25 species both ornamental and food value.

The evaluation of conservation status of the fishes and the results of the present study also revealed that 25.36% of the fishes belonged to the lower risk near threatened (LRnt), 29.71% to 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). 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,* a critically endangered species was found in this region. *Tenualosa toil,* the Chinese herring, was also found at Chhat Bhelakopa (Site-4) during monsoon only.

In the present study an attempt had been made to explore the available indigenous ornamental fish fauna of West Bengal. Ornamental fishes were dominant over the food fishes. All the three types of feeding habits of fishes namely carnivorous, omnivorous and herbivorous were available in this region. About 97 species of fishes are carnivorous, 28 species are omnivorous and 13 species are herbivorous fish.

Similar findings had been reported by **Ghosh and Lipton (1982)** where, 172 species were noted from North East India; **Choudhury (2005)** reported 297 fish species; **Goswami *et al.*, (2012)** recorded 422 species from North East India; **Mahapatra *et al.*, (2015)** reported 190 fish species from West Bengal; **Acharjee *et al*. (2013, 2014a and 2014b)** reported 65 species from Teesta river, 25 species from river Relli and 20 species of loaches from Darjeeling Himalaya; **Dey *et al.*, (2015f)** reported 141 species from the three districts of Eastern Himalayan region; **Dey and Sarkar (2015)** recorded 107 species from Torsa river; **Das (2015)** recorded 105 species from Torsha and **Debnath (2015)** recorded 73 species from Gadadhar river, Cooch Behar, respectively.