

SUMMARY AND CONCLUSION

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The present thesis entitled “**Breeding behaviour, embryonic development and barcoding of the ornamental loaches (Cobitidae: Cypriniformes) of Terai region of West Bengal, India**” comprises of 7 chapters (Introduction, Review of Literature, Materials and Methods, Results, Discussion, Summary and Conclusion and Bibliography) was executed for a period of three years (August 2012 to July 2015). With the following objectives of the study, 1) to estimate the limnochemistry of River Kaljani, 2) standardize breeding behaviour, 3) embryonic development, 4) captive breeding techniques and 5) barcoding of Endangered and Vulnerable *Botia* loaches. The following important findings of the thesis are presented:

- ❖ Four sampling area in the river Kaljani namely Amlaguri, Chhatoa, Jaigir Chilakhana and Chhat Bhelakopa were selected for collection of water samples and fishes. Physico-chemical parameters were studied in river Kaljani and Captivity (in aquariums) or Laboratory for rearing of *Botia* loaches.
- ❖ The average Physio-Chemical parameters of River Kaljani recorded were air temperature (30.5⁰C), water temperature (28.45⁰C), pH(7.82), Specific Conductivity (151.27 $\mu\text{S cm}^{-1}$), Total Dissolved Solids (135 mg L^{-1}), Dissolved Oxygen (10.98 mg L^{-1}), Free Carbon Dioxide (3.76 mg L^{-1}), Total Alkalinity (69.78 mg L^{-1}), Total Hardness (24.0 mg L^{-1}), Ammonium-nitrogen ($\text{NH}_4\text{-N}$) (0.017 mg L^{-1}), Nitrite-nitrogen ($\text{NO}_2\text{-N}$) (0.009 mg L^{-1}), Nitrate-nitrogen ($\text{NO}_3\text{-N}$) (0.312 mg L^{-1}) and Phosphate-phosphorous ($\text{PO}_4\text{-P}$) (0.101 mg L^{-1}).
- ❖ The mean of Laboratory (captive) Physico- Chemical parameters were air temperature (29.54⁰C), pH(7.88), Specific Conductivity (246.14 $\mu\text{S cm}^{-1}$), Total Dissolved Solids (255 mg L^{-1}), Dissolved Oxygen (6.3 to 7.6 mg L^{-1}), Free

carbon dioxide (6.67 mg L^{-1}), Total Alkalinity (54.68 mg L^{-1}), Total Hardness (27.29 mg L^{-1}), Ammonium-nitrogen ($\text{NH}_4\text{-N}$) (0.002 mg L^{-1}), Nitrite-nitrogen ($\text{NO}_2\text{-N}$) (0.003 mg L^{-1}), Nitrate-nitrogen ($\text{NO}_3\text{-N}$) (0.215 mg L^{-1}) and Phosphate-phosphorous ($\text{PO}_4\text{-P}$) (0.172 mg L^{-1}).

- ❖ In the present study, for both river Kaljani and Captive study water was soft, alkaline in nature with high Dissolved Oxygen and medium productive condition. The temperature was good for growth of the fishes of Kaljani river water and Laboratory water (Captive condition). TDS of the sampling water ranged from 80 mg L^{-1} to 260 mg L^{-1} which was within limits (**BSI, 1991**). Lowest value of Free Carbon Dioxide was found in July due to high rainfall and highest in December probably due to high decomposition load in river Kaljani. The average concentrations of ammonium-nitrogen (0.017 mg L^{-1}), nitrite-nitrogen (0.009 mg L^{-1}) and nitrate-nitrogen (0.312 mg L^{-1}) were negligible. Phosphate-phosphorous concentration in the Kaljani river and captive condition were also within normal range (0.101 mg L^{-1}).
- ❖ The average Gonado-somatic Index (GSI) data of *Botia* species revealed to be *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* the lowest. The Coefficient of Correlation (r) between Gonado-somatic Index of female and male of *Botia* species were, *Botia almorhae* (0.87), *Botia dario* (0.99), *Botia lohachata* (0.949) and *Botia rostrata* (0.817). The results showed significance at $p \leq 0.01$ and were positively correlated. It was further revealed that GSI increased with increase of the gonad size of *Botia* species. The peak GSI values were found only during breeding season. This confirmed that *Botia* species breeds only once a year. *Botia*

almorhae, *Botia lohachata*, *Botia rostrata* spawned from June to August whereas; in *Botia dario* it was from May to July.

- ❖ Condition Factor or K- factor in Captive condition for *Botia almorhae* was 1.390, *Botia dario* was 1.788., *Botia lohachata* was 1.538 and *Botia rostrata* was 1.399. The values indicated good general condition of fish as 'K' was greater than 1.0. Among the four *Botia* loaches *Botia dario* had highest Condition Factor and this was also revealed by its health condition (robustness).
- ❖ 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, and 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 $p \leq 0.01$. *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 show positive allometric growth and that the fish grows in proportion to the length in Captive condition.
- ❖ Average fecundity of *Botia almorhae*, *Botia dario*, *Botia lohachata* and *Botia rostrata* were 18539, 22573, 18053 and 18698 respectively and fertilization rate 90.03%, 82.09 %, 95.98 % and 67.60 % respectively
- ❖ In the present study it was observed that among the four *Botia* loaches *Botia dario* had high fecundity and *Botia rostrata* the lowest fecundity. It was found that individual fecundity increased with body weight and length.

- ❖ *Botia* loaches are near to the door of extinction due to indiscriminate fishing for its high ornamental value. These loaches do not breed spontaneously in captivity. Breeding technique was developed with the help of synthetic hormone in captivity. Four different doses of WOVA-FH hormone (0.5 ml/kg as 1st dose, 0.25 ml/kg as 2nd dose, 0.025ml/Fish as 3rd dose and 0.0125 ml/fish as 4th dose) were used, and the best response to reproduction was obtained from the dosage of WOVA-FH of 0.025 ml/ fish. The higher fertilization, hatching and survival rates were found in fish injected with 0.025 ml/fish in Set-ups- 3, 7, 11 and 15. Same dose of WOVA-FH hormone was injected to both male and female
- ❖ The latency period of *Botia* species were; *Botia almorhae* (05.00 to 05.30 hours), *Botia dario* (5 to 6 hours), *Botia lohachata* (4 to 5 hours) and *Botia rostrata* (4.30 and 05.00 hours) for fish injected with a dosage of 0.025ml WOVA-FH per fish. It was observed that latency period of *Botia* species was shorter than other species. *Botia dario* had the highest latency period than the other three species.
- ❖ 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 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 vent the region more frequently, c) fighting between the males, d) male chasing the female, e) male and female fish were embraced together and swam and f) Cannibalism behaviour.
- ❖ 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 was divided into eight stages-Zygote, Cleavage, Blastula, Gastrula, Segmentation, Pharyngula, Hatching and Early larval period.

In the present study, egg incubation period ranged as follows: *Botia almorhae* (15.30 to 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 the other species. The first cleavage occurred at 28 minutes for *Botia almorhae*, 25 minutes for *Botia dario*, 24 minutes for *Botia lohachata* and 26 minutes for *Botia rostrata* after the eggs of *Botia* species were fertilized. First cleavage formed in *Botia* species within 28 minutes and this development took less time than other ornamental fishes reported.

- ❖ At first, larvae were fed with *Paramecium* sp. then *Artemia* after 3 days. The larvae consumed small sized zooplanktons of *Botia* species in captivity. 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 with live zooplanktons and Tank-C with boiled minced meat. The growth rates were similar in Tank B and Tank C. Lowest growth rate was observed in Tank-A.
- ❖ After captive breeding of wild *Botia* species, F₁ generation of *Botia* loaches were aqua-ranched into the natural environment of the river system.
- ❖ The pre-spawning phase or developing phase of ovary was found to be during March to May. Developing stage of testis of *Botia* species was 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 was milky whitish, long and flat, narrower behind, ribbon-like and increased in size. Spawning phase of testis of *Botia* species was found during May to September. Post-spawning phase of ovary of *Botia almorhae*, *Botia lohachata* and *Botia rostrata* was 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 was found to be from October to February.

- ❖ 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*.
- ❖ 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.
- ❖ The present study permitted to study the Ichthyodiversity of river Kaljani, Cooch Behar district, West Bengal, 138 fish species were recorded which belonged to 31 families. The most dominant of the fish families contributing to the study was Cyprinidae: 50 species and Sisoridae: 14 species. The less dominant family than Cyprinidae was Bagridae contributing 11 species and Cobitidae: 8 species. 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 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, respectively. Month wise availability of fish species were high in the months of November (2012) to April (2013) and also 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. *Tenuulosa toil*, a Chinese herring, was also found at Chhat Bhelakopa (Site-4) only during monsoon. About 97 species of fishes are carnivorous, 28 species are omnivorous and 13 species are herbivorous.

It may be concluded from the present study, that Physico-Chemical parameters of River Kaljani in Cooch Behar district was not polluted and good for propagation of *Botia* loaches which are highly sensitive to temperature and water pollution. *Botia* species can be easily matured and bred successfully under captive conditions with the help of synthetic hormone WOVA-FH. The present study also 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. *Botia* species are near to the door of extinction due to indiscriminate fishing for its high ornamental value. So, establishment of proper sanctuaries in the selected areas of Terai and Dooars rivers, floodplain and reservoirs is recommended for conservation of this species. This thesis is useful for fish breeders, aquarium keepers and those involved in or interested in the study of indigenous fish.