Skewed sex ratio induced imperilment of Himalayan golden mahseer *Tor putitora:* a bottleneck for captive propagation

Abstract

Getting sufficient number of female *Tor putitora* in wild or in captive conditions is a bottleneck for its sustainable management. In this study, presence of 84.62% male and 15.38% female in the riverine environment was observed while 85.25% male and 14.75% female was found in the lacustrine environment.There was 78.12% male and 21.88% female population in the hatchery produced siblings. Further, *T. putitora* fry (30 dpf) when treated with 17βestradiol (150 mg/kg feed) for 30 days resulted into production of 69.5% female while rearing it at 23 ±1 °C without aby other treatment brought about41.5% females. The skewed sex-ratio and low female populations of *T. putitora* has been understood to be an important factor for the imperilment of Himalayan golden mahseer and its propagation in captivity.

Keywords: golden mahseer; sex ratio; sex-reversal; propagation; sustainability

Running head: Skewed sex ratio in *Tor putitora*

Introduction

Golden mahseer,*Tor putitora*, a flagship aquaculture species in the Himalayan region has a high demand for food, sports, and recreation. It is the king of the Himalayan fishes and bears high economic and ecological value. In the past forty years in India and trans-Himalayan countries, many studies have been conducted on the reproduction and seed production of golden mahseer for its rehabilitation and conservation (Sehgal, 1991; Shrestha, 2002; Sarma et al., 2016). Mahseer hatchery technology has now been developed and significantly improved yet captive maturation and brood raising arestill a big challenge.Breeding mahseer still relies on wild-collected gravid females from natural sources such as lakes, rivers, and reservoirs (Sarma et al. 2016; Akhtar et al., 2017; Akhtar et al., 2018). In addition,the captive development of female broodstock is still a constraintfor its propagation(Singh, 2007; Sarma et al., 2016). Recently, the issue of inducing gonadal maturity and spawning of female Himalayan golden mahseer in captive conditions through manipulations of environmental conditions, including temperaturehas been addressed and published (Akhtar et al., 2017,2018, 2020). In this study, the presence of a skewed sex ratioof *T. putitora* was studied in the riverine and lacustrine environments.

Material and methods

Data on sex ratio of wild captured mahseer,*Tor putitora* was generated from Ladhiya and Ramnagar river streams of Kumaon region and also from Bhagirathi, Mandakini and Nanakini river streams of Garhwal region of Uttarakhand. At the same time, sex ratio of T. putitora was also studied from different lakes of Kumao which were Bhimtal, Sat-tal and Naukuchiatal.Further, sex ratio of the hatchery produced by single female siblings was also studied.The methodology of studying the sex ratio in the river streams and lakes was followed as reported earlier (Singh, 2007; Singh & Kapila, 2007) while determination of male and female sex of golden mahseer was based on standard methods reported earlier (Singh, 2013; Singh & Singh, 2013).

Results

The results showed that 89.24% male and 10.76% female population was found in the Ladhiya river;there was 81.43% male and 18.57% female populationin the Ramnagar river of the Kumaon region. In the Garhwal region of the Uttarakhand state, 83.92% males and 16.08% females wereobserved in the Bhagirathi river; 83.64% males and 16.36% females in the Mandakini river; 84.87% males and 15.13% females in the Nandakini river. The average male and female population in the rivers of Kumaon and Garhwal was found 84.62 and 15.38% respectively (Fig. 1). The average male population in the lakes of Kumaon namely the Nainital, Bhimtal, Sat-tal and Naukuchiatal was further observed to dominate by males where it was 85.25% (Fig. 1). In the hatchery produced siblingsofsingle female, it was further observed that there was a 78.12% male population.

Discussion

Nowadays a variety of environmental factors e.g., water temperature, pH, salinity,photoperiod, and population density are recognised as highly responsible for phenotypic sex in fish (Devlin &Nagahama, 2002; Stelkens&Wedekind, 2010; Yamamoto et al., 2019). The low level of females in the riverine and lacustrine population of *T. putitora*and also at the hatchery level has been attributable to the environmental sex determination in this fish and this is how thesex ratio has deviated from the Mendelian sex ratioof 1:1 consequently an equal number of male and female fish are not available. The findings of this studyreport thatthe presence of available females dependson the environmental conditions. Freshwater *T. putitora* is an ectotherm and metabolically sensitive fish to environmental temperature (Akhtar et al. 2018). It is most likelythat climate-induced changes in reproductive physiology in *T. putitora*might be triggering the skewed sex ratiowhich causesits population tovary across geographic regions due to local adaptations of the fish.

Considering the problem of a skewed sex ratio, an attempt was made to produce all-females population of *T. putitora* using a hormonal sex reversal technique. Hatchery-produced fry (30 dpf) of*T. putitora*at ICAR Directorate of Coldwater Fisheries Research, Bhimtal was treated with 17βestradiol (150 mg/kg feed) for 30 days in 2x2m troughs in triplicate. The results of this study showed that the male predominant population (78.12%) was reversed into 69.5% female populations(Fig. 2). At the same time, these sex reversed fishes have shown improved performance of the fish for growth. The possibility of hormonal sex inversion for obtaining all-female population has also been achieved indirectly by integrating hormonal sex reversal with genetic engineering (Devlin and Nagahama, 2002; Singh, 2013). For this approach, the fry of *T. putitora* when treated with 17α-Methyltestosterone, sex reversed monosex male can be obtained. Such androgenised neo-males (XX) are when crossed with normal females (XX), all-female population have been achieved. The achieved sex reversed female *T. putitora* will definitely help reproductive management and stock improvement of this endangered fish. Karyomorphological studies in *T. putitora* has reported that the fish is gonochoristic and presents a simple heterogametic species where Y and X chromosomes are identified with the presence of XX:XY system of sex determination mechanism (Devlin and Nagahama, 2002).

Further, the effect of temperature on sex determination in *T. putitora*has also been studiedwhich showed that maintaining 30 dpf fry of *T.putitora* at 23±1°C in glass aquaria in triplicate five degreesabove the ambiancetemperature(control value) shifted skewed sex-ratio towards normal i.e., it was close to 1:1 sex-ratio. In this case, there was 41.5% female population observedas compared to the normal 27.88% female. The observation was significant (P>0.05) when compared with the control value.Since the temperature has been documented to be significantly modulated by the aromatase activity (Devlin and Nagahama, 2002; Singh, 2013; Singh and Singh, 2013), thermosensitive sex change in *T. putitora*has also been understood by the findings of this study. The thermosensitive gonadal sex differentiation as discovered in *T. putitora* from the results of this study corroborateswith an increasing number of reports on temperature-dependent sex determination in cyprinids (Devlin and Nagahama, 2002; Stelkens&Wedekind, 2010; Yamamoto et al. 2019).

In the light of findings of this study, the sex-determination mechanism in *T. putitora*isunderstood to be dependent on temperature (Devlin and Nagahama, 2002; Singh, 2013). It is also obvious that higher ambient temperature might down-regulate the sex-determining gene cyp19a1a for females, and thus may reverse the male sex-biased population (Singh 2013; Singh and Singh 2013). It is clear thatlowertemperature from the ambiancemay cause hypermethylation of *cyp19a1a* gene and suppresses its expression for femaleness and thus may resultin the production of more the male population (Devlin and Nagahama, 2002; Singh, 2013; Singh and Singh, 2013).Hormonal sex determination for feminization and androgenisation is well-documented (Devlin and Nagahama, 2002; Singh 2013). However, the insight into the sex genes that elucidate the mechanism of maintaining males and female phenotype of *T. putitora* is yet to be explored in detailto elucidate biased sex ratio in *T. putitora*.The findings strongly support the evidence of temperature-dependent sex determination in *T. putitora*. However, it is important to know how sex genes respond to the environmental stress and temperature,therefore, the transcriptomics profile of gonadal and brain tissue must be studied in detailto answer such questions like sex genes have been studied and reported in *Tor tambra* (Komwit et al. 2022).

Conclusion

In nature, Himalayan golden mahseer exhibited normally the presence of less than 20% female population which was significantly deviated from the expected Mendelian sex ratio of 1male:1female. The skewed sex ratio in *Tor putitora* has been confirmed owing to degrading aquatic environment particularly the climate induced changes in temperature. It was thus, temperature dependent sex determination in *T. putitora* has been confirmed. Nevertheless, it is suggested that transcriptomics profile of gonadal and brain tissue of *T. putitora* in relation to environmental stress and temperature must be studied in future to get a more information on sex determining genes.

References

Akhtar, M.S, Ciji, A., Sarma, D., Rajesh, M, Kamalam, B.S., Sharma P., Singh, A.K.(2017).Reproductive dysfunction in females of endangered golden mahseer (*Tor putitora*) in captivity. *Animal Reproduction Science*Appeared online 18 May, 2017

Akhtar, M.S., Rajesh, M., Ciji, A., Sharma, P., Kamalam, B.S., Patiyal, R.S., Singh, A.K.**,**Sarma, D. (2018). Photo-thermal manipulations induce captive maturation and spawning in endangered golden mahseer (*Tor putitora*): a silver-lining in the strangled conservation efforts of decades. *Aquaculture* 497: 336-347.

Akhtar, M.S., Rajesh, M., Kamalam, B.S., Ciji, A. (2020). Effect of photoperiod and temperature on indicators of immunity and wellbeing of endangered golden mahseer (*Tor putitora*) broodstock. *Journal of Thermal Biology* 93: 102694

Devlin, R.H. &Nagahama, Y. (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences, *Aquaculture*, 208 (3–4): 191-364.

Komwit, S., Deachamag, P., Wonglapsuwan, M., (2022). The first de novo genome assembly and sex marker identification of PluangChomphu fish (*Tor tambra*) from Southern Thailand.*Computational and Structural Biotechnology Journal* 20: 1470–1480

Sarma, D., Singh A.K.,Akhtar, M.S., (2016). *Mahseer in India: Resources, breeding, propagation, conservation, policies and issues.*Published by ICAR-Directorate of Coldwater Fisheries Research, Bhimtal (Nainital), India 63p. ISBN987-81-931230-0-3

Sehgal, K.L. (1999). Coldwater fish and fisheries in the Indian himalayas: rivers and streams. In *Fish and Fisheries of higher altitude Asia FAO Fisheries technical paper 385 FAo Rome*, pp. 41–63.

Shrestha, T.K. (2002). Ranching mahseer (*Tor tor* and *Tor putitora*) in running waters of Nepal. In *Coldwater Fisheries in Trans Himalayan countries, FAO Fisheries Technical paper 431*, pp. 297–300.

Singh, A.K., Kapila, R. (2007). Sex control in endangered upland golden mahsseer, *Tor putitora* (Hamilton 1822) for reproductive management. Proceedings of the 7th Indian Fisheries Forum (Eds.) C. Vasudevappa, Y. Basavraju, D. Seenappa, S. Ayyappan and S. Ravichandra Reddy. Asian Society of Fisheries, Indian Branch, Mangalore, ICAR, UAS(B) KVAFSU(B) India, pp 165–172.

Singh, A.K. (2013). Introduction of modern endocrine techniques for the production of monosex population of fishes. *General & Comparative Endocrinology* 181:146–155.

Singh A.K. and Ruchi Singh (2013). *In vivo* response of melatonin, gonadal activity and biochemical changes during aromatase inhibited sex reversal in common carp *Cyprinus carpio* (L). *Animal Reproduction Science* 136, 317- 325.

Stelkens, R.B., Wedekind, C. (2010). Environmental sex reversal, Trojan sex genes, and sex ratio adjustment: conditions and population consequences. *Molecular Ecology* 19: 627–646

Yamamoto, Y., Ricardo, S. Hattori., Reynaldo Patiño, Strüssmann CA., (2019). Chapter Two- Environmental regulation of sex determination in fishes: Insights from Atheriniformes, Editor(s): Blanche Capel, Current Topics in Developmental Biology, Academic Press, 134:49-69,

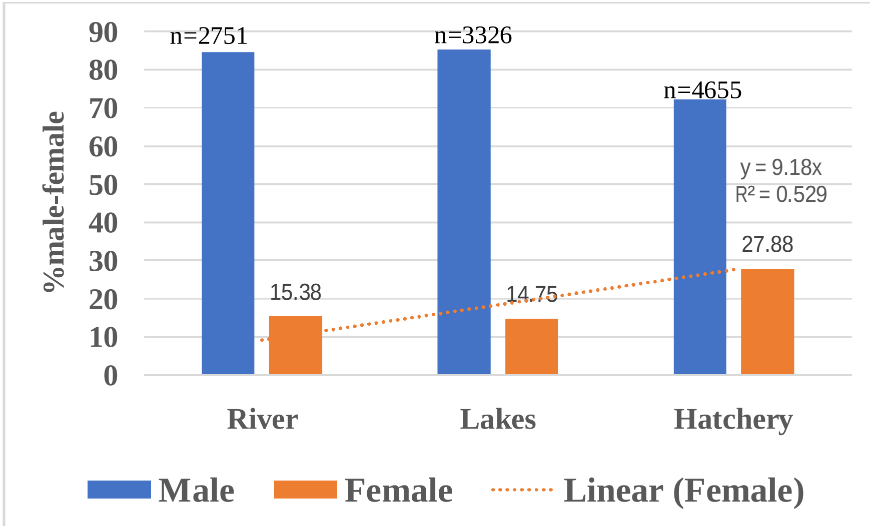

Figure 1: Average sex ratio of *Tor putitora* observed in river streams, lakes and Hatchery produced stock

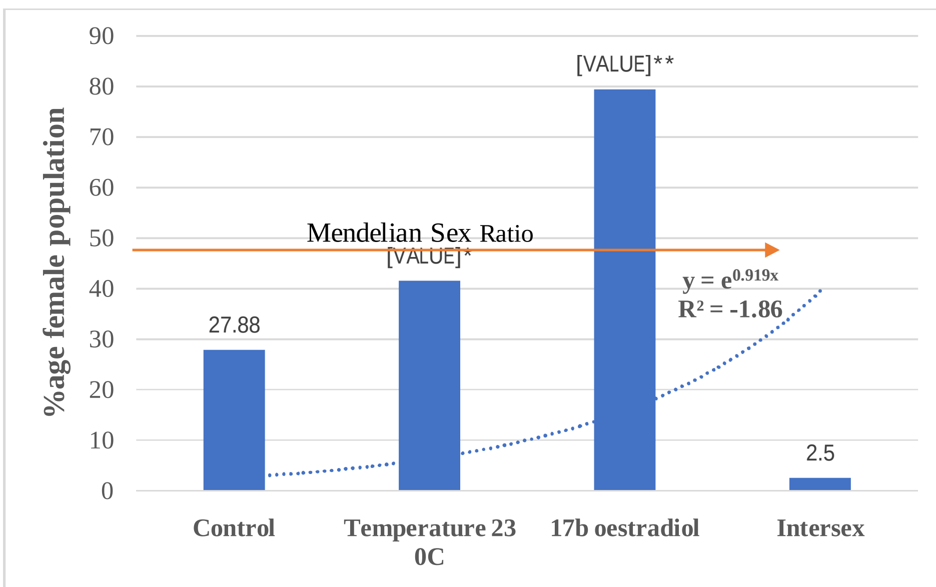

Figure 2: Oestradiol and temperature induced feminisation in *Tor putitora*(Significance level was $*p<0.05$ for temperature and $**p<0.01$ for 17b oestradiol as compared to the control)