Short communication

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 17β estradiol (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 about 41.5% 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

Introduction

Golden mahseer *Tor putitora* , a flagship aquaculture species in the Himalayan region has 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 has been conducted on 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 is still a big challenge. Breeding mahseer still relies on the 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, captive development of female brood stock is still a constrain for 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 temperature has been addressed and published (Akhtar et al., 2017; 2018; 2020).

In this study, presence of skewed sex ration of *T. putitora* was studied in the riverine and lacustrine environments. 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 population in the Ramnagar river of the Kumaon region. In Garhwal region of the Uttarakhand state, 83.92% males and 16.08% females was observed 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 (Figure 1). The average male population in the lakes of Kumaon namely the Nainital, Bhimtal, Sat-tal and Naukuchia tal was further observed to dominate by males where it was 85.25% (Figure 1). In the hatchery produced single female siblings, it was further observed that there was 78.12% male population. The methodology of studying sex ratio in the river streams and lakes was followed as documented earlier (Singh, 2007; Singh & Kapila, 2007) while determination of male and female sex of golden mahseer was done using standard methods reported in previous studies (Singh, 2013; Singh & Singh, 2013).

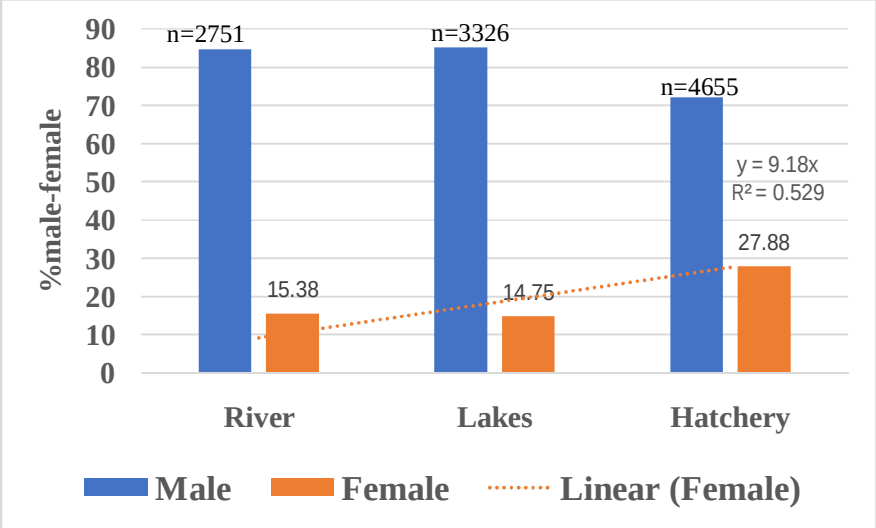

Figure 1: Average sex ratio of *Tor putitora* observed in river streams, lakes and Hatchery produced stock

Now a days a variety of environmental factors e.g., water temperature, pH, salinity, photoperiod, 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 the sex ratio has deviated from the Mendelian sex ration of 1:1 consequently equal number of male and female fish are not available. The findings of this study report that presence of available females depend on the environmental conditions. Freshwater *T. putitora* is ectotherm and metabolically sensitive fish to environmental temperature (Akhtar et al. 2018). It is most likely that climate induced changes in reproductive physiology in *T. putitora* might be triggering the skewed sex-ratio which causes its population to vary across geographic regions due to local adaptations of the fish.

Considering the problem of skewed sex ratio, an attempt was made to produce all-females population of *T. putitora* by using hormonal sex reversal technique. Hatchery produced fry (30 dpf) of *T. putitora* at ICAR Directorate of Coldwater Fisheries Research, Bhimtal was treated with 17β estradiol (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 (Figure 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 studied which showed that maintaining 30 dpf fry of *T.putitora* at 23±1°C in glass aquaria in triplicate five degree above the ambience temperature (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 observed as compared to the normal 27.88% female. The observation was significant ($p>0.05$) when compared with the control value. Since 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, corroborate with increasing number of reports on temperature dependent sex determination in cyprinids (Devlin and Nagahama, 2002; Stelkens & Wedekind, 2010; Yamamoto et al. 2019).

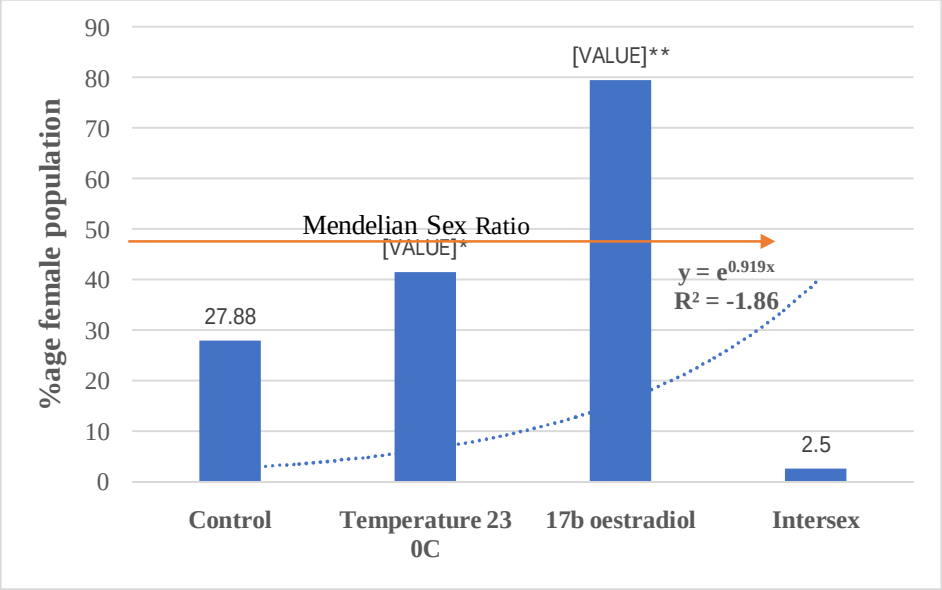

Figure 2: Oestradiol and temperature induced feminisation in *Tor putitora* (Significance level was *p<0.05 for temperature and **p<0.01 for 17b oestradiol as compared to the control)

In the light of findings of this study, sex determination mechanism in *T. putitora* is understood 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 female, and thus may reverse the male sex-biased population (Singh 2013; Singh and Singh 2013). It is clear that lower temperature from the ambience may cause hypermethylation of *cyp19a1a* gene and suppresses its expression for femaleness and thus may result in production of more number of 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 mechanism of maintaining males and female phenotype of *T. putitora* is yet to be explored in details to 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, transcriptomics profile of gonadal and brain tissue must be studied in details to answer such questions like sex genes have been studied and reported in *Tor tambra* (Komwit et al. 2022).

Statements and Declarations

Consent to participate - Not Applicable

Consent to publish - Not Applicable

Ethical Approval - the study did not require any ethics approval

Authors hereby confirm that the study does not require any ethics approval or grant an exemption based on institutional and national standards for the care and welfare of animals.

Data Availability Statement

The data that support this study are available in the article. Data sharing is not applicable as no new data were generated or analysed during this study.

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