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# Skewed Sex Ratio Induced Imperilment of Himalayan Golden Mahseer *Tor putitora*: A Bottleneck for Captive Propagation

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***Author's contribution***

*The sole author designed, analyzed, interpreted and prepared the manuscript.*

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## 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 were 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 any 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.*

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## 1. 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 [1-3]. Mahseer hatchery technology has now been developed and significantly improved yet captive maturation and brood raising are still a big challenge. Breeding mahseer still relies on wild-collected gravid females from natural sources such as lakes, rivers, and reservoirs [3-5]. In addition, the captive development of female broodstock is still a constraint for its propagation [6,3]. 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 [4,5,7]. In this study, the presence of a skewed sex ratio of *T. putitora* was studied in the riverine and lacustrine environments.

## 2. MATERIALS 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 Kumaon which were Bhimtal, Sat-tal and Naukuchia tal. Further, sex ratio of the hatchery produced single female siblings was also studied. The methodology of studying the sex ratio in the river streams and lakes was followed as reported earlier [6] while determination of male and female sex of golden mahseer was based on standard methods reported earlier [3,8].

## 3. 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 population in the Ramnagar river of the Kumaon region. In the Garhwal region of the Uttarakhand state, 83.92% males and 16.08% females were 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 (Fig. 1). The average male population in the lakes of Kumaon namely the Bhimtal, Sat-tal and Naukuchia tal was further observed to dominate by males where it was 85.25% (Fig. 1). In the hatchery produced siblings of single female, it was further observed that there was a 78.12% male population.

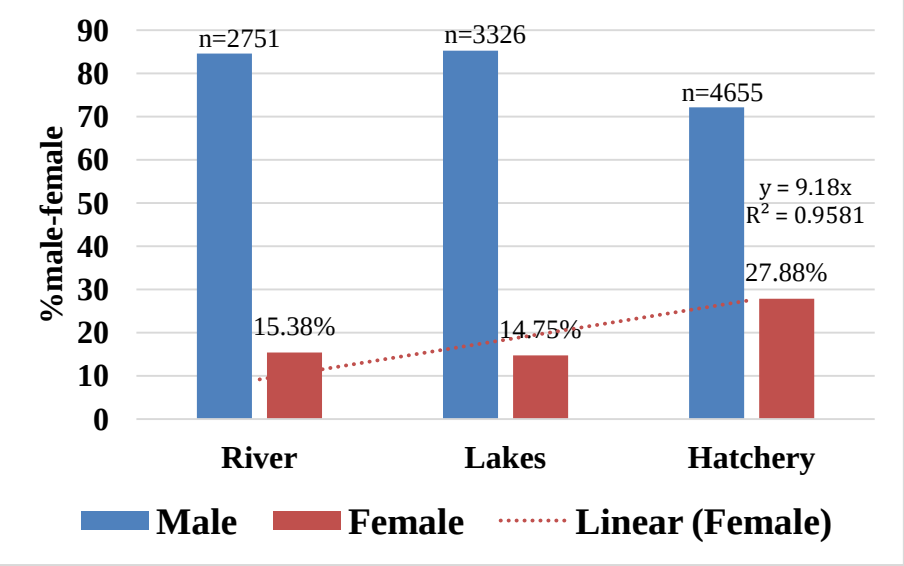


**Fig. 1. Average sex ratio of *Tor putitora* observed in river streams, lakes and hatchery produced stock**

## 4. 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 [9,10,11]. 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 that is why the sex ratio has deviated from the Mendelian sex ratio of 1:1 consequently an equal number of male and female fish are not available. The findings of this study indicatre that the presence of available females depends on the environmental conditions. Freshwater *T. putitora* is an ectotherm and metabolically sensitive fish to environmental temperature [5]. 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 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 [8,9]. For this approach, the fry of *T. putitora* when treated with 17⟨-methyltestosterone, sex reversed monosex male was obtained. Such androgenised neo-males (XX) were when crossed with normal females (XX), all-female population were achieved. The achieved sex reversed female of *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 [9].

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 degrees above the ambiance 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.

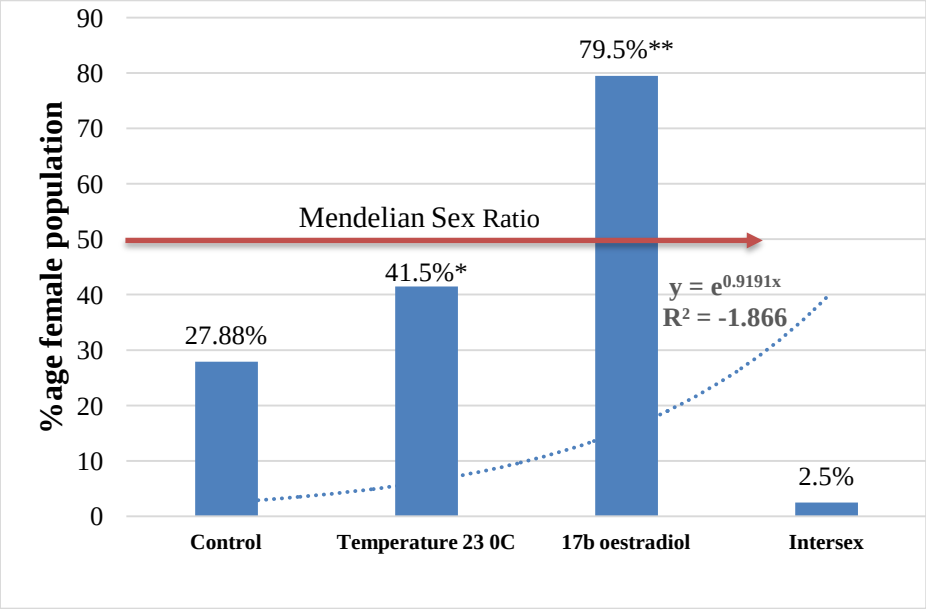


**Fig. 2. 17β-Oestradiol and temperature induced feminisation in *Tor putitora* (Significance level was *p<0.05 for temperature and **p<0.01 for 17β oestradiol as compared to the control)**

Since temperature is documented to significantly modulate aromatase activity [8,12,9], 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* corroborates with an increasing number of reports on temperature-dependent sex determination in cyprinids [9-11].

In the light of findings of this study, the sex-determination mechanism in *T. putitora* is clearly dependent on temperature [8,9]. It is also obvious that temperature above ambient might down-regulate the sex-determining gene cyp19a1a, and thus may reverse the male sex-biased population [8,12]. It is apparent that lower temperature from the ambiance may cause hypermethylation of *cyp19a1a* gene which suppresses its expression for femaleness resulting in the preponderance of male population [8,12,9]. Hormonal sex determination for feminization and androgenisation is well-documented [8,9]. However, the insight into the sex genes that elucidate the mechanism of maintaining males and female phenotype in *T. putitora* is yet to be explored in detail to elucidate biased sex ratio in *T. putitora*. The findings of this study 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 detail to answer such questions like sex genes that have been studied and reported in *Tor tambra* [13].

## 5. CONCLUSION

In nature, Himalayan golden mahseer exhibit normally the presence of less than 25% female population which significantly deviate from the expected Mendelian sex ratio of 1male:1female. The skewed sex ratio in *Tor putitora* observed in this study confirms that this deviation could be due to degrading aquatic environment particularly the climate induced changes in temperature. The findings of this study also suggest that temperature dependent sex determination exists in *T. putitora*. Nevertheless, it is proposed that transcriptomics profile of gonad and brain tissue of *T. putitora* in relation to environmental stress and temperature should be studied in future to get more detailed information on sex determining genes.

## COMPETING INTERESTS

Author declares that there exist no competing interests.

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