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Reproductive dysfunction in females of endangered golden mahseer (*Tor putitora*) in captivity

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ABSTRACT

The present study was undertaken to gain insight on the physiological basis underlying the constraints in attaining maturity of endangered golden mahseer (*Tor putitora*) in captivity. Selected hormone levels and stress biomarkers were analysed in wild and captive reared brooders to address the above objectives. As compared to their captive counterparts, plasma 17β-estradiol was significantly ($p < 0.05$) higher in wild caught females. A concurrent trend was observed for plasma vitellogenin, aromatase, 17α, 20β-dihydroxy progesterone (17α, 20β DHP), luteinizing hormone (LH) and11-ketotestosterone (11-KT) indicating a weak hormone response in captive females that potentially hindered maturity. To the contrary, the plasma11-KT levels were not significantly different between wild and captive males. Plasma 17α, 20β DHP level was found significantly ($p < 0.05$) higher in wild caught females compared to females reared in captivity. However, both males of wild and captivity registered significantly higher 17α, 20β DHP than captive females. Plasma 11-KT level was significantly higher in males compared to females. However, the captive females had higher level of 11-KT than captive males. Stress biomarkers *viz.,* cortisol, superoxide dismutase (SOD) and glutathione peroxidase (GPx) were also estimated both in wild and captive brooders. There was no significant difference in plasma cortisol levels of wild and captive reared brooders. However, plasma GPx and SOD activity were significantly higher in captive reared *T. putitora* as compared to wild brooders counterpart manifesting prevailing oxidative stress in captivity. Overall results showed endocrine and stress differences between wild and captive reared brood fishes during early spawning period which highlighted the endocrine failure of female reproductive maturity in captivity.

1. Introduction

The brain–pituitary–gonad axis regulates the reproductive function in vertebrates, including fish (Zohar, 1989; Imanagaa et al., 2014). External and internal signals are primarily integrated in the brain by the hypothalamic neurons producing the gonadotropin-releasing hormones (GnRHs) (Mylonas et al., 2010). The environmental triggers to stimulate GnRHs lead the synthesis and secretion of gonadotropins in the pituitary which are critical modulators of gonadal maturation through the mediation of gonadal steroids (Yaron et al., 2003; Zohar et al., 2010). Optimal function of the reproductive endocrine system is critical for fish to complete gonadal development, maturation and successful spawning (Guzmán et al., 2013). Under captive conditions, many fish species exhibit some level of reproductive dysfunction (Zohar, 1989; Zohar and Mylonas, 2001; Mylonas et al., 2010) and are more prevalent in female broodstocks. These reproductive dysfunctions can vary from inconsistent spawning as reported in salmonids to complete failure to undergo vitellogenesis as in Japanese eel and Mediterranean amberjack (Kagawa et al., 2005; Mylonas et al., 2010; Guzmán et al.,

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2013). The most common type of reproductive dysfunction involves the absence of final oocyte maturation, ovulation and hence spawning (Zohar and Mylonas, 2001; Mananos et al., 2009) which can be overcome by hormonal interventions. Generally, cyprinids reared under controlled conditions manifest reproductive dysfunctions leading to the inability to undergo final oocyte maturation and ovulation (Podhorec and Kouril, 2009). These dysfunctions most likely result from a combination of captivity-induced stress (Pankhurst and Van der Kraak, 1997) and lack of suitable natural spawning environment (Zohar, 1989; Ohta et al., 1997). A number of investigations advocate a negative effect of confinement associated stress directly on the gonadotropin-releasing hormone system (Guzmán et al., 2013; Zohar and Mylonas, 2001). Failure to spawn in captivity has also been reported to be linked with inhibited LH release from the pituitary (Mylonas et al., 1998) likely due to non conduscive environmental cues. Therefore, the impairment of brain-pituitary endocrine system under captive conditions may adversely affect the normal functioning of the reproductive process (Mylonas et al., 2010).

Golden mahseer (*Tor putitora),* is an important cyprinid commonly known as 'king of game fish' (Sarma et al., 2016b). It is considered as a cultural icon in its native distribution encircling the Trans-Himalayan region and it commands diverse economic, recreational and conservational value in Asian nations (Nautiyal, 1984; Langer et al., 2001; Akhtar et al., 2013a; Oliver et al., 2007; Dinesh et al., 2010; Akhtar et al., 2014; Sarma et al., 2016a). However, in recent past due to anthropogenic pressure, pollution, environmental degradation and indiscriminate fishing, the population of golden mahseer in the natural water bodies had declined alarmingly (Sehgal, 1999; Nautiyal, 1994; Oliver et al., 2007; Dinesh et al., 2010). Besides man-made constraints like habitat fragmentation and over-exploitation, the inherent factors like delayed maturity, low fecundity, long hatching period and slow growth rate are also responsible for the decline of golden mahseer population (Nautiyal, 1994; Singh, 2013). As a result, it is now identified as a critically endangered species (IUCN, 2014) as depletion of *T. putitora* populations has been reported from various parts of Asia (Hussain and Mazid 2001).

To enhance natural population of golden mahseer, investigations on developing protocols for captive propagation have been carried out in most of the Trans-Himalayan countries over a number of years to obtain better knowledge on spawning biology, ecological aspects and behaviour of *T. putitora* in its natural habitats (Nautiyal et al., 1994; Ingram et al., 2005; Sarma et al., 2010; Akhtar et al., 2013b; Sharma et al., 2016). This resulted in the development of seed production and hatchery technology of golden mahseer at ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal, India and elsewhere using gravid brooders collected from wild (Sarma et al., 2016a; Ogale, 2002; Sehgal, 1999; ShyamSunder et al., 1993) during the months of May-June and August-September coinciding its bi-modal breeding seasons (Sehgal, 1987). Breeding and seed production of golden mahseer at DCFR mahseer flow through hatchery is being done for over last 20 years using wild females collected from lakes and rivers which is proven as a non-sustainable practice. The non-availability of golden mahseer matured brooders particularly females in captivity has been playing a major bottleneck in its rehabilitation and conservation programme (personal observation). Like females of many other economically important fishes (Zohar, 1989), golden mahseer females fail to complete ovarian development and maturation when reared in captivity. To address this issue, induced spawning through administration of ovaprim (salmon GnRH with domperidone), has been attempted earlier with very limited success (Pandey et al., 1998) or no success (Sehgal, 1991). Conversely, Sehgal (1991) attempted induced spawning of golden mahseer under captive conditions several times but did not succeed.

So far, to the best of our knowledge, no study has been undertaken to understand the probable factors underlying failure of gonadal maturation of golden mahseer in captive conditions. Therefore, for the first time, comparative assessment of sex steroids and related indices together with stress biomarkers has been attempted in wild and captive reared golden mahseer to elucidate its reproductive dysfunction in captivity so that effective strategies could be made to develop brooders in captive conditions.

2. Materials and methods

2.1. Experimental fish and rearing

Wild mature golden mahseer brood fishes (n = 12 with 1:1 sex ratio) were caught live using sportfishing gear (hook and line) during early spawning season (first week of May) from Bhimtal lake (latitude 29°20′58.720″N – 29°20′11.536″N and longitude 79°33′15.447″E – 79°33′29.745″E) of mid-Himalayan region with the help of local fisherman. The captured fishes were kept in floating cages installed in the same lake to acclimatize for a period of one week. During the acclimation period, water temperature, pH and dissolved oxygen were in the range of 22.3–23.6 °C; 7.8–8.2; 6.2–7.2 mg/L respectively. The fish were fed with formulated diet containing 35% crude protein and 8% lipid twice daily @ 3% body weight. Among the wild captured brood fishes, six females (average weight: 873.45 ± 89.42 g, mean ± SE and 4^+ to 6^+ years age) and six males (average weight: 561.52 ± 69.31 g, mean ± SE and 3^+ to 5^+ years age) were used for the study.

Captive brooders of golden mahseer (*T. putitora*) were reared in earthen ponds of ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal, Uttarakhand, India. These brooders were raised from advanced fingerlings obtained from DCFR Mahseer Hatchery where seed production of golden mahseer is done every year. The ponds were supplied with freshwater from a bore-well and a slow flow was maintained to ensure optimum water quality during the rearing period. The water quality parameters such as water temperature, pH and dissolved oxygen were in the range of 23.5–24.1 °C; 7.63–8.76 and 6.7–8.2 mg/L respectively. The fish were fed daily *ad libitum* with dry feed pellets containing 35% crude protein and 8% lipid prepared at the feed mill of ICAR-DCFR. For the study, six females (average weight: 913.21 ± 76.34 g, mean ± SE and 4^+ to 6^+ years age) and six males (average weight: 587.46 ± 71.38 g, mean ± SE and 3^+ to 4^+ years age) were used. The age of the fish under study was determined by counting the annual growth rings (annuli) on scale following the method of Nikolsky (1963) using scale reader (Sipcon Profile Projector SP-300, Ambala Cantonment, India). The water quality parameters were analysed using multi-parameter water testing apparatus (Hach