Effects of Hatchery Rearing on Brain Structure, Aggression and Stress Hormones Levels of Mahseer (*Tor putitora*)

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Effects of Hatchery Rearing on Brain Structure, Aggression and Stress Hormones Levels of Mahseer (*Tor putitora*)

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By

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CERTIFICATE

This dissertation "Effects of Hatchery Rearing on Brain Structure, Aggression and Stress Hormone levels of Mahseer *(Tor putitora*" submitted by Mr. Shahzad Ahmad is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy in Fisheries and Aquaculture. .

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Declaration

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

SHAHZAD AHMAD

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SHAHZAD AHMAD

Abstract

ABSTRACT

Captive bred and reared fish are often used for conservation and replenishment of natural stock, but it is assumed that these fish are fraught with danger and shows low survival in a natural environment, may be due to behavior deficiencies. Therefore this study was designed to screen the possible influences of captive rearing environment on the growth of brains, hypothalamic-pituitary-interrenal (HPI) axis and aggression of mahseer *Tor putitora*. Eco-morphological and physiological approaches were used to investigate the possible impact. Adult hatchery reared *Tor putitora,* average weight 250-350 gm were purchased from Mahseer Hatchery Hattian, Attock whereas wild counterparts were captured from River Poonch and River Harro. They were kept for 15 days in circular tank under flow through system for acclimatization. After acclimatization, both wild and hatchery reared fishes were captured with dip net, immediately anesthetized and blood samples were collected from the caudal vein for the plasma cortisol and testosterone levels. After noting length, the brain of all these fish was dissected out for ecomorphological study. The area of the whole brain and the dorsally visible substructures (olfactory bulbs, OB; telencephalon, TE; optic tectum, OT and cerebellum, CE) were measured on the digital images using Image J 1.41. For comparing the stress response, both wild and captive reared mahseer which were held in circular tanks under flow through system were subjected to stress by continuous chasing for about 5 min. Experiment was conducted in replicate and water samples were collected before exposure to stress and at different interval of post stress. The results indicated the significantly ($P<0.0001$) small brain to body size ratio and reduced optic tectum in captive reared mahseer as compared to their wild counterpart. The cerebellum and telencephalon of captive reared mahseer were also comparatively reduced but statistically comparable to wild mahseer. The plasma cortisol level of wild mahseer after mild handling stress was also significantly ($P<0.0001$) higher in comparison to the status in captive reared mahseer. Furthermore, both populations showed considerable differences in pre-stress and post-stress water borne cortisol levels. The values were significantly higher in wild population as compared to captive reared mahseer. Wild mahseer showed typical stress response with rapid increase in cortisol and quick recovery (within 4 hrs) after exposure to stress whereas captive reared mahseer showed atypical stress response with gradual but continuous increase in cortisol even after the cessation of sampling (6 hrs). However, significantly ($P < 0.05$) higher plasma testosterone levels was observed in captive reared mahseer compared to wild counterpart

Introduction

INTRODUCTION

Freshwater fish, the main group of vertebrates worldwide are threatened with extinction (Gray *et al.*, 2011), thus creating an alarming situation that need urgent conservation measures. Multiple factors including habitat destruction, overexploitation and biological invasions are contributing in this menace (Gozlan *et al.*, 2005). Conservation of freshwater fish is a complex challenge that requires a combination of proactive strategies and supported on an ongoing basis (Dahanukar *et al.*, 2011; Dudgeon *et al.*, 2006). To make conservation measures successful, the political will of national and regional authorities and the participation of local communities is required (Kottelat *et al.*, 2012). Despite of fact, many of Asian counties have the largest accumulation of endemic freshwater fish (de Silva *et al.*, 2007), but many of these are threatened (Vishwanath *et al.*, 2011) and some probably are extinct (Raghavan and Ali, 2012 and 2013).

The genus *Tor* of Mahseer is the endemic cyprinids of mainland Asia, icons of culture economic interest, recreation and conservation in their natural range (Siraj *et al.*, 2007; Nguyen *et al.*, 2008). According to Stone (2007), Mahseer can accomplish a large size, therefore find a place among the 20 "mega fish" of the world and called the "tiger of the water" (Nautiyal, 2006) and considered as the hardest fighting fish in the world. There is no reliable estimate of the number of species of Tor but five (*T. khudri, T. kulkarni, T. malabaricus, T. mussullah* and *T. putitora*) are considered as "endangered" and two (*T. tor* and *T. progenius*) are consider as "nearly theatened" in red List of Theatened Species (IUCN, 2012; Qureshi, 2011; Jha and Rayamajhi, 2009; Rehman *et al.*, 2005). In 1976 National Commission on Agriculture (NCA) draw attention to the dilemma of the Mahseers and call for their conservation. A number of studies indicated that overhunting and change in habitat leads to ruthless decrease in population of different *Tor* species, as well as the golden Mahseer (*T. putitora)* and the *Tor* Mahseer (*Tor tor)* in the Himalayan Rivers (Bhatt *et al.*, 2000 and 2004).

From last few years, the natural population of *Tor* specie have shown a continuous declining trend due to multiple factors including change in natural environment due to natural disasters (prolong drought, floods, sedimentation and soil erosion of mountainous estuaries) and artificial changes (building of dams, overflow and waste water system, indiscriminate use of pesticide and discarding of industrial and agriculture waste, overexploitation and illegal fishing

methods). These factors result in drastic changes in water regime and not only ruined the breeding landscape but also caused havoc to the accessibility of broodfish including hatchlings, fry and fingerlings (Hussain and Hossain, 1999).

In recent years government of Pakistan has realized the economic importance of Mahseer, game as well as food fish and taken step for their conservation. It established hatcheries in the Punjab and Kpk for the artificial propagation of this species. Moreover, for replenishment of the river and other natural bodies, restocking program was also initiated that involve the release of artificially propagated hatchery reared fish in natural bodies. In spite of ample efforts, the results are not encouraging and captures of this species from natural reservoirs are continuously declining (Personal observation and communication with Punjab Fisheries). It seems that hatchery reared fish in the natural environment underwent mortality.

Animals including fish are bred and reared in captivity for various purposes including meat production, trade, laboratory use and replenishment of natural stock. Under captivity, fish are subjected to various aquaculture practices including capturing, crowding, handling etc, therefore growing concern about their welfare in captivity has been developed successively (Huntingford *et al.*, 2006). Generally, if intentional selection practices are ignored then artificial environments can be considered as 'extreme' forms of the natural environment. Typically, in captivity (at farm or hatcheries) stocking densities are comparatively very high while food is easily available (Siikavuopio *et al.*, 1996) the chance of predation is negligible whereas physical conditions are kept within the range that support high growth performance. Thus mass production of fish at farm or hatcheries involved the control or semi-control condition, therefore their environment is different from their natural habitat. Such sterile environments at hatcheries thus leave negative impact on the behavior or physiology of fish. Many scientists have reported the behavior (Jonsson *et al.* 2001), physiological (Zuberi *et al.*, 2011; Jonsson *et al.* 2001; Fleming *et al.* 2002) morphological (Näslund *et al.*, 2012; Vehanen and Huusko, 2011; Burns *et al.*, 2009; Kihslinger *et al.*, 2006) and ecological traits (Vehanen *et al.*, 2009) differences between various captive reared fish and their wild counterparts. Previously, it was thought that such difference may be due to unintentional selection pressures in the artificial environment (Price, 1999) but later on it was confirmed that in captivity, the effect of artificial rearing

environment resulted in altered phenotypic trait (Näslund *et al.*, 2012; Mayer *et al.*, 2011; Burns *et al.*, 2009; Kihslinger *et al.*, 2006)

The natural or artificial selection over generation effect the structure and size of the animals brain (Kihslinger *et al.*, 2006). Many investigators observed variation in brain size among domesticated and wild animals after many generation of artificial selection in captivity like Sheep (*Ovis aries*), Norwegian rats (*Rattus norvegicus*), cats (*Felis catus*) and pigs (*Sus scrofa*) generally have 8-33% smaller brains than their wild counterparts (McCarter, 2012; Diamond, 2002). The forebrain usually appeared smaller in size (Kruska, 1988). It is confirmed that in the captive environment, the variation in the brain size occurred due to genetic changes that is the end product of selection over several generations (Kihslinger *et al.*, 2006). In captivity, inbreeding depression, the pleiotropic effects of artificial selection, increased levels of reproduction and relaxed selection pressure are the main factors for effect the genetic makeup of an organism over several generations (Kihslinger *et al.*, 2006).

Fish is a vertebrate whose brain grow continuously thoughout their life (Kotrschal and Palzenberger, 1992) and show greater impact of rearing environment (Näslund *et al.*, 2012; Kihslinger *et al.*, 2006; Michael *et al.*, 2003; Marchetti and Nevitt, 2003). Although, developmental plasticity in fishes is considered as a possible contributing factor to neural phenotype, but the extent to which brain growth can be influenced by surrounding environment is mainly unidentified (Lema *et al.*, 2005; Hofman and Fernald, 2000). Majority of scientists reported the larger brain of wild fish as compared to their captive counterparts (e.g. *Poecilia reticulata*, Burns *et al.*, 2009; Michael *et al.*, 2003; *Oncorhynchus tshawytscha*, Kihslinger *et al.*, 2006).

Generally, animals live in enrich environment have larger brain structures, higher neuron generation rates and learning capacity as compare to animal having un-enrich place (Van Praag *et al.*, 2000). Usually, what make environment enrich is a species specific term because every species is unique in its requirement for optimum performance but generally it is related to environment that is complex in term of structural variability, foraging opportunities and social interaction and provide an individual greater opportunity to learn, explore and interact with other inter-specific and intra-specific animals (Newberry, 1995).

In fish, brain morphology is plastic, therefore enrich environment effect the development of central nervous system not only in early stages when neural and body development take place but also show effect in the later stages of life (Kolb and Whishaw, 1998; Rosenzweig and Bennett, 1996). These effects include neurogenesis i.e., increase in number and size of neurons, dendrite branching, cortical thickness, increase in weight and volume of brain (Kolb and Whishaw, 1998). Thus, it is suggested that this environmental impact on brain development in teleost, greatly influence the behavior of fish and choice of an individual to find living place in adulthood (Zaunreiter *et al.*, 1991; Kotrschal and Palzenberger, 1992).

Majority of fish, as compare to most other vertebrates, shows indeterminate somatic growth that also appears in the central neural system (Kotrschal *et al.*, 1998). Fish do not seem to have final morphology of the adult brain. The brain structures may be less limited and may be affected by spatial and temporal constraint (Kotrschal *et al.*, 1998). Moreover, cranium of fish generally allows larger brain volumes (except perhaps in younger individuals and smaller species) and significant portion of the available space is often filled with fat lymphatic tissue (Kotrschal *et al.*, 1998).

Generally, it is considered that cognitive ability is related to the overall brain size of an animal (Bshary *et al.*, 2002; Lefebvre and Sol, 2008; Ito *et al.*, 2007), but this hypothesis need validation (Healy and Rowe, 2007). According to Shumway (2008), in fish although different substructures of brain are multifunctional to some extent, but their overall size normally related to its ecological niche. Hence, the sizes of a various substructures normally reflect the relative importance of the senses or behavioral traits that it regulates (Ito *et al.* 2007). Many investigators find a relationship between size of particular area of brain and their function within and between the species and suggested that a small change in size can show pronounced effect (Gonzalez-Voyer and Kolm, 2010; Kolm *et al.*, 2009). Many interspecific studies showed how selection pressure effect the evolution of brain with respect to fish size (Pollen *et al.*, 2007; Gonzalez-Voyer *et al.*, 2009) and observed positive correlation among the complexity of habitat and telencephalon size in sympatric Ectodine cichlids (Pollen *et al.*, 2007). Moreover, intraspecific studies reported the changes in brain size with respect to social and sexual behavior (Kolm *et al.*, 2009; Gonda *et al.*, 2009).

The relationship between behavior and brain size is extensively studied in birds and mammals (Lefebvre and Sol, 2008) as compared to fish. However, in some fish scientists find out the correlation between the size of different substructure of brain and behavior (Wilson and McLaughlin, 2010; Park and Bell, 2010; Burns *et al.*, 2008). Fish as compare to mammals and birds show continuous growth of body and the throughout adulthood (Zupanc 2006). This indeterminate growth in fish could allow plasticity in brain development that is not possible in taxa where determinate growth is prominent. Thus, environmental deprivation in early life stages in fish leads to smaller brain size and resulted in behavior deficiencies, but environmental enrichment can compensate these deficits.

In captivity, environmental enrichment is considered as a means to improve the biological functioning of animals (Brown and Day, 2002). It increases neurogenesis in rodents and less stereotypic behavior in a large number of vertebrates (Shyne, 2006; Van Praag *et al.*, 2000). Whilst in fish, several researchers find relationship between neural, behavioral, and cognitive effects (Spence *et al.*, 2011; Kihslinger *et al.*, 2006; Braithwaite and Salvanes, 2005). Conversely, some scientist could not observed these correlation and suggested that the enrichment effect may be vague or lacking (Brockmark *et al.*, 2010; Brydges and Braithwaite, 2009), while according to Fairhurst *et al.* (2011) certain enrich environment may cause distress. These discrepancies in results explain the species specific effect of environmental enrichment. Scientist used different species and range of enrichment in different studies. Moreover, when sample size is small and cognitive challenges are rare then enrichment effects cannot be detected. However, beside all these discrepancies, environmental enrichment in captivity is still a promising method for modulating more natural behavior of fish (Rodewald *et al.*, 2011; Roberts *et al.*, 2011; Salvanes and Braithwaite, 2005).

In addition to brain size, many other phenotypic traits also differ among wild and captive reared individuals. These differences include change in feeding and sexual behaviors, growth rate, timing of sexual maturity and anti-predator behavior (Hard *et al.*, 2000; Flagg *et al.*, 2000; Gross, 1998; Fleming *et al.*, 1997). According to Jonsson *et al.* (2003), these differences may possibly effect the survival of hatchery reared fish upon released into wild. Therefore, production of fishes in hatcheries for fisheries or conservation purpose is fraught with danger

(Daniels and Watanabe, 2010). They show low survival rates and present lower return to anglers than wild counterpart (El Balaa and Blouin-demers, 2011; Ebner and Thiem, 2009).

Many researchers used the simple eco-morphological and physiological approach to screen for possible influence of hatchery rearing practice on the brain and hypothalamic-pituitary-interrenal (HPI) axis (Zuberi *et al.*, 2011; Breves and Specker, 2005; Marchetti and Nevitt, 2003) whereas some studies revealed that brain structure often reflect the manner in which a species has adapted to particular environment or selection regime (Vargas *et al* ., 2000; Ishikawa *et al.*, 1999; Kotrschal *et al.*, 1998; Huber *et al.*, 1997). Marchetti and Nevitt (2003) has reported that hatchery rearing practices influence the growth and development of brain sub structures i.e optic tectum and telencephalon, area that are often linked to aggression , feeding behavior , cognitive behavior and reproduction in fish (Vonderschen, 2002).

Wild and hatchery-reared fish of the same stock may respond differently to artificial fish culture conditions. Norman (1987) showed that hatchery stocks of Atlantic salmon (*Salmo salar*) are less aggressive than wild stocks while Maisse *et al.* (1983) observed that hybrid brown trout of wild male and domestic female parents are more susceptible to costia (*Costia necatrix*) than wild fish of the female strain. Genetic differences between hatchery and wild steelhead trout (*Oncorhynchus mykiss*) were demonstrated by Reissenbichler and McIntyre (1977) where hatchery fish had the highest survival and growth rates than their wild counterpart. Chilcote *et al.* (1986) furthermore, showed that the reproductive success rate of hatchery steelhead trout was only 28% that of wild fish. These studies suggest that differences may exist between hatchery and wild fish of the same stock.

The natural habitat is generally very demanding and wild fish are persistently provoked to a number of latent stressors. The main apparent cause of stress is abrupt disclosure to predatory attack. Wild animals counter to such attack with the flight or fight response with the release of certain hormones directly in to the blood stream and effect many organs the body. One of the major components of this response is the release of the stress hormone cortisol (Barton, 2002). Like wild fish, commercially important species of fish are also exposed to various stressors in their lives. In captivity, they underwent stressful aquaculture practices like seining, crowding, confinement; transportation etc while after release in wild environment faced other challenging conditions. Moreover, in contrast to natural environment hatchery-rearing tanks

provides safe and constant housing and provide excellent protection from predators, and have negative effect on fish behavior and physiology (Zuberi *et al.*, 2011) so it is unsurprising that a large proportion of post-release mortality occurs though predation (Brown and Day, 2002; Olla *et al.*, 1998).

One of the key components of physiological response to stress is the release of stress hormone cortisol (Barton, 2002). Cortisol level in the blood normally rise quickly following exposure to a stressor and then decay over a number of hours before returning to their basal level (Barton, 2000). Such a quick response is essential to ensure that the animal display the suitable avoidance behaviors such as escaping. Likewise, recovery period is also very important, because persistent levels of stress are harmful in term of excess of energy expenditure. In fish, neuroendocrine system with release of catecholamines and glucocorticoids play main role in controlling stress response by stimulating the hypothalamic-pituitary-interrenal axis (HPI) and sympathetic chomaffin which result in initiating cascade of physiological and metabolic changes (Perry and Bernier, 1999; Wendelaar-Bonga, 1997).

According to Iwama *et al.* (2006) stress responses in fish occurs in three stages, primary, secondary and tertiary. The primary stress responses involve the activation of natural alarm system and trigger the release of catecholamine, adrenaline / noradrenalin from medulla of adrenal gland and corticosteroid hormones from adrenal cortex. In stress, catecholamine helps the organism to show immediate physical reactions by accelerating breathing and heart rate while cortisol regulate the metabolism and increase blood glucose level though gluconegenesis and provide energy to every cell. The secondary stress response involves successful acclimation of animal with the use of energy that often results in suppression of growth process. The tertiary response appears when stressful condition surpasses the acclimation tolerance limits and fish become exhausted. Tertiary responses involve mal-adaptation, suppression of digestive process, retardation of growth and impairment of immune system resulted in vulnerability to pathogen (Iwama *et al.*, 2006).

Generally, cortisol is the main end product of the physiological response to stress (Barton, 2000; Fridell *et al.*, 2007) and consider as an indicator of fish welfare (North *et al.*, 2006; Turnbull *et al.*, 2005; Varsamos *et al.*, 2006). Normally, the most common method of evaluating the status of cortisol in fish is though blood analysis (Barton *et al.*, 2005; Haukenes

and Barton, 2004; Quigley and Hinch, 2006), but in small fishes, blood volume is not enough for assessing the status of hormone (Pottinger *et al.*, 2002; Sink *et al.*, 2007). Therefore, whole-body cortisol levels are in practice for evaluating the stress response of fry or small fish (Ramsay *et al.*, 2006; Zuberi *et al.*, 2014). Although both these techniques are extensively use but they are associated with problems like capturing or handling of fish for sample collection makes it difficult to obtain baseline level of hormone. Thus to avoid these problems, non-invasive methodology, that involve extraction of hormone from medium in which fish are present have been introduced (Scott and Ellis, 2007). Many scientists used the non-invasive methodology for measuring the water-borne cortisol levels from large and small enclosures having *Melanoteania duboulayi* (Zuberi *et al.*, 2011), *Cyprinus carpio* and *Oncorhynchus mykiss* (Ellis *et al.*, 2004; Ruane and Komen, 2003).

Scott *et al.* (2001) was first who proposed a concept release of free cortisol measurement from the water, a non invasive indicator of stress. It is suggested that cortisol is released from anterior region of the gills (a passive 'leakage') due to concentration gradient among plasma and surrounding water. Measurement of cortisol though non invasive method provides several advantages over plasma sample, such as water sample can easily collected without disturbing fish, repeated sampling over time from the same population become possible, thus reducing the number of animals and tanks required, and experiments related to stress can be performed on fish that are too small to be bled (Scott and Ellis, 2007; Ellis *et al.*, 2004; Scott *et al.*, 2001).

The magnitude of stress response in term of corticosteroid release is variable among different fish species and even in strains of fish (Barton, 1997). Most fishes show typical stress response with the release of cortisol that reach to its peak value after 1 h and followed rapid recovery (Iwama *et al.*, 2006). However variation exists in time of stress responses and recovery period. According to Scheck *et al.*(2000) physiological recovery period can be prolong and extend to 10 days to 2 weeks , if the stressor persist but are not lethal. In red drum cortisol level rise rapidly during handling processes, but return to basal level after 48 hrs (Robertson *et al.*, 1988) while in Common dentex (*Dentex dentex*) cortisol and glucose level enhanced immediately and decrease to baseline level after 8 hs. Wild rainbow fish (*Melanoteania duboulayi*) also show typical elevation of cortisol after 0.5 h, peak after 2 hrs and return to basal

2005; non-human primates: Muller and Wrangham, 2004; Cristobal-Azkarate *et al.*, 2006; birds: Wingfield *et al.*, 1990; fish: Oliveira *et al.*, 1996) and revealed that testosterone level is associated with situational cues, mainly cues signaling intra-sexual competition and dominant behaviors (Wingfield *et al.*, 1990).

In human as well as in fish, winner showed high level of testosterone as compared to looser after encounter (Einum & Fleming 2001). Similarly in some cichlids like *Neolamprologus* species subordinates frequently show submissive behaviors towards high-ranking group members, thus have low level of testosterone (Galhardo and Oliveira, 2014.)

In vertebrates, testosterone acts as a prohormone that can exert its effects either on estrogen receptors after aromatization to estradiol or on androgen receptors after conversion to 5-alpha-dihydrotestosterone. Many scientists observed and reported that major effect of testosterone on aggression occur after aromatization (Huffman *et al.*, 2013; review; Simpson, 2001; Schlinger *et al.*, 1990). For example, in African cichlid fish, *Astatotilapia burtoni* subordinate males show increase level of aromatase expression as compare to dominant males in the magnocellular and gigantocellular regions of the preoptic area. In other animals like quail and rat testosterone induced aggression is concomitant with increased level of aromatization and nuclear estrogen receptor activity in the hypothalamic/ preoptic area, whereas aromatase inhibitor block the aggressive behavior and decrease the nuclear activated estrogen receptors (Naftoli *et al.*, 1990)

Testosterone can also act via genomic or non-genomic mechanisms by stimulating the synthesis of proteins or modulating neural activity respectively (review: Simpson, 2001). The non-genomic mechanisms involve the changes in electrical potential and permeability of nerve cell membrane probably though cyclic adenosine monophosphate activation that result in the release of neurotransmitters into the synapse (Szego, 1984). It reveals that androgen and estrogen receptors are also found along neurotransmitter pathways. Hence testosterone is playing role in modulating the levels of different neurotransmitters that involve in showing effects on aggressive behavior (Simpson, 2001).

It has been reported by many investigator that in vertebrates not only corticosteroids respond to stress but also sex steroids. For example in mammals (Huhman *et al.*, 1991), birds (Wilson *et al.*, 1979), reptiles (Moore *et al.*, 1991) and amphibians (Licht *et al.*, 1983) stress

decrease the androgen level. Whilst an inverse relation has been found between cortisol and androgen levels during smoltification and sexual maturation of salmonid fishes (Pickering *et al.*, 1987). It was also reported that in teleost fish exogenous cortisol affects the reproductive function and gametes development (Barry *et al.*, 1995; Foo and Lam, 1993; Carragher and Sumpter, 1990).

Efforts have been made to elucidate the mechanisms though which stress influence reproductive role though cortisol action. Though *in vitro* cultures Carragher and Sumpter (1990) found that cortisol suppressed secretion of both estradiol and testosterone from female follicles of rainbow trout. Generally, cortisol show direct impact on gonadal steroid secreting tissues rather than the pituitary-gonadal axis thus either suppresses the steroids common precursor or slows down the mechanism of secretion of hormones (Carragher and Sumpter (1990).

In teleost, the androgens, testosterone (T), or 11 ketotestosterone (11KT) effect is studied mostly with respect to sex steroid and sexual behavior. However in some Cichlids role of androgens is well explained in the context of social activities of male, both in terms of organization (Groothuis and Ros, 1993) and activation (Wapler-Leong and Reinboth, 1974; Fernald, 1976). Moreover, in certain species of tilapia (*Oreochomis mossambicus*) administration of androgen in early ontogeny can even cause sex reversal of the individual (Hunter and Donaldson, 1983).

Some scientists reported the role of androgen in social activities like communications among conspecifics and parental care (Magee *et al.*, 2006; Ros *et al.*, 2004; Páll *et al.*, 2002a). Effect of communications on androgen levels among conspecifics reveals a type of two-way interaction among androgens and behavior (Villars, 1983). For example territorial males of stoplight parrotfish (*Sparisoma viride*) in a natural population have elevated level of androgen than non-territorial males, but androgen concentration in non territorial male increase from territorial males during territory acquisition. Moreover, in experiments, introduction of other male showing agonistic behavior can also affect the level of androgen in fish (Cardwell and Liley, 1991a). Similarly, many investigators reported the peak levels of circulating androgens like testosterone and 11-ketotestosterone (11 KT) in large teleost fish, during the parade and territorial defense phases of parental care (Páll *et al.*, 2002a; Ros *et al.*, 2004; Magee *et al.*, 2006).

each of the duplicate images, to the nearest 0.001 mm. The mean area from these measurements was analyzed. During dissection, in some samples, a few substructures of brains were missing like one of the paired olfactory bulbs. In these cases the area of that sub-structure was calculated as twice the area of the remaining one.

Stress response

This part of the study was conducted at Mahseer Hatchery Hattian, Attock. The fish of hatchery were used as captive reared mahseer while wild fish were collected from the same sites Poonch River, Azad Kashmir and River Harro from Attock. Wild fish were transported alive by using hauling tanks and kept in earthen ponds for 15-20 days in order to acclimatize them. During acclimatization period fish were provided 40% protein commercial fish feed.

Experimental design

After acclimatization, both wild and hatchery reared fish were stocked at a stocking density of 5 g/ L in circular tanks. Experiment was conducted in replicate i.e., three tank for wild and 3 tanks for hatchery reared fish. They were housed in a circular tank under the flow through system and acclimatized up to six days. Flow through system was maintained at a constant flow rate of 1 L/min in all circular tanks with the help of stand pipes. The water was aerated with stone diffusers while fish were remained at ambient temperature. Circular tanks water was daily replaced with fresh well water by means of the flow through system as well as with siphoning so that the fish were completely accustomed with daily handling. Water quality parameters like ammonia, pH and oxygen level have been regularly monitored, while 12:12 light / dark photoperiod was used throughout the experiment. Fish remained in a flow through system for 6 days and get 40% protein commercial fish feed at the rate of 4% body weight.

Sample collection

On the day of experiment, fish were not provided feed and whole water was exchanged with well water. For obtaining pre-stress water borne cortisol level, 500 ml water was collected without disturbing the fish from each tank after 1 hr in a sterilized mineral water bottles through the outlet of flow through system, while for plasma basal level, one fish from each tank was quickly captured with hand net, anesthetized with clove oil (0.03 ml/L) and blood sample was

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After acclimatization, both wild and hatchery reared fish were stocked at a stocking density of 5 g/ L in circular tanks. Experiment was conducted in replicate i.e., three tank for wild and 3 tanks for hatchery reared fish. They were housed in a circular tank under the flow through system and acclimatized up to six days. Flow through system was maintained at a constant flow rate of 1 L/min in all circular tanks with the help of stand pipes. The water was aerated with stone diffusers while fish were remained at ambient temperature. Circular tanks water was daily replaced with fresh well water by means of the flow through system as well as with siphoning so that the fish were completely accustomed with daily handling. Water quality parameters like ammonia, pH and oxygen level have been regularly monitored, while 12:12 light / dark photoperiod was used throughout the experiment. Fish remained in a flow through system for 6 days and get 40% protein commercial fish feed at the rate of 4% body weight.

Sample collection

On the day of experiment, fish were not provided feed and whole water was exchanged with well water. For obtaining pre-stress water borne cortisol level, 500 ml water was collected without disturbing the fish from each tank after 1 hr in a sterilized mineral water bottles through the outlet of flow through system, while for plasma basal level, one fish from each tank was quickly captured with hand net, anesthetized with clove oil (0.03 ml/L) and blood sample was

collected. The blood plasma was separated and stored by following the same procedure as described above.

After capturing one fish from each tank, the physical stress was induced in other wild and captive reared fish by continuous chasing for about 5 min with hand net. Immediately after stress, 500 ml of water sample was collected from each tank through the outlet pipe. By using the same technique, further water samples were collected at 1, 2, 4, 6 hr after chasing stress. Water samples were stored at -20°C until extraction.

Water-borne cortisol analysis

The method reported by Zuberi *et al.* (2011) was adopted for extraction of water-borne cortisol. Briefly, before extraction water samples were removed from the freezer, thawed and maintained at room temperature. Then filtered by using Whatman filters to remove particulate material, and pass through millipore filter 0.45 µm by using Millipore filtering unit. The LiChrolut® RP-18 solid phase extraction cartridge (500 mg, 3 ml, 40 - 63 µm, standard PP Merck) were used for the extraction of hormone from water sample. The cartridges were primly integrated to a 20-port vacuum manifold and after that activated by passing 4 ml HPLC grade methanol (CH_3OH), subsequently pass 4 ml double distilled water. The water samples were then passed through the cartridge. After passing water sample cartridges were washed with double distilled water and free cortisol was extracted from the columns into 10 ml glass test tube by three repeated (2 ml) washes with ethyl acetate. The 6 ml of collected solvent was removed at 45°C using a nitrogen gas stream. The test tubes were sealed with parafilm and stored at 4°C until analysis.

Cortisol Assay

Cortisol level was analyzed by ELISA, using Amgenix MicroLISA™ – Cortisol kit USA.

Principal of Test

Cortisol is a solid phase competitive ELISA which is based on a principle where competition for binding sites between sample cortisol and cortisol enzyme conjugate takes place. The sample and conjugate are loaded into the wells coated with antibody (anti-Cortisol monoclonal). Washing buffer is used to wash off unbind cortisol and conjugate. Then by adding

the substrate, color appears. The concentration of color is inversely proportional to the sample concentration. A standard curve is prepared by concerning color intensity depend on the concentration of cortisol.

Assay Procedure

First of all, reagents and all plasma samples were removed from freezer and kept at room temperature. The plasma samples were thawed, centrifuged and the supernatant was used for analysis while the water-borne cortisol residue was dissolved in ELISA buffer before use. Reagents were slightly shaken before use and then 25µl of cortisol standards and samples were pipette out and pour in wells of Elisa plate. After that, 200 µl of cortisol enzyme conjugate was loaded to each well and allow them to stand for 10 sec. and then incubated at room temperature for 60 min.

After incubation, liquid from all wells was removed. Then well were washed by adding 300 µl of 1X washing buffer and repeated that process three times. The remaining water droplets were removed by tabbing the plate on absorbent paper. Then 100 µl TMB substrate was pipette out to each well and incubated at room temperature for 15 min. After that stop solution of 50 µl was poured to each well in order to stop the reaction. The ELISA plate was gently shaken to mix the solution. At the end, ELISA Reader was used to read the absorbance at 450 nm within 20 min after adding stop solution.

Cortisol release rate

The cortisol release rate was calculated by following the equation described by Ellis *et al.* (2004).

Cortisol release rate = $[(V (C_t - C_o e^{-kt}) kt]/ 1-e^{-kt})] / w$

Where,

V = Water volume

C_t = Final hormone concentration

Co = Initial hormone concentration

K = Rate of decrease due to dilution over time t

W = the total weight of the fish in the sample.

e = 2.718 **(exponential constant)**

Testosterone Assay

Testosterone level was analyzed by using Amgenix MicroLISA™ – Testosterone EIA kit, USA.

Principle of Test

The EIA for Testosterone is a competitive assay in which testosterone in a sample, standard, control and Testosterone-HRP conjugate compete for a single antibody (rabbit anti-Testosterone). Ten µl each of standard, control and sample are loaded in the wells for incubation, then 100 µl testosterone-HRP conjugate reagents along with 50 µl rabbit anti-Testosterone reagent was also loaded in each well and allowed it to incubate for 90 min at 37°C. The competition is between Testosterone HRP and testosterone in sample standard and control so that Testosterone HRP is responsible for coloration. Therefore, greater the HRP conjugate, intensive will be the color which is the indication of low level of testosterone in the sample, standard, control and vice versa.

The washing of wells removes the unbound testosterone peroxidase conjugate. Then TMB reagent is added in each well, allowed for 20 min incubation that result in blue color. The reaction is stopped by adding 1N HCl stop solution into each well. By plotting standard concentration verses absorbance a standard curve is obtained. From that standard curve, we can calculate the concentration of testosterone in the sample and control.

Assay Procedure

First of all reagents of EIA kit were removed from the refrigerator and kept at room temperature. All samples were removed from the freezer and thaw them at room temperature. Before using all samples were centrifuged and the supernatant was used for analysis. Samples and standard, 10 µl each was dispensed into the wells of Elisa plate. After that 100 µl of testosterone-HRP Conjugate reagent was added into all wells. Then 50 µl of rabbit anti-

Testosterone reagent was added into all wells. Then mixed for 30 sec and incubated at 37°C for 90 min.

After that the reagent from all wells were removed by washing and flicking all the wells 5 times with dH_2O. Then 100 µl of TMB Reagent was added into all well and mixed for 10 sec. The EIA plate was incubated at room temperature (18-25°C) for 20 min. In order to stop the reaction 100 µl stop solution was dispensed to each well and gently mixed for 30 sec. At the end, ELISA Reader was used to read the absorbance at 450 nm within 20 min after mixing stop solution.

Statistical Analysis

Data obtained from the experiment was expressed as mean ± SEM. The results were analyzed by using Statistic Version 8.1. Results were analyzed by ANOVA and simple T test. Values of $P < 0.05$ were considered statistically significant.

Results

RESULTS

Brain size:

Total brain area and different substructure of brain were measured in order to study the effects of rearing environment on mahseer (*T. putitora)* (Table 1). The telencephalon area of hatchery reared mahseer was 22.83 ± 1.28 mm^2 was slightly less but statistically comparable to wild mahseer, 25.46 ± 2.13 mm^2. Similarly, cerebellum size of hatchery reared (31.81 ± 3.15 mm^2) and wild fish (29.16 ± 4.14 mm^2) also showed no significant difference. However, optic tectum size of wild mahseer was considerably ($P < 0.01$) larger (70.34 ± 2.69 mm^2) as compared to hatchery reared mahseer (56.33 ± 2.64 mm^2) (Fig 1)

The total body length and total brain area ratio showed that brain size of wild fish was significantly ($P < 0.0001$) higher than hatchery reared mahseer (*T. putitora)* (Table 2, Fig 2). The wild mahseer showed 28 % larger brain as compared to hatchery reared mahseer.

Water-borne cortisol levels:

Water borne cortisol was considerably higher in wild fish as compared to hatchery reared fish (Table 3). The non significant interaction between Population and Treatment showed that response of both populations to the treatments is varied. It was observed that water borne cortisol was higher in the wild fish than hatchery reared fish after acute stress and no difference was observed in the control treatment. The significant three-way interaction between time, population and treatment is indicative of the different patterns displayed by fish from both populations in both treatments over time.

The pre stress basal levels of water-borne cortisol in hatchery reared and wild mahseer were 37.75 ± 1.75 ng/L and 60.50 ± 3.43 ng/L respectively (Table 3). The water borne cortisol immediately after 5 min acute stress (chasing with dip net) increased to 39.93 ± 2.28 ng/L and 69.15 ± 3.92 ng/L in hatchery reared and wild fish population respectively. Afterward, the post stress levels showed continuous increasing trend up to 6 hr in captive reared mahseer and 4 hr in wild mahseer and reached to their peak levels (Captive, 63.04 ± 1.15 ng/L; wild, 91.03 ± 1.38 ng/L). Then decreasing trend was observed (Fig 3). The pre stress and post stress water borne cortisol levels were significantly ($P < 0.0001$) higher in wild mahseer as compared to hatchery reared counterpart (Table 4, Fig 4).

Cortisol release rate

Water supplying to circular tanks (experimental tanks) contain no cortisol. Cortisol release rate was significantly higher in wild mahseer as compared to hatchery reared fish after acute stress response (Table 5). The significant relationship was found between Population and treatment indicated that the two populations show different responce to the treatments. The repeated measure was also significant indicating that cortisol release rates changed over time. The significant three-way interaction between time, population and treatment is indicative of the different patterns displayed by fish from both populations in both treatments over time.

The pre stress cortisol release rate in hatchery and wild mahseer (*T. putitora*) were 0.003 ± 0.27 ng/g/h and 0.017 ± 0.54 ng/g/h respectively (Table 5), increased immediately after stress and followed the same trend as observed for water-borne cortisol level. In wild *T. putitora*, the post stress cortisol release rate reached to peak level (5.22 ± 0.22 ng/g/h) after 4 hr of stress and then declined and reached to 1.96 ± 0.11 ng/g/h at 6 hr, while in captive reared, the highest level (4.35 ± 0.18 ng/g/h) was attained after 6 hr of acute stress (Fig 6). The cortisol release rate levels were higher in wild as compared to captive reared fish (Table 6, Fig 7).

Plasma cortisol levels:

Plasma cortisol levels after mild capturing stress, in hatchery reared and wild mahseer (*T. putitora)* were 383.33 ± 18.30 ng/mL and 460.82 ± 38.24 ng/mL respectively (Table 7). Mean plasma cortisol concentration was significantly ($P < 0.0001$) higher in wild mahseer as compared to their captive counterpart (Fig 8).

Plasma testosterone levels:

Plasma testosterone levels in hatchery and wild reared *T. putitora* are shown in Table 8. A significantly ($P < 0.05$) higher mean plasma testosterone concentration was observed in hatchery reared mahseer as compared to wild (Fig 9).

Table 1: Comparison of total area of telencephalon, optic tectum, cerebellum with reference to whole brain of hatchery reared and wild mahseer (*T. putitora*).

	Total area (mm^2)		
Population	**Telencephalon**	**Optic tectum**	**Cerebellum**
Captive	22.83± 1.28	56.33 ± 2.64	31.81 ± 3.15
Wild	25.46 ± 2.13^{ns}	$70.34 \pm 2.69^{**}$	29.16 ± 4.14^{ns}

Values are presented as mean ± SEM (n=15).
*P< 0.05; **P< 0.01; ***P < 0.0001
ns = non significant

Table 2: Comparison of brain to body size ratio of wild and captive reared mahseer (*T. putitora*).

Population	Body length (cm)	Total brain length (mm)	Body size: Brain size
Captive	37.49 ± 0.21	18.50 ± 0.29	0.003
Wild	36.62 ± 0.39	18.45 ±0.40	0.005***

Values are presented as mean ± SEM (n=15).
*P< 0.05; **P< 0.01; ***P< 0.0001

Table 3: Comparison of pre-stress and post-stress water-borne cortisol levels (ng/L) of hatchery reared and wild mahseer (*Tor putitora*) at different time periods after stress (n=3).

Time (hrs)	Water-borne cortisol (ng/L)	
	Captive	**Wild**
Basal	37.79 ± 1.75^{h}	60.50 ± 3.43^{ef}
0	39.93 ± 2.88^{h}	69.15 ± 3.92^{d}
1	51.17 ± 3.37^{g}	85.77 ± 3.84^{b}
2	49.77 ± 1.79^{g}	80.72 ± 1.70^{c}
4	57.06 ± 0.81^{f}	91.03 ± 1.38^{a}
6	63.04 ± 1.15^{e}	70.95 ± 0.70^{d}

Data are represented as Mean ± SEM. Means followed by the different letter within the columns are significantly different ($P<0.05$). (ANOVA followed by LSD test).

Table 4: Comparison of basal and peak cortisol levels of hatchery reared and wild mahseer (*T. putitora)* after acute stress.

	Water-borne cortisol (ng/L)	
Population	**Pre-stress**	**Post-stress**
Captive	37.79 ± 1.75	60.50 ± 3.43
Wild	63.04 ± 1.15***	91.03 ± 1.38***

Values are presented as mean ± SEM.
*P < 0.05; **P < 0.01; ***P < 0.0001

Table 5: Comparison of pre-stress and post-stress cortisol release rate (ng/g/h) of hatchery reared and wild mahseer (*Tor putitora)* at different time periods after stress (n=3).

Time (hrs)	Cortisol release rate (ng/g/h)	
	Captive	Wild
Basal	0.03 ± 0.02^{f}	0.017 ± 0.03^{f}
0	0.35 ± 0.29^{f}	1.39 ± 0.62^{e}
1	2.17 ± 0.53^{d}	4.06 ± 0.61^{b}
2	1.95 ± 0.28^{d}	3.28 ± 0.27^{c}
4	3.03 ± 0.12^{c}	5.22 ± 0.22^{a}
6	4.35 ± 0.18^{b}	1.96 ± 0.11^{d}

Data are represented as Mean ± SEM. Means followed by the different letter within the columns are significantly different ($P<0.05$). (ANOVA followed by LSD test).

Table 6: Comparison of basal and peak levels of cortisol release rate (ng/g/h) of hatchery reared and wild mahseer (*T. putitora)* after acute stress

	Cortisol release rate (ng/g/h)	
Population	**Pre-stress**	**Post-stress**
Captive	0.27 ± 0.01	4.22 ± 0.16
Wild	0.66 ± 0.08**	5.37 ± 0.19**

Values are presented as mean ± SEM.
*P< 0.05; **P< 0.01; ***P< 0.0001

Table 7: Comparison of plasma cortisol levels (ng/ml) of captive reared and wild mahseer (*T. putitora)*

Population	Cortisol levels (ng/ml)
Captive	383.33 ± 18.30
Wild	460.82 ± 38.24***

Values are expressed as mean ± SEM (n=15).
*P< 0.05; **P< 0.01; ***P< 0.0001 captive vs wild

Table 8: Comparison of plasma testosterone levels (ng/ml) of hatchery reared and wild mahseer (*T. putitora)*

Population	Testosterone levels (ng/ml)
Captive	0.66 ± 0.15
Wild	0.19 ± 0.05*

Values are presented as mean ± SEM (n=15).
*P< 0.05; **P< 0.01; ***P< 0.0001. captive vs wild

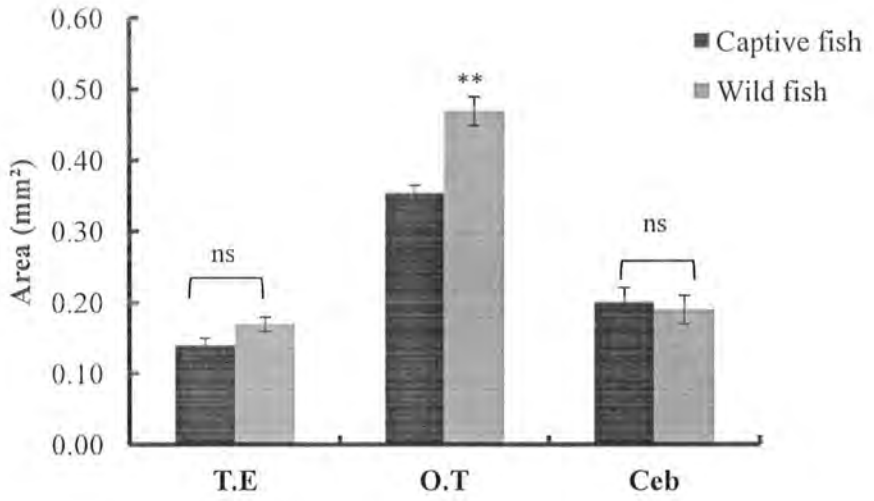

Fig 1: Telencephalon(T.E), optic tectum (O.T) and cerebellum (Ceb) size (mm^2) of hatchery reared and wild mahseer (*Tor putitora)*with refrence to whole brain area . Values are presented as mean ± SEM (n=15).

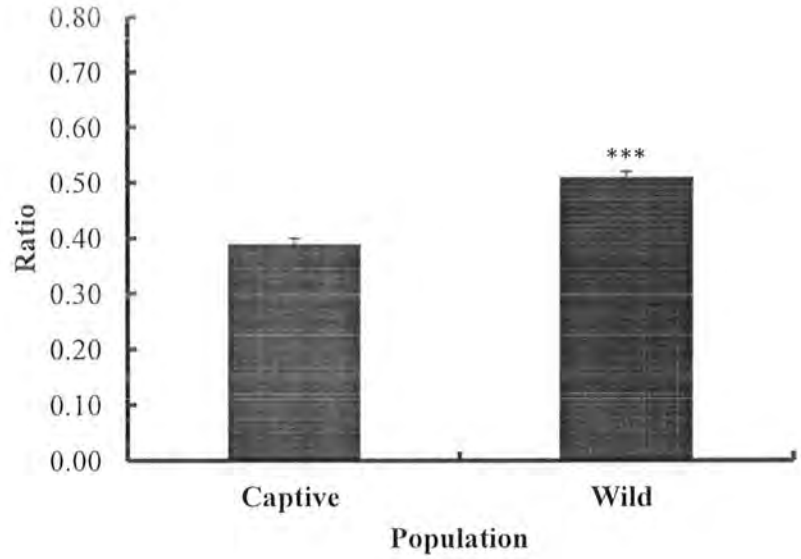

Fig 2: Comparison of brain to body size ratio of wild and captive reared mahseer (*T. putitora).*

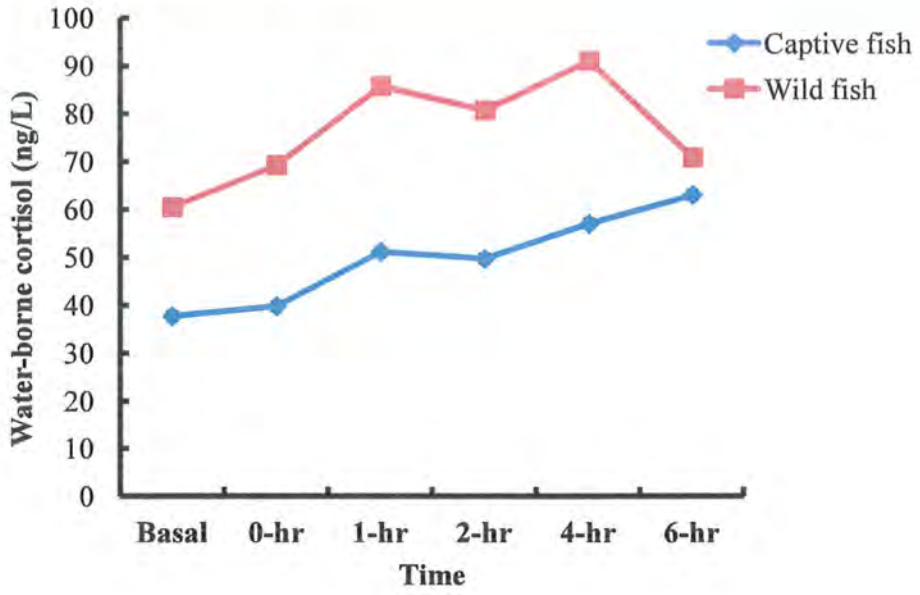

Fig 3: Pre-stress and post stress water-borne cortisol (ng/L) from captive and wild mahseer (*Tor putitora*). Values are presented as mean ± SEM (n=3).

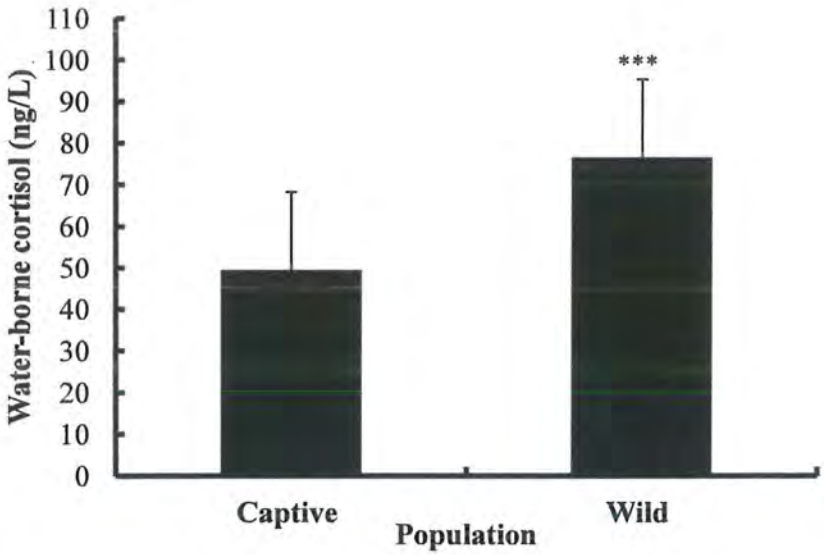

Fig 4: Comparison of peak levels of water-borne cortisol (ng/L) from captive and wild mahseer (*Tor putitora*) after acute stress.

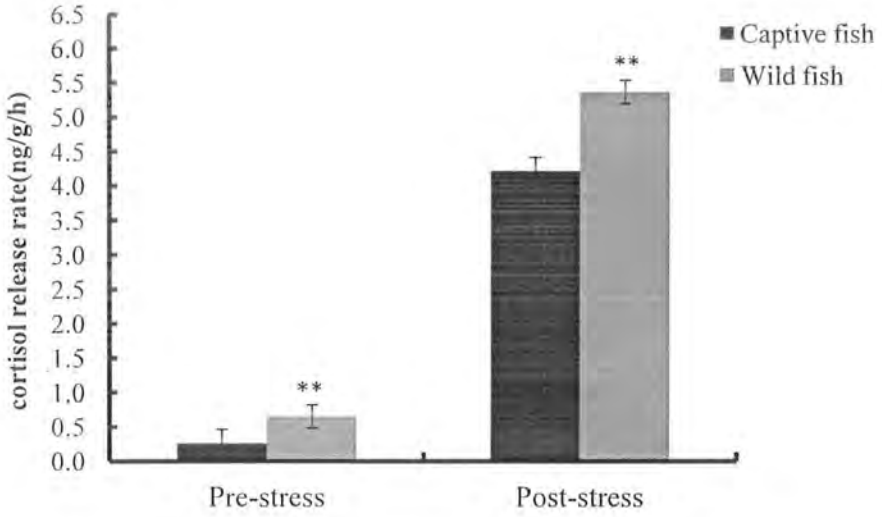

Fig 7: Comparison of pre-stress and post-stress (Peak level) cortisol release rate (ng/g/h) of captive and wild mahseer (*Tor putitora*).

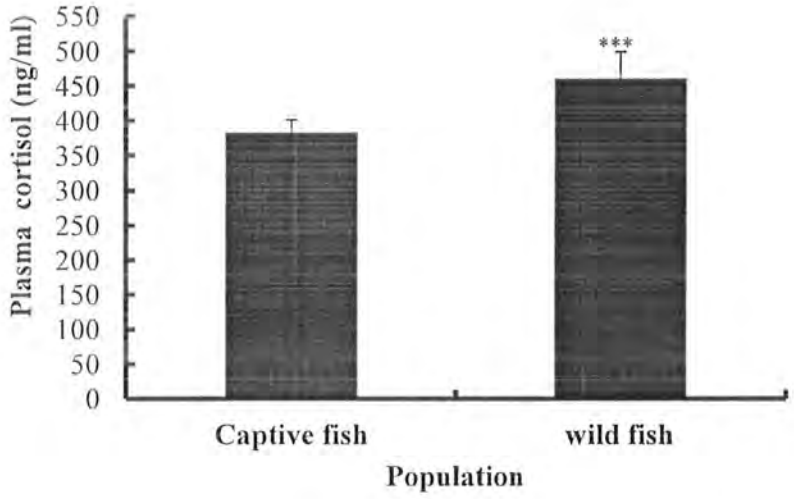

Fig 8: Plasma cortisol levels (ng/ml) of captive and wild mahseer (*Tor putitora*). Values are presented as mean ± SEM (n=15).

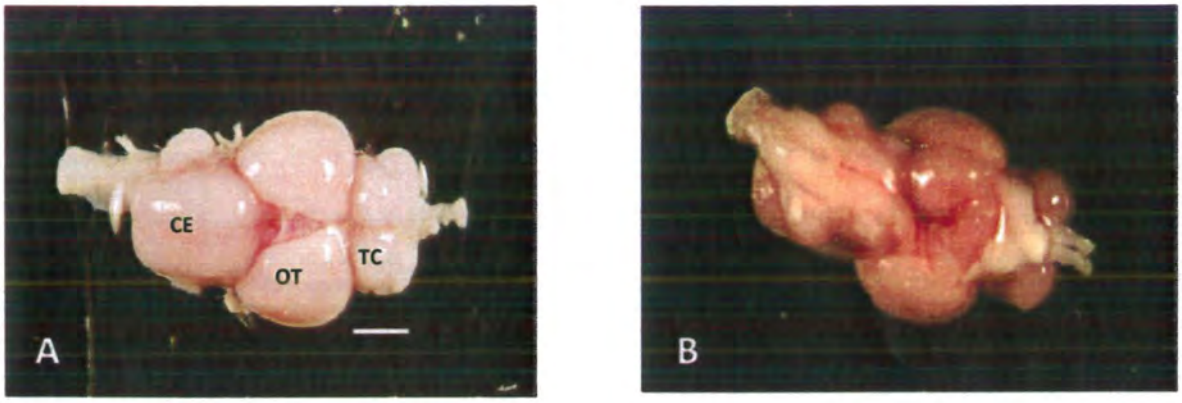

Fig 12: Photograph of hatchery reared mahseer (*T. putitora*) brain (A) Dorsal view (B) Ventral view. TC, telencephalon; OT, optic tectum; CE, cerebellum. Scale bar = 1mm.

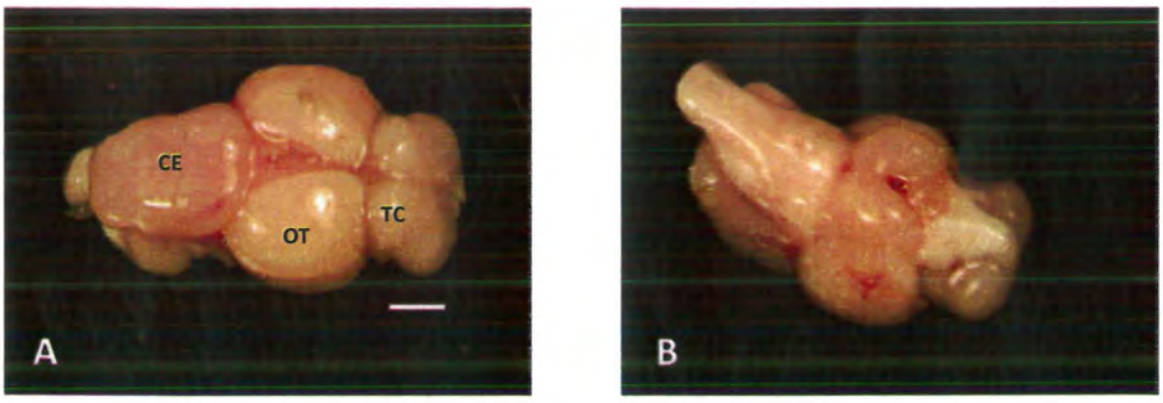

Fig 13: Photograph of wild mahseer (*T. putitora)* brain (A) Dorsal view (B) Ventral view. TC, telencephalon; OT, optic tectum; CE, cerebellum. Scale bar = 1mm.

Discussion

DISCUSSION

The results of present study indicated the effect of rearing environment on the development of brain and their sub-structure, physiological stress response and testosterone level of Mahseer (*T. putitora*) and support the view that due to developmental plasticity the rearing environment plays significant role in shaping the behavior, morphology and physiological of fish (Näslund *et al.*, 2012; Kihslinger *et al.*, 2006; Michael *et al.*, 2003; Marchetti and Nevitt, 2003).

In the present study, wild fish showed 28% larger brain as compared to hatchery reared Mahseer. Our results are in agreement with many other reports (Näslund *et al.*, 2012; Burns *et al.*, 2009; Kihslinger *et al.*, 2006; Michael *et al.*, 2003) where captive reared *S. salar*, *P. reticulata*, *O. mykiss* and *O. tshawytscha* showed smaller brain as compared to their wild counterparts. In addition to fish, many other domesticated animals like sheep (*Ovis aries*), Norwegian rats (*Rattus norvegicus*), cats (*Felis catus*) and pigs (*Sus scrofa*) also showed 8-33% smaller brains as compared to their wild counterparts (McCarter, 2012; Diamond, 2002). Conversely, Can *et al.* (2012) observed larger brain of hatchery reared Atlantic salmon (*Salmo salar*) compared to stream-run *Salmo salar* and suggested that different environmental conditions play important role in the development of brain size of fish in addition to structural intricacy.

According to many investigators variation in brain size of captive animals occurred due to inbreeding depression, the pleiotropic effects of artificial selection, increased levels of reproduction and relaxed selection pressure over generation (Ebinger and Rohs, 1995; Kruska, 1996; Kruska, 1988) while other scientists suggested that significant differences in the size of whole brain and different brain sub structure is the result of the developmental consequence of rearing conditions (Kihslinger *et al.*, 2006).

Generally, it has been assumed that most fish in comparison to other vertebrates shows indeterminate somatic growth that also become visible in the central neural system (Kotrschal *et al.*, 1998). Thus brain structures in fish may be less limited and may be affected by spatial and temporal constraint (Kotrschal *et al.*, 1998) and show greater impact of rearing environment (Näslund *et al.*, 2012; Kihslinger *et al.*, 2006; Michael *et al.*, 2003; Marchetti and Nevitt, 2003) as compared to vertebrates that showed determinate growth. Several scientist reported the

plasticity of fish brain (increase in number and size of neurons, dendrite branching, cortical thickness) and suggested that rearing environment effect the development of central nervous system both in early stages when neural and body development take place and at the later stages of life (Kolb and Whishaw, 1998; Rosenzweig and Bennett, 1996). Though, developmental plasticity in fishes is suggested as a potential causative factor to neural phenotype, but the extent of influence of surrounding environment on brain growth still need further research (Lema *et al.*, 2005; Hofman and Fernald, 2000).

In fish, different substructures of brain are multifunctional to some extent but their overall size normally related to its ecological niche (Shumway, 2008). The sizes of these various substructures usually related to the particular behavioral traits, sense and physiological response of an individual (Ito *et al.* 2007). Several scientist suggested that smaller variation in size of these area can show pronounced effect on various traits of an organism (Gonzalez-Voyer and Kolm 2010; Kolm *et al.* 2009). In the present study the size of telencephalon and cerebellum of captive reared fish was slightly less but statistically comparable to their wild counterpart However, the size of optic tectum of wild Mahseer was considerably ($P < 0.01$) larger as compared to hatchery reared Mahseer. It seems that conventional hatchery rearing environment profoundly affect the development of different sub-structure of brain of Mahseer *T. putitora*. Kihslinger *et al.*, (2006) also observed the influence of rearing environment on the development of different brain structure of Chinook salmon *Oncorhynchus tshawytscha* and reported the significantly larger volume of olfactory bulb and telencephalon relative to body size in wild fish compared to captive reared fish. Similarly, Marchetti and Nevitt (2003) also reported smaller olfactory bulbs and telencephalons relative to body size of captive reared rainbow trout (*Oncorhynchus mykiss*) as compared to wild fish.

In addition to fish, environmental stimuli also effect the development of brain in other taxa like in birds, the rate of experience changes in the volume of the hippocampus by inducing neurogenesis (Patel *et al.*, 1997). Similarly in mammals cell proliferation and dendritic arborization in the brain increased when organism exposed to enriched environments as compared to animals only exposed to conventional hatchery environment (Van Praag *et al.*, 2000; Faherty *et al.*, 2003). In birds, the rate of experience- induced neurogenesis has been

linked to changes in the volume of the hippocampus (Patel *et al.*, 1997), suggesting that changes in neurogenesis can generate changes in brain size.

The optic tectum in fishes is the primary visual center that is well developed in fish which have strong visual behavior (Ben-Tov *et al.*, 2013). The development of optic tectum in different fish species depend upon its habitat and shooting and feeding behavior. Golden Mahseer *T. putitora* is a major *Tor* that is dwelling in mid hills stretches of Himalayan region and inhabits the rapid streams with rocky bottom, riverine pools and lakes. It usually likes the somewhat clear water where its visual behavior is well developed. Hence, optic tectum is well developed in wild population as compared to conventional hatchery reared fish Mahseer.

It is well documented that in addition to habitat, other factors like social status and temperature are influencing the size or number of neuroendocrine cells in the forebrain of fish (Semsar and Godwin, 2003; Miranda *et al.*, 2003;) and may also contribute in shaping neural phenotype. The sterile hatchery environment or stressful rearing condition like crowding can also hinder the growth of brain, brain growth could be hindered by stressful conditions (e.g. crowding) in captivity. For example Burgess and Coss (1982) observed depressed growth of dendrites and arborization in jewel fish under crowded rearing conditions. In other group of animals poor nutrition and maternal deprivation also play role in reducing the size of brain by affecting the neurogensis (Mirescu *et al.*, 2004). No such data is available for in literature related to fish. The results of our study reveal that captive rearing environment of Mahseer is somewhat sterile and different from natural environment, therefore showed negative impact on development of brain.

The results of present study also indicated that both captive reared and wild population showed difference in extent of stress response when exposed to acute stress (chasing with dip net). In fish, cortisol is extensively used as an indicator of stress, it rapidly increase in stressful condition (Zuberi *et al.*, 2011; Barton, 2002; Wendelaar Bonga, 1997) like aquaculture practices netting, crowding, handling and live hauling (Vijayan *et al.*, 1997; Arends *et al.*, 1999). Cortisol secretion is the major end result of physiological stress response and it regulates energy metabolism (Wendelaar Bonga, 1997) and effect fitness of organism.

Our results demonstrated the considerable difference in pre-stress water-borne cortisol levels and release rate in wild and captive reared Mahseer. Similarly, significant ($P< 0.0001$) difference in post-stress water-borne cortisol levels and release rate in wild and hatchery reared fish were also observed. The wild Mahseer showed typical stress response that is increased in water borne cortisol level after 1 h acute stress followed by rapid recovery (Zuberi *et al.*, 2011; Iwama *et al.*, 2006; Barton, 2002) while captive reared Mahseer showed atypical response. The cortisol level in captive population increase slowly and showed continuous increasing trend even after 6 hrs. The experiment was conducted in replicate under the similar environmental condition by using similar stress stimulus; therefore we are certain that variation in response appeared due to physiological difference of both populations.

The results of acute stress response of wild Mahseer were similar to those observed in other fish species where the stress response normally appear between 30 min and 4 hr after exposure to a stressor (Zuberi *et al.*, 2011; Barton, 2002; Scott *et al.*, 2008). For example, wild caught rainbowfish (*Melanoteania duboulayi*) showed marked increase in cortisol release rate within 30 min after exposing stress by chasing with simulated predator (Zuberi *et al.*, 2011) and showed recovery trend within 4 hr similar to observed in case of wild Mahseer population compared to captive reared Mahseer. Similarly, in rainbow trout, *Oncorhynchus mykiss* Walbaum plasma cortisol levels peaked within 30 min after the onset of mild confinement stress (Pottinger and Moran, 1993) while water cortisol level peaked at 2 hr post mild handling stress followed by recovery (Ellis et *al.*, 2004). Moreover, in carp, *Cyprinus carpio* L cortisol levels returned to basal levels after 4 hrs of exposure to stress by capturing and holding in nets (Pottinger, 1998).

In acute stress the release of cortisol is temporary and it provide energy to cope with environment, while prolong release of this hormone resulted in adverse effects. The present study showed that in captive reared Mahseer, water borne cortisol level remained high even after 6 h of post stress. This result is in accordance to the stress response of captive reared *Melanoteania duboulayi* (Zuberi *et al.*, 2011) where water-borne cortisol increased after 4 hr of stress exposure and remained at elevated level. Our results of gradual increased in water-borne cortosl for an extended period in captive reared Mahseer further showed the odd physiological response of hatchery reared fish.

Captive reared *T. putitora* besides showing difference in pattern of cortisol release rate, also showed vitiation in concentration of pre-stress and post stress water-borne cortisol. The wild fish showed significantly ($P < 0.0001$) higher level of pre-stress water-borne cortisol concentration (60.50 ± 3.43 ng/ml) and release rate (0.017±0.03 ng/g/h) as compared to their hatchery reared counterpart. The post stress cortisol level also followed the same trend, quantitatively low in captive reared fish compared to wild Mahseer. The peak water-borne cortisol concentration in the wild Mahseer (91.03 ± 1.38 ng/L) was about 45% higher as compared to observe in captive reared Mahseer (63.04 ± 1.15 ng/L). Wild and captive reared population of *Melanoteania duboulayi* also followed the same quantitative difference in cortisol release rate and concentration (Zuberi *et al.*, 2011).

According to many scientist variations in stress response and cortisol levels after experiencing a stressful event is heritable factor (Fevolden *et al.*, 1991; Fevolden *et al.*, 1993; Pottinger and Mosuwe 1994; Fevolden *et al*, 1999) while stress responses heritability (h^2) may vary noticeably in population and species under consideration. Many scientist suggest that even a single generation under hatchery conditions can showed profound effect on the behavior of fish (Álvarez and Nicieza, 2003; Salonen and Peuhkuri, 2006) while repeated stressors can increase the heritability of plasma cortisol (Fevolden *et al.*,1999). In hatchery-reared Atlantic salmon and rainbow trout heritability value of stress response were 0.05 and 0.27 respectively, while it increased to 0.56 in rainbow trout after repeated stressors (Fevolden *et al.*, 1999). Moreover magnitude of corticosteroid response is different among species and depends upon the duration and severity of stressor, environmental conditions and developmental stage of fish (Ellis, 2004; Barton, 2002).

In fish bile, urine and gills are three main ways though which free steroids are cleared from plasma and released into the water (Vermeirssen and Scott, 1996; Sorensen *et al.*, 2000). Mostly cortisol is released from anterior region of the gills (a passive 'leakage') due to concentration gradient among plasma and surrounding water (Scott *et al.*, 2002). Many investigators adopted both invasive (From blood) and non invasive (though water) techniques for examining the difference in corticosteroids response to stressors in various fish species and showed variable results with respect to the cortisol concentration and release rate. This is due to fact that magnitude of corticosteroid response in different fish species depends upon the duration

and severity of stressor, environmental conditions and developmental stage of fish (Ellis, 2004; Barton, 2002).

In the present study we used both invasive and non invasive approaches to screen the possible effect of rearing environment on the physiology of fish. The plasma level of cortisol in wild Mahseer was 460.82 ± 38.24 ng/ml, significantly ($P < 0.0001$) higher than observed in captive reared Mahseer (383.33 ± 18.30 ng/ml). These findings support over non invasive results because it is well documented that in fish water-borne cortisol concentration and release rate is correlated with the plasma cortisol concentration (Ellis *et al.*, 2004). Furthermore, the results further provide evidence to the negative impact of captive rearing environment on the behavior and stress response of fish.

The concentration of plasma cortisol observed in both population falls within the range as observed in other fish species. Blood sampling itself is a stressful event that involves capturing and handling of fish. Therefore the plasma cortisol level in both wild and captive reared fish were higher than observed in sea raven (*Hemitripterus americanus*), 260 ng/ml at 4 hr after intense stress (Moon and Vijayan, 1994). Similarly, hatchery reared rainbow trout (*Salmo gairdneri*) also showed lower levels of plasma cortisol (155 ng/L) during net confinement as compared to captive reared Mahseer. However, peak level of cortisol 480 ng/ml during net confinement in wild rainbow trout was similar as we observed in wild Mahseer after mild handling stress. The increased in plasma cortisol depend on various factors including fish species, population, strength, duration and environmental factor. Because of these fact different species and population showed variable stress response even against similar stressor (Zuberi *et al.*, 2011; Woodward and Strange, 1987). Some fish species showed low level of stress response while in others like striped bass (*Morone saxatilis*), plasma cortisol levels even reached to 2000 ng/ml (highest levels documented) during transport (Mazik *et al.*, 1991). Although variation in plasma level depends on various factors but in the present study the difference reveal the population effect because we adopted the same blood collecting methodology for both wild and captive reared fish.

Captive rearing environment can cause morphological, physiological, behavioral and genetic differences that in turn influence the fitness of fish during restocking program (Kelley *et al.*, 2006; Heath *et al.*, 2003). It may cause behavior deficiencies due to intentional or un

intentional processes that effect the survival of species in wild environment (Brown and Laland, 2001; Snyder *et al.*, 1996). For example in captivity intensive culture system is in practice for the enhanced production by selecting different trait but this practice indirectly effect the other associated trait like higher growth rate related to aggression (Price, 1988). It is well known that captive bred fish display more aggressive behavior than wild counterpart. In captivity fish are engaged in aggressive interactions as compared to natural environment where wild fish are preoccupied with searching for food (Kelly *et al.*, 2006). Majority of the scientist related the aggressive behavior of captive bred fish with stocking density, sex, habitat structure etc while some suggest the involvement of biological factor like testosterone in modulating aggressive behavior (Carré and McCormick, 2008; Simpson, 2001).

In the present study, the mean plasma testosterone concentration in hatchery reared fish was significantly ($P < 0.05$) higher as compared to wild fish. Many investigators showed the strong relationship between testosterone level and aggression / dominant behaviors (Trainor and Nelson, 2007;Simpson, 2001). In animal aggression is considered as a response of some of stimuli which conveys deleterious stimuli to other individual. Although the animals can show various forms of aggression like fear-induced, predatory, irritable, territorial, maternal and inter-male (Moyer, 1968) but androgens only showed association with territorial and inter-male aggression and do not show any involvement with other type of aggression like predatory attack (Bermond *et al.*, 1982). Later on many scientists working on different species of vertebrates and invertebrates also provided the evidence and support of view that testosterone is associated with inter-male aggression and territorial defense (insects: Trumbo, 2007; Scott, 2008; rodents: Oyegbile and Marler, 2005; non-human primates: Muller and Wrangham, 2004; Cristobal-Azkarate *et al.*, 2006; birds: Wingfield *et al.*, 1990; fish: Oliveira *et al.*, 1996). According to them testosterone level is generally associated with situational cues, mainly cues signaling intra-sexual competition and dominant behaviors (Wingfield *et al.*, 1990).

Rearing environment can promote aggressive behavior in fishes (reviewed: Ruzzante, 1994). For example, rainbow trout (*Oncorhynchus mykiss*) showed increased aggressive and dominant behavior, when fish provided structurally enriched hatchery tanks or stream environments (Berejikian *et al.*, 2001). According to Ruzzante (1994) in captivity selective processes can favor aggressive behavior like if aggression favors the individual to gain more

food in feeding completion (e.g. feeding regime). It is also reported that in high density tank, fish become habituated to take food from particular part of tank/pond, thus dominant fish adopted to defend that particular area for receiving food. In this situation the appearance of aggression is the result of un-intentional selection (Kelley *et al.*, 2006). However, intentional selection for fast growth in salmonids, may affect the endocrine regulation systems which in turn could affect the appearance of other behaviors like risk-sensitive behaviors and aggression (Fleming *et al.*, 2002).

In the present study, we collected the hatchery reared fish from Mahseer hatchery Hattain where semi-intensive culture system in earthen pond is in practice. It seems that at hatchery, the habitat structure, water quality and other environment condition are different as compared to natural environment, therefore captive reared Mahseer showed difference in the physiological stress response and testosterone levels. The difference between hatchery reared and wild fish can be decrease by changing the structure of habitat (Mikheev *et al.*, 2005). In literature, scientist showed variable effect of habitat complexity on the behavior of fish results like male butterfly splitfins showed more aggressive behavior in the structured environment compared to unstructured one (Kelley *et al.*, 2011) while zebrafish and brown trout showed a decrease in aggression with increasing habitat complexity (Sundbaum and Naslund, 1998). Moreover, Mikheev *et al.* (2005) reported increased aggression in perch (*Perca fluviatilis*) in the presence of a shelter. From the studies it appears that stocking density, culture condition, feeding practices and habitat complexity are the major contributing fact in modulating the aggression in fish but all these factors are directly related to fish species because every species is unique in its requirement.

In our study, plasma testosterone levels (0.66 ± 0.15 ng/ml) in hatchery reared Mahseer (*T. putitora*) were comparable to those reported by Ismail *et al.* (2011) in captive Mahseer (*Tor tambroides*) (0.83 ng/ml).Conversely, depending upon the fish species, plasma testosterone levels show remarkable variation as reported in various literatures. For example, the testosterone level was 0.125-0.117 ng/ml in rohu *Labeo rohita* (Sen *et al.*, 2002) and 0.06 ng/ml in giant Mekong catfish (*P.gigas*) (Manosroi *et al.*, 2003). The variation may be due to species difference and the stage of collection of samples because it is sex related hormone and showed seasonal variation (Ismail *et al.*, 2011; Zuberi *et al.*, 2002). Although in the present study both wild and captive reared fish were collected in the same time of the year and showed variation in

testosterone level but still further extensive research is required to screen the possible role of this hormone in the aggression of fish.

We also observed inverse relationship between plasma testosterone and cortisol level in both wild and captive reared. In wild population plasma cortisol level was high but testosterone level was low as compared to captive reared counterpart where testosterone level was high and cortisol level was low. It seems that cortisol level suppress the level of testosterone. This suppressive effect of cortisol was also observed in mature male brown trout *Salmo trutta* L (Pickering *et al.*, 1987). According to Saez *et al.* (1977) the increase plasma corticosteroids in stress conditions can block the synthesis and release of androgens in plasma. There are many factors that can elevate or suppress the secretion of testosterone like social interaction, pH, toxin seasonal variation etc (Truscott *et al.*, 1983; Freeman *et al.*, 1983; Lee and Gerking., 1980). Freeman *et al.* (1983) observed higher plasma testosterone concentrations and 11-ketotestosterone levels in Atlantic salmon (*Salmo salar*) inhabited Medway River having pH 5.6 as compared to dwelling in Westfield River where pH was 4.7. Testosterone, a sex steroid, is directly involved in reproductive behavior. In view of the involvement of number of factors in the status of testosterone more comprehensive experiment are required to confirm the relationship of testosterone and aggressive behavior in Mahseer.

The results of present study reveal the impact of rearing environment in shaping the behavior, development of brain and physiological stress response of fish. The smaller brain to body size ratio, reduced optic tectum, atypical stress response and high level of testosterone in captive reared Mahseer as compared to wild counterpart indicated the difference in both population and support the data that showed the negative impact of hatchery rearing environment

References

REFRENCES

Alvarez, D., Nicieza, A.G. (2003). Predator avoidance behavior in wild and hatchery-reared brown trout: the role of experience and domestication. J Fish Biol 63(6):1565-1577.

Barcellos, L.J.G., Kreutz, L.C., Quevedo, R.M., Fioreze, I., Rodrigues, L.B., Soso, A.B., Ritter, F., Conrad, J., Cericato, L., Fagundes, M., Lacerda, L.A., Terra, S. (2004). Hematological changes in jundia (Rhamdia quelen Quoy & Gaimard, Pimelodidae) provoked by usual Aqua practices, with emphasis on immunosuppressive effects. Aqua 237: 229-236.

Arends, R.J., Mancera, J.M., Munoz, J.L., Wendeler-Bonga, S.E. (1995). Effects of selected hormones and male cohorts on final oocyte maturation, ovulation, and steroid production in walleye *Sitzosedion iitreum.* Aqua 138: 331-347.

Barton, B. A. (2000). Salmonid fish differ in their cortisol and glucose response to handling and transport stress. Nor. Am J Aquat. 62: 12-18.

Barton, B.A. (1997). Stress in finfish: Past, present and future - A historical perspective. Cambridge Univ. Press, Cambridge, UK. pp. 1-33.

Barton, B.A. (2002). Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids. Integ and Comp Biol 42: 517-525.

Barton, B.A., Iwama, G.K. (1991). Physiological changes in fish from stress in Aqua with emphasis on the response and effects of corticosteroids. Ann Rev Fish Dis 1: 3-26.

Barton, B.A., Ribas, L., Acerete, L., Tort, L. (2005). Effects of chronic confinement on physiological responses of juvenile gilthead sea bream, Sparus aurata, to acute handling. Aquacult. Res. 36: 172-179.

BenTov,P. R., Doron,M. E., Iavnilovitch, E., Rishal, I., Dagan, S,Y., Tsoory, M., Coppola, G., McDonal, M.K.,Gomes, C., Geschwind, D,H., Twiss, J.L., Yaron, A.,Fainzilber, M. (2012). Subcellular knockout of importin βl perturbs axonal retrograde signaling. Neuron 75:294-305

Berejikian, B.A., Tezak, E.P., Schoder S.L., Knudsen, C.M., Hard, J.J. (1997). Reproductive behavioral interactions between wild and captively reared coho salmon *(Oncorhynchus kisutch).* ICES J Mar Sci 54: 1040-1050.

Bermond, B., Mos, J., Meelis, W., Vander Pod, A.M., Kruk, M.R. (1982). Aggression induced by stimulation of the hypothalamus: Effects of androgens. Pharmacol Biochem Behav. 16: 41-45.

Bhatt, J.P., Nautiyal, P., Singh, H.R. (2000). Population structure of Himalayan Mahseer, a large cyprinid fish in the regulated foothill section of the River Ganga. Fish Res 44: 267-271.

Bhatt, J.P., Nautiyal, P., Singh, H.R. (2004). Status (1993-1994) of the endangered fish Himalayan Mahseer *T. putitora* (Hamilton) (Cyprinidae) in the mountain reaches of the River Ganga. J Asian Fish Soc 17: 341-355.

Billard, R., Gillet, C. (1981). Stress, environment and reproduction in teleost fish. Academic Press, New York. pp. 185-208.

Billy, A.J., Liley, N.R. (1985). The effects of early and late androgen treatments on the behavior of *Sarotherodon mossambicus* (Pisces: Cichlidae). Horm Behav 19: 311-330.

Braithwaite, V.A., Salvanes, A.G.V. (2005). Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild populations. Proc. Biol. Sci. 272(1568): 1107-1113.

Breves, J.P., Specker, J.L. (2005). Cortisol stress response of juvenile winter flounder *(Pseudopleuronectes americanus,* Walbaum) to predators. J Exp Mar Bio and Ecol 325: 1-7.

Breves, J.P., Specker, J.L. (2005). Cortisol stress response of juvenile winter flounder *(Pseudopleuronectes americanus,* Walbaum) to predators. J Exp Mar Bio and Ecol. 325: 1-7.

Brockunark, S., Adriaenssens, B., Johnsson, J.I. (2010). Less is more: density influences the development of behavioural life skills in trout. Proc. R. Soc. Lond. B Biol. Sci. 277(1696): 3035-3043.

Brown, C., Laland, R. (2002). The future of stock enhancements: lessons for hatchery practice from conservation biology. Fish and Fisheries 3: 79-94.

Brown, C., Day, R. (2002). The future of stock enhancements: bridging the gap between hatchery practice and conservation biology. Fish and Fisheries 4: 79-94.

Brydges, N.M., Braithwaite, V.A. (2009). Does environmental enrichment affect the behaviour of fish commonly used in laboratory work. Appl Anim Behav Sci 118: 137-143.

Bshary, R., Wickler, W., Fricke, H. (2002). Fish cognition: a primate's eye view. Animal Cognition 5: 1-13.

Burgess, J.W and Coss, R.G. (1982). Crowded jewel fish show changes in dendritic spine density and spine morphology. Neurosci Lett 17: 277-281.

Burns, J.G., Saravanan, A., Rodd, F.H. (2009). Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology 115(2): 122-133.

Cardwell, J.R. Liley, N.R. (1991). Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viride* (Scaridae). Horm Behav 25: 1-18.

Carragher, J.F., Sumpter, J.P. (1990). The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. Gen Comp Endocrinol 77: 403-407.

Carragher, J.F., Sumpter, J.P., Pottinger, T.G., Pickering, A.D. (1989). The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. Gen Comp Endocrinol 76: 310-321

Carre, J.M., McCormick, C.M. (2008). Aggressive behaviour and change in salivary testosterone concentrations predict willingness to engage in a competitive task. Horm Behav 54: 403-409.

Chandra, K.J. (1977). Studies on the biology of Tor spp. in natural and artificial waters of Bangladesh. MSc thesis, Bangladesh Agricultural University, Mymensingh. p. 47.

Chandra, K.J., Aminul Hague, A.K.M. (1982). Studies on the biology of Tor spp. in natural and artificial waters of Bangladesh: II—Taxonomy. Bangladesh J Fish 2-5: 59-64.

Chapman, L., Albert, J., Galls, F. (2008). Developmental Plasticity, Genetic Differentiation, and Hypoxia-induced Trade-offs in an African Cichlid Fish. Open Evo Jour 2: 75-88.

Chilcote, M.W., Leider, S.A., Loch, J.J. (1986). Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Trans Am Fish Soc 115: 726-735.

Clemens, H.P., Inslee, T. (1968). The production of unisexual broods of *Tilapia mossambica* sex-reversed with methyltestosterone. Trans Am Fish Soc 97: 18-21.

Costantini, D. (2011). On the measurement of circulating antioxidant capacity and the nightmare of uric acid. Methods Ecol Evol 2: 321-325.

Cox, R.M., John-Adler, H.B. (2005). Testosterone has opposite effects on male growth in lizards *(Sceloporus* spp.) with opposite patterns of sexual size dimorphism. J Exp Biol 208: 4679-4687.

Cristobal-Azkarate, J., Chavira, R., Boeck, L., Rodriguez-Luna, E., Vea, J.J. (2006). Testosterone levels of free-ranging resident mantled howler monkey males in relation to

the number and density of solitary males: a test of the challenge hypothesis. Horm Behav 49:261-267.

Dahanukar, N., Paingankar, M., Rant, R.N., Kharat, S.S. (2012). Fish fauna of Indrayani River, Northern Western Ghats, India. Jour Threat Taxa 4(1): 2310-2317.

Daniels, H.V., Watanabe, W.O. (2010). Practical Flatfish Culture and stock Enhancement. Blackwell Publishing USA. pp. 303-317.

de Silva, S.S., Abery, N.W., Nguyen, T.T.T. (2007). Endemic freshwater finfish of Asia: distribution and conservation status. Divers Distrib 13: 172-184.

Diamond, J. (2002). Evolution, consequences and future of plant and animal domestication Nature 418: 700-707.

Dudgeon, D., Arthington, A., Gessner, M., Kawabata, Z., Knowler, D., Leveque, C., Naiman, R., Prieur-Richard, A., Soto, D., Stiassny, M., Sullivan, C. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev 8(12): 163-182.

Ebinger, P., Rohs, M. (1995). Domestication and plasticity of brain organization in mallards *(Anas platyrhynchos)*. J Brain Res 36: 219-228.

Ebner, B.C., Thiem, J.D. (2009). Monitoring by telemetry reveals differences in movement and survival following hatchery or wild rearing of endangered fish. Marine and Freshwater Research 60: 45-57.

El Balaa, R., Blouin-Demers, G. (2011). Anti-predator behavior of wild-caught vs captive-breed freshwater angelfish, *Pterophyllum scalare*. Jour App Ichthyo 426: 1439-1740.

Einum, S., Fleming, LA. (2001) Implications of stocking: ecological interactions between wild and released salmonids. Nordic J Freshwater Res 75: 56-70.

Ellis, T., James, J.D., Stewart, C., Scott, A.P. (2004). A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. J Fish Biol 65: 1233-1252.

Faherty, C.J., Kerley, D., Smeyne, R.J. (2003). A Golgi Cox morphological analysis of neuronal changes induced by environmental enrichment. Brain Res Dev Brain Res 141: 55-61.

Fairhurst, G.D., Frey, M.D., Reichert, J.F., Szelest, I., Kelly, D.M., Bortolotti, G.R. (2011). Does environmental enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. Fish Biol 64:851-862.

Fernald, R.D. (1976). The effect of testosterone on the behavior and coloration of adult male cichlid fish *(Haplochomis burtoni* Gunther). Horm Res 7: 172-178.

Fevolden, S.E., Refstie, T., Gjerde, B. (1993). Genetic and phenotypic parameters for cortisol and glucose stress response in Atlantic salmon and rainbow trout. Aqua 118: 205-216.

Fevolden, S.E., Roed, K.H., Fjalestad, K.T., Stien, J. (1999). Post-stress levels of lysozyme and cortisol in adult rainbow trout *(Oncorhynchus mykiss);* heritabilities and genetic correlations. J Fish Biol 54: 900-910.

Flagg, T. A., Berejikian, B. A., Colt, J. E., Dickhoff, W. W., Harrell, L. W., Maynard, D. J., Nash, C., E., Strom, M. S., Iwamoto, R. N., Mahnken, C. V. W. (2000). Ecological and behavioral impacts of artificial production strategies on the abundance of wild salmon populations: A review of practices in the Pacific Northwest. NOAA Technical Memorandum. environmental sentinel: effects if stressors on whole-body physiological indices. J. Fish Bio1.61:207-229.

Fleming, I.A., Gross, M.R. (1993). Breeding success of hatchery and wild coho salmon *(Oncorhynchus kisutch)* in competition. Ecol App 3: 230-245.

Fleming, I.A., Agustsson, T., Finstad, B., Johnsson, J.I., Bjornsson, B.T. (2002). Effects of domestication on growth physiology and endocrinology of Atlantic salmon *(Salmo salar).* Can J Fish and Aqua Sci. 59:1323-1330.

Fleming, L.A., Lamberg, A., Jonsson, B. (1997). Effects of early experience on the reproductive performance of Atlantic salmon. Beh Ecol 8(5): 470-480.

Foo, J.T.W., Lam, T.J. (1993). Serum cortisol response to handling stress and the effect of cortisol implantation on testosterone level in the tilapia, *Oreochromis mossambicus.* Aqua 115: 145-158.

Ford, M.J. (2002). Selection in captivity during supportive breeding may reduce fitness in the wild. Conserv Biol 16: 815-825.

Fostier, A., Jalabert, B., Billard, R., Breton, B., Zohar, Y. (1983). The gonadal steroids. In Fish Physiology, Vol. 9A. Academic Press, New York. pp. 277-372.

Frankham, R. (2008). Genetic adaptation to captivity in species conservation programs. Mol Ecol 17: 325-333.

Freeman, H. C., Sangalang, G. B., Burns, G., and McMenemy. M. (1983). The blood sex hormone levels in, sexually mature male Atlantic salmon (Sahno salar) in the Westfield River (pH 4.7) and the Medway River (pH 5.6), Nova Scotia Sci Total Environ 32: 87-91.

Fridell, F., Gadan, K., Sundh, H., Taranger, G.L., Glette, J., Olsen, R.E., Sundell, K., Evensen, 0. (2007). Effect of hyperoxygenation and low water flow on the primary stress

response and susceptibility of Atlantic salmon *(Salmo salar)* to experimental challenge of IPN virus. Aqua 270: 23-35.

Froese, R., Pauly, D. (1999). Fish Base 99: *Concepts, structure, et sources des donnee.* ICLARM, Los Banos, Laguna, Philippines. p. 324.

Galhardo, L., Oliveira, R. F. (2014). The effects of social isolation on steroid hormone levels are modulated by previous social status and context in a cichlid fish. horm and behav 65: 1-5

Goncharova, N.D., Shmaliy, A.V., Bogatyrenko, T.N., Koltover, V.K. (2006). Correlation between activity of antioxidant enzymes and circadian rhythms of corticosteroids in *Macaca mulatta* monkeys of different ages. Exp Gerontol 41: 778-783.

Gonda, A., Herczeg, G., Merila, J. (2009a). Adaptive brain size divergence in nine-spined sticklebacks *(Pungitius pungitius).* J Evol Biol 22: 1721-1726

Gonda, A., Herczeg, G., Merila, J. (2009b). Habitat-dependent and —independent plastic responses to social environment in the nine-spined stickleback (Pungitius pungitius) brain. Proceedings of the Royal Society of London. Series B; Biological Sciences 276: 2085-2092.

Gonda, A., Herczeg, G., Merila, J. (2009). Habitat-dependent and —independent plastic responses to social environment in the nine-spined stickleback *(Pungitius pungitius)* brain. Proceedings of the Royal Society of London. Series B: Biological Sciences 276: 2085-2092.

Gonzalez-Voyer, A., and Kolm, N. (2010). Sex, ecology and the brain: evolutionary correlates of brain structure volumes in Tanganyikan cichlids. 5(12):14355-14360.

Gonzalez-Voyer, A., Winberg, S., Kolm, N. (2009). Social fishes and single mothers: brain evolution in African cichlids. Proceedings of the Royal Society of London B 276: 161-167.

Gozlan, R. E., St-Hilaire, S., Feist, S. W., Martin, P., Kent, M. L. (2005). Biodiversity-Disease threat to European fish. Nature 435: 1046.

Gray, R., The Telegraph, 30 July 2011; http://www.telegraph.co.uIc/earth/wildlife/ 8672417 / Third-of-freshwater-fish-threatened- with-extinction.html (accessed 10 February 2013).

Greenberg, N., Wingfield, J.C. (1987). Stress and reproduction: reciprocal relationships. Plenum, New York, pp. 461-503.

Gross, M. (1998). One species with two biologies: Atlantic salmon (Salmo salar) in the wild and in Aqua. Can J Fish Aquat Sci 55: 131-144.

Gross, M.R. (1998). One species with two biologies: Atlantic salmon *(Salmo salar)* in the wild and in Aqua. Can J Fish Aquat Sci 55: 131-144.

Hakansson, J., Jensen, P. (2005). Behavioural and morphological variation between captive populations of red junglefowl (Gallus gallus) — possible implications for conservation. Biol Conserv 122: 431-439.

Hard J.J., Berejikian, B.A., Tezak, E.P., Schroder, S.L., Knudsen, C.M., Parker. L.T. (2000). Evidence for morphometric differentiation of wild and captively reared adult coho salmon: a geometric analysis. Env Biol Fish 58: 61-73.

Haukenes, A.H., Barton, B.A. (2004). Characterization of the cortisol response following an acute challenge with lipopolysaccharide in yellow perch and the influence of rearing density. J Aggress Behav 22: 37-45

Healy, S.D., and Rowe, C. (2007). A critique of comparative studies of brain size. Proc Biol Sci 274: 453-464.

Heath, D.D., Heath, J.W., Bryden, C.A., Johnson, R.M., Fox, C.W. (2003). Rapid evolution of egg size in captive salmon. Science 299:1738-1740.

Hebb, D.O. (1947). The effects of early experience on problem solving at maturity. Am Psychol 2: 306-307.

Heggberget, T.G., Staurnes, M., Strand, R., Husby, J. (1992). Smoltification in salmonids. NINA-Norsk- instituttfor-naturforsking-forrsleingsrapport 31: 3-42.

Hofman, H. A. and Fernald, R. D. (2000). Social status controls somatostatin neuron size and growth. J Neurosci 20: 4740-4744.

Huber, R., Rylander, M.K. (1992). Brain morphology and turbidity preference in Notropis and related genera (Cyprinidae. Teleosteii). Env Biol Fish 33: 153-165.

Huber, R., Staaden, M.J., Kaufman, L.S., Liem, K.F. (1997). Microhabitat use, trophic patterns and the evolution of brain structures in African cichlids. Brain Behav Evol 50: 167-182.

Huffman, L.S., Mitchell, M.M., O'Connell, L.A., Hofmann, H.A. (2012). Rising StARs: behavioral, hormonal, and molecular responses to social challenge and opportunity. Horm Behav 61: 631-641.

Huffman, L. S., Lauren A., O'Connell, H., Hofmann, A. (2013). Aromatase regulates aggression in the African cichlid fish *Astatotilapia burtoni.* Physiol behav 112-113: 77-83.

Huhman, K.L., Moore, T.O., Ferris, C.F., Mougey, E.H., Meyerhoff, J.L. (1991). Acute and

repeated exposure to social conflict in male golden hamsters—increases in plasma POMC peptides and cortisol and decreases in plasma testosterone. Horm Behav 25: 206-216.

Hunter, G.A., Donaldson, E.M. (1983). Hormonal sex control and its application to fish culture. Academic Press, New York. pp. 223-303.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandoe, P., Turnbull, J.F. (2006). Current issues in fish welfare. J Fish Biol 68: 332-372.

Hussain, M.G., Hossain, M.A. (1999). Controlled breeding technology and step for conservation of gene pool of certain endangered fish species of Bangladesh. Fish Newsl 7: 2-3.

Ishikawa, Y., Yoshimoto, N., Ito, H. (1999). Different brain morphology from different genotypes in a single teleost species, the medaka *(Oryzias latipes).* Brain Behav Evol 53: 2-9

Ismail, M.F.S., Siraj, S.S., Daud, S.K., Harmin, S.A. (2011). Association of annual hormonal profile with gonad maturity of mahseer *(Tor tambroides)* in captivity. J Biol 170:125-130.

Ito, H., Ishikawa, Y., Yoshimoto, M., Yamamoto, N., 2007. Diversity of Brain Morphology in Teleosts: Brain and Ecological Niche. Brain Behav Evolut 69: 76-86.

IUCN. (2012). The IUCN Red List of Threatened Species, Version 2 http://www.iucnredlistorg (downloaded on 17 October 2012).

Iwama, G.K., Afonso, L.O.B., Vijayan,M.M. (2006). Stress in fish, In: Evans, D.H., Claiborne, J.B.m (Eds.), The Physiology of Fishes, Third ed. CRC Press, Boca Raton, Florida, pp. 319-342.

Jha, B.R. and Rayamajhi, A. (2009). *Tor putitora* In: IUCN 2011. IUCN Red list of threaten species. Version 2011.1. www.iucnredlistorg

Jonsson, J.I., Hojesjii, J., Fleming, I.A. (2001). Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. Can J Fish Aquat Sci 58: 788-794.

Jonsson, N., Jonsson, B. and Hansen, L. P. (2003). The marine survival and growth of wild and hatchery-reared Atlantic salmon. J App! Ecol 40: 900-911.

Kelley, J.L., Magurran, A.E., Garcia, C.M. (2006). Captive breeding promotes aggression in an endangered Mexican fish. Biol Conservat 133:169-177.

Kelley, M.R., Lou, M., Reed, A., Su, D., Delaplane, S., Borch, R.F., Nyland, R.L, Gross, M.L. Georgiadis, M. (2011). Functional analysis of new and novel analogs of E3330 that block the redox signaling activity of the multifunctional AP endonuclease/ redox signaling enzyme APE1/ Ref-1. Antioxid Redox Signal 14: 1387-1401.

Kihslinger, R.L., Lema, S.C., Nevitt, G.A. (2006). Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus tshawytscha.* Comp Biochem Physiol Part A Mol Integr Physiol 145: 145-151.

Kime, D.E., Manning, N.J. (1982). Seasonal patterns of free and conjugated androgens in the brown trout *Salmo trutta.* Gen Comp Endocrinol 48: 222-231.

Kolb, B. Whishaw, I. Q. (1998). Brain plasticity and behavior. Annual Review of Psychology 49: 43-64.

Kolb, B., Whishaw, I.Q. (1998). Brain plasticity and behavior. Ann Rev of Psych 49: 43-64. **Kolm, N., Gonzalez-Voyer, A., Brelin, D., Winberg, S. (2009).** Evidence for small scale variation in the vertebrate brain: mating strategy and sex affect brain size and structure in wild brown trout *(Salmo trutta).* Jour Evo Bio 22: 2524-2531.

Kotrschal, K., Palzenberger, M. (1992). Neuroecology of cyprinids (Cyprinidae, Teleostei): Comparative, quantitative histology reveals diverse brain patterns. Environ Biol Fish 33: 135- 152.

Kotrschal, K., Van Staaden, M.J., Huber, R. (1998). Fish brain: evolution and environmental relationship. Rev Fish Biol Fisheries 8: 373-408.

Kottelat, M. et al. (2012). In The Status and Distribution of Freshwater Biodiversity in IndoBurma (compilers: Allen, D. J. et al.), IUCN, Cambridge, UK and Gland, Switzerland, p. 158.

Kramer, B., Molenda, W., Fiedler, K. (1969). Behavioural effect of the antiandrogen cyproterone acetate (Schering) in *Tilapia mossambica* and *Lepomis gibbosus.* Gen Comp Endocrinol 13: 515.

Kruska, D. (1988). Mammalian domestication and its effect on brain structure and behavior. In: Intelligence and Evolutionary Biology (Jerison, H. J. & Jerison, I., eds). Springer-Verlag, Berlin, pp. 211-250.

Kruska, D. (1996). The effect of domestication on brain size and composition in the milk *(Muska vison).* Jour Zool (London) 239: 645-661.

Kruska, D. (1988). Mammalian domestication and its effect on brain structure and behavior. Springer-Verlag Berlin. pp. 211-250.

Lee, R. M., and Gerking, S. D. (1980). Survival and reproductive performance of the desert pupfish, Cyprinodorz n. nevadensis (Eigenmann and Ei-genmann), in acid waters. I Fish Rio 17: 507-515.

Lefebvre, L., Sol, D. (2008). Brains, lifestyles and cognition: Are there general trends? Brain Behav Evol 72: 135-144.

Lema, S. C., Hodges, M. J., Marchetti, M. P. Nevitt, G. A. (2005). Proliferation zones in the salmon telencephalon and evidence for environmental influence on proliferation rate. Comp. Biochem Physiol 141:327-335.

Licht, P., McCreery, B.R., Barnes, R., Pang, R. (1983). Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana.* Gen Comp Endocrinol 60: 252-258.

Liley, N.R., Breton, B., Fostier, A., Tan, E.S.P. (1986). Endocrine changes associated with spawning behavior and social stimuli in a wild population of rainbow trout *Salmo gairdneri.* Gen Comp Endocrinol 62(1): 145-156.

Lacy, N.R., Olsen, K.H., Foote, C.J. (1991). Reproductive pheromones in rainbow trout, *Oncorhynchus mykiss,* and kokanee salmon, *0. nerka.* FishSymp 91, Sheffield. pp. 188-190.

M.W. Chilcote, K.W. Goodson, and M.R. Falcy. (2011). Reduced recruitment performance in natural populations of anadromous salmonids associated with hatchery-reared fish. Can J Fish Aquat Sci 68: 511-522.

Magee, S.E., Neff, B.D., Knapp, R. (2006). Plasma levels of androgens and cortisol in relation to breeding behavior in parental male bluegill sunfish, *Lepomis macrochirus.* Horm Behav 49: 598-609.

Maisse, G., Pinson, A., Maurice, L. (1988). Caracterisation de l'aptitude a la congelation du sperme de truite arc-en-ciel *(Salmo gairdneri)* par des criteres physico-chimiques. Aquatic Living Resources 1: 45-51.

Marchetti, M.P., Nevitt, G.A. (2003). Effect of hatchery rearing on brain structures of rainbow trout, *Oncorhynchus mykiss.* Env biol Fish 66: 9-14

Mathews, F., Orros, M., McLaren, G., Gelling, M., Foster, R. (2005). Keeping fit on the ark: assessing the suitability of captive-bred animals for release. Biol Conserv 121:569-577.

Mayer, I., Meager, J., Skjxraasen, J., Rodewald, P., Sverdrup, G., Fernii, A. (2011). Domestication causes rapid changes in heart and brain morphology in Atlantic cod *(Gadus morhua).* Environ Biol Fishes 92: 181-186.

Maynard, D., Riley, S., Berejikian, B., Flagg, T. (2001). Development of a Natural Rearing System to Improve Supplemental Fish Quality. Anim Behav 71: 265-277.

Mazik, P. M., B. A. Simco, and N. C. Parker. (1991). Influence of water hardness and salts on survival and physiological characteristics of striped bass during and after transport. Trans Am Fish Soc 120:121-126.

McCarter, S.F. (2012). Neolithic. Routledge, New York.

McGinnity, P., Prodohl, P., Ferguson, K., Hynes, R., O'Maoileidigh, N., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J., Cross, T. (2003). Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London Series B Biol Sci 270: 2443— 2450.

Michael, T.P., Salome, P.A., Yu, H.J., Spencer, T.R, Sharp, E.L., McPeek, M.A, Alonso, J.M, Ecker, J.R and Mcclung, C.R. (2003). Enhanced fitness conferred by naturally occurring variation in circatdian clock. Science 302: 1049-1053.

Mijkherjee, M., Praharaj, A., Das, S. (2002). Conservation of endangered fish stocks though artificial propagation and larval rearing in West Bengal. India Aquacult Asia 7: 8-11.

Mikheev, V.N., Pasternak, A.F., Tischler, G., Wanzenbock, J. (2005). Contestable shelters provoke aggression among 0 + perch, *Perca fluviatilis*. Environmental Biology of Fishes 73: 227-231.

Miranda, R., Meyerson, L.A., Myers, R.R., Lovallo, W.R. (2003). Altered affective modulation of the startle reflex in alcoholics with antisocial personality disorder. Alcohol Clin Exp Res 27: 1901-1911.

Mirescu, C., Peters, J. D., Noiman, L., Gould, E. (2006). Sleep deprivation inhibits adult eurogenesis in the hippocampus by elevating glucocorticoids. PNAS 103: 19170 —19175.

Miura, T., Yamauchi, K., Takahashi, H., Nagahama, Y. (1992). The role of hormones in the acquisition of sperm motility in salmonid fish. J Exp Zool 261: 359-363.

Monosroi, J., Scipalakit, P., Manosroi, A. (2003). Factor affecting the biotransformation of chlormadinone acetate to delmadinone acetate. J Mol Cataly Enzym 23: 37-42.

Moore, I.T., Hopkins, W.A. (2009). Interactions and trade-offs among physiological determinants of performance and reproductive success. Integr Comp Biol 49: 441-451.

Moore, M.C., Thompson, C.W., Marler, C.A. (1991). Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in the tree lizard, *Urosaurus ornatus*. Gen Comp Endocrin 81: 217-226.

Moyer, K.E. (1968). Kinds Of Aggression And Their Physiological Basis. Communica In Behav Biol 2: 65-87.

Muller,M. N., Wrangham. R. W. (2004). Dominance, aggression and testosterone in wild chimpanzees: a test of the 'challenge hypothesis'. Animal Behavior 67: 113-123.

Naftoli, F., Garcia-Segura, L.M., Keefe, D. (1990). Estrogen effects on the synaptology and neural memebranes of the rat hypothalamic arcuate nucleus. Biol Reprod 42: 21-28.

Nautiyal, P. (2006). Rising awareness and efforts to conserve the Indian mahseer. Curr Sci 91: 1604.

Nelson, R.J. (2000). An Introduction to Behavioral Endocrinology. Sinauer Associates, Sunderland, Massachusetts, USA.

Newberry, R. C. (1995). Environmental enrichment: Increasing the biological relevance of captive environments. Appl Anim Behav Sci 44:229-243.

Nguyen, T.T.T., Na-Nakorn, U., Sukmanomon, S., Ziming, C. 2008. A study on phylogency and biogeography of Mahseer species (Pisces, Cyprinidae) using sequences of thee mitochondrial DNA gene regions. Mol Pyhlo Evo 48: 1223-1231.

Norman, L. (1987). Stream aquarium observation of territorial behaviour in young salmon *(Salmo salar* L.) of wild and hatchery origin — Salmon Research Institute Report, Swedon, 2. p.7.

Norris, D.O., Hobbs, S.L. (2006). The HPA axis and functions of corticosteroids in fishes. In: Reinecke, M., Zaccone, G., Kapoor, B.G. (Eds.), Fish Endocrinology. Science Publishers,

North, B. P., Turnbull, J. F., Ellis, T., Porter, M. J., Migaud, H., Bron, J. & Bromage, N. R. (2006). The impact of stocking density on the welfare of rainbow trout, Oncorhynchus mykiss. Aqua. 255: 466-479.

011a, B.L., Davis, M.W., Ryer, C.H. (1998). Understanding, how the hatchery environment represses or promotes the development of behavioural survival skills. Bull Mar Sci 62: 531-550.

Oliveira, R. F., Almada, V.C. (1999). Male displaying characters, gonadal maturation and androgens in the cichlid fish, *Oreochomis mossambicus.* Acta Ethol 2: 67-70

011a, B.L., Davis, M.W., Rayer, C.H. (1994). Behaviour deficit in hatchery-reared fish: potential effects on survival following release. Aquacult Fish Man Suppl 1: 19-34.

Oyegbile, T.0 and Marler, C.A. (2005). Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. Horm Behav 48(3): 259-67.

Ozon, R. (1972). Steroids in Non-mammalian Vertebrates. Academic Press, New York. pp. 329-389.

Pall, M.K., Mayer, I., Borg, B. (2002a). Androgen and behavior in the male thee-spined stickleback, *Gasterosteus aculeatus:* I. Changes in 11-ketotestosterone levels during the nesting cycle. Horm Behav 41: 377-383.

Pall, M.K., Mayer, I., Borg, B. (2002b). Androgen and behavior in the male thee-spined stickleback, *Gasterosteus aculeatus:* **II.** Castration and 11-ketoandrostenedione effects on courtship and parental care during the nesting cycle. Horm Behav 42: 337-344.

Park, P.J., and Bell, M.A. (2010). Variation of telencephalon morphology of the threespine stickleback *(Gasterosteus aculeatus)* in relation to inferred ecology. J Evol Biol 23: 1261-1277

Patel, S.S., Balfour, J.A., Bryson, H.M. (1997). Forfomycin tromethanine, A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infection. Drugs 53: 637-656.

Perry, F.S., Bernier, J.N. (1999). The acute humoral adrenergic stress response in fish: facts and fiction. Aqua 177: 285-295.

Petersson, E., Jarvi, T. (1997). Reproductive behavior of sea trout *(Salmo trutta)* the consequences of sea-ranching. Behav 134: 1-22.

Pickering, A.D. (1992). Rainbow trout husbandry: management of the stress response. Aqua 100: 125-139

Pickering, A.D., Chistie, P. (1981). Changes in the concentrations of plasma cortisol and thyroxine during sexual maturation of the hatchery-reared brown trout, *Salmo trutta* L. Gen Comp. Endocrinol 44: 487-496.

Pickering, A.D., Pottinger, T.G. (1989). Stress responses and disease resistance in salmonid fish: Effects of chonic elevation of plasma cortisol. Fish Physiol Biochem 7: 253-258.

Pickering, A.D., Pottinger, T.G., Carragher, J.F., Sumpter, J.P. (1987). The effects of acute and chonic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. Gen Comp Endocrinol 68: 249-259.

Pickering, A.D., Pottinger, T.G., Chistie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time course study. J Fish Biol 20: 229-244.

Pisolkar, M.D., Karamchandani, S.J. (1984). Fishery and biology of *Tor tor* (Hamilton) from Govindgarh lake (Madhya Pradesh). J Inland Fish Soc India 13: 15-24.

Pollen, A.A., Dobberfuhl, A.P., Scace, J., Igulu, M.M., Renn, S.C.P., Shumway, C.A., Hofmann, H.A. (2007). Environmental Complexity and Social Organization Sculpt the Brain in Lake Tanganyikan Cichlid Fish. Brain Behav Evol 70: 21-39.

Pottinger, T.G., Calder, G.M. (1995). Physiological stress in fish during toxicological procedures: a potentially confounding factor. Environ Toxicol Water Qual 10: 135-146.

Pottinger, T.G., Moran, T.A. (1993). Differences in plasma cortisol and cortisone dynamics during stress in two strains of rainbow trout *(Oncorhynchus mykiss).* J Fish Biol 43: 121-130.

Pottinger, T.G., Mosuwe, E.M. (1994). The corticosteroidogenic response of brown and rainbow trout alevins and fry during a "critical" period. Gen Comp Endocrinol 95: 350-362.

Pottinger, T.G., Pickering, A.D. (1985). Changes in skin structure associated with elevated androgen levels in maturing male brown trout, *Salmo trutta* L. J Fish Biol 26.

Price, E. 0. (1999). Behavioral development in animals undergoing domestication. Appl. Anim. Behav Sci. 65: 245-271.

Price, E.O. (1988). Behavior genetics and the process of animal domestication. In: Grandin, T. (Ed.), Genetics and the Behavior of Domestic Animals. Academic Press, New York, pp. 31-65

Qureshi, T.A. (2011). MAHSEER: An endangered FISH. Article in greaterkashmir.cominews

Raghavan, R., Ali, A. (2012). Hypselobarbus lithopidos. In IUCN Red List of Threatened Species. Version 2012.2; www.iucnredlist.org (accessed 16 February 2013).

Rahman M.A., Mazid, M.A., Rahman, M.R, Khan,M.N., Hossain,M.A.,. Hussain, M.G. (2005). Effect of stocking density on survival and growth of critically endangered mahseer, Tor putitora (Hamilton), in nursery ponds. Aqua 249: 275— 284.

Ramsay, J.M., Feist, G.W., Varga, Z.M., Westerfield, M., Kent, M.L., Scheck, *C.B.* (2006). Whole-body cortisol is an indicator of crowding stress in adult zebrafish, *Danio rerio.* Aqua 258: 565-574.

Reisenbichler, R.R and McIntyre. J.D. (1977). Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri.* J. Fish. Res. Board Can 34: 123-128.

Roberts, L.J., Taylor, J., Garcia de Leaniz, C. (2011). Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. Biol. Consery 144: 1972-1979.

Robertson, L., Thomas, P., Arnold, C.R. (1988). Plasma cortisol and secondary stress response of cultured red drum *(Scianops ocellatus)* to several transportation procedures. Aqua 68:115-130.

Rodewald, P., HyvArinen, P., Hirvonen, H. (2011). Wild origin and enriched environment promote foraging rate and learning to forage on natural prey of captive reared Atlantic salmon parr. Ecol Freshw Fish 20: 569-579.

Ros, A.F.H., Bruintjes, R., Santos, R.S., Canario, A.V.M., Oliveira, R.F. (2004). The role of androgens in the trade-off between territorial and parental behavior in the Azorean rock-pool blenny, *Parablennius parvicornis*. Horm Behav 46: 49-497.

Rosenzweig, M.R., Bennett, E.L. (1996). Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 78: 57-65.

Ruane, N.M., Komen, H. (2003). Measuring cortisol in the water as anindicator of stress caused by increased loading density in common carp *(Cyprinus carpio)*. Aqua 218: 685-693.

Ruzzante, D.E. (1994). Domestication effects on aggressive and schooling behaviour in fish. Aqua 120: 1-24.

Saez, J. M., Morera, A. M., Haour, F., and Evain, D. (1977). Effects of in vivp administration of dexa- methasone, corticotropin and human chorionic gonadotropin on steroidogenesis and protein and DNA synthesis of testicular interstitial cells in prepubertal rats. Endocrin 101: 1256-1263.

Salonen, A., Peuhkuri, N. (2006). The effect of captive breeding on aggressive behaviour of European grayling, *Thymallus thymallus*, in different contexts. Anim Behav 72: 819-825.

Salvanes, A., Braithwaite, V. (2005). Exposure to variable spatial information in the early rearing environment generates asymmetries in social interactions in cod *(Gadus morhua)*. Behav Ecol Sociobiol 59: 250-257.

Sangiao-Alvarellos, S., Polakof, S., Arjona, F.J., Garcia-Lopez, A., del Rio, M.P.M., Martinez-Rodriguez, G., Miguez, J.M., Mancera, J.M., Soengas, J.L. (2006). Influence of testosterone administration on osmoregulation and energy metabolism of gilthead sea bream *Sparus auratus*. Gen Comp Endocrinol 149: 30-41.

Schlesinger, W.H., Reynolds, J.F., Cunningham, G.L., Huenneke, L.F., Jarrell, W.M., Virginia, R.A ., Whitford, W.G. (1990). Biological feedbacks in global desertification. Sci 247: 1043-1048.

Scott, A.P., Pinillos, M., Ellis, T. (2001). Why measure steroids in fish plasma when you can measure them in water?/. Goos, H.J.Th., Rastogi, R.K., Vaudry, H., Pierantoni, R. (Eds.), Perspective in Comparative Endocrinology: Unity and Diversity. Monduzzi Editore, Bologna. pp.1291-1295.

Scott, P.A., Ellis, T. (2007). Measurement of fish steroids in water-a review. Gen. Comp. Endocrinol 153: 392-400.

Semsar, K and Godwin, J. (2004). Multiple Mechanism of phenotype development in the bluehead wrasse. Hormones and Behavior 45 :345-353.

Sen,U., Mukherjee, D., Bhattacharyya, S.P., Mukherjee,D. (2002). Seasonal changes in plasma steroid levels in Indian major carp *Labeo rohita:* influence of homologous pituitary extract on steroid production and development of oocyte maturational competence. Gen Comp Endcrinol 128: 123-134.

Shestha, C., Rai, A.K., Gurung, T.B., Mori, K. (1990). Successful artificial induced spawning of Himalayan Mahseer *(T. putitora* Hamilton) in Pokhara Valley, Nepal. In: Hirano, R., Hanyn, I. (Eds.), Proceedings of the Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp.573— 575.

Shumway, C. A. (2008). Habitat complexity, Brain and Behavior. Brain Behaviour and Evolution 72: 123-134.

Shyne, A. (2006). Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. Zoo Biol. **25: 317-337.**

Siikavuopio, Baardvik, B.M. and Jobling, M. (1996) Domestication effects on fin nipping, survival and growth in hatchery-reared Arctic charr, *Salvelinus alpines* (L.), in competition with wild conspecifics. Aqua Research 27: 205-211.

Simpson, K. (2001). The Role of Testosterone in Aggression. MJM 6: 32-40.

Sink, T., Kumaran, S., Lochmann, R. T. (2007). Development of a whole-body cortisol extraction procedure for determination of stress in golden shiners *(Notemigonus crysoleucas).* Fish Physiol Biochem **33: 189-193.**

Siraj, S.S., Z. Kamaruddin., M.K.A., Satar and MS Kamarudin. (2007). Mahseer: The Biology, Culture and Conservation, Malaysian Fisheries Society, Serdang, Malaysia, p. 235.

Slater, C.H., Scheck, C.B., Swanson, P. (1994). Plasma profiles of the sex steroids and gonadotropins inmaturing female spring chinook salmon *(Oncorhynchus tshawytscha).* Comp Biochem Physiol 109: 167-175.

Snyder, N.F.R., Derrickson, S.R., Beissinger, S.R., Wiley, J.W., Smith, T.B., Toone, W.D., Miller, B. (1996). Limitations of captive breeding in endangered species recovery. Conser Biol 23: 20-34.

Sorensen, P.W., Scott, A.P. & Kihslinger, R.L. (2000). How common hormonal metabolites function as relatively specific pheromonal signals in the goldfish. —In: Proceedings of the 6th international symposium on reproductive physiology of fish, Bergen, Norway, July 4-9,1999 (Norberg, B., Kjesbu, 0.S., Taranger, G.L., Andersson, E. & Stefansson, S.O., eds), p. 125-128.

Sorensen, P.W., Stacey, N.E., Chamberlain, K.J. (1989). Differing behavioral and endocrinological effects of two female sex pheromones on male goldfish. Horm Behav 23: 317-332.

Spence, R., Magurran, A., Smith, C. (2011). Spatial cognition in zebrafish: the role of strain and rearing environment. Anim Cogn 14: 607-612.

Squires, E.J. (2010). Applied Animal Endocrinology, 2nd ed. CAB International, Cambridge, spotless starlings. Am Nat 172: 42-53.

Stone, R. (2007). The last of the leviathans. Science 316:1684– 1688.

Sumpter, J.P., Dye, H.M., Benfey, T.J. (1986). The effect of stress on blood ACTH, a-MSH and cortisol levels in salmonid fishes. Gen Comp Endocrinol 62: 377-385.

Sundbaum, K., Naslund, I. (1998). Effects of woody debris on the growth and behaviour of brown trout in experimental stream channels. Can J Zoo 76: 56-61.

Szego, C,M. (1984). Mechanism of hormone action: parallels in receptor mediated signal propagation for steroid and peptide effectors. Life Sciences 35: 2383-2396.

Talwar, P.K., Jhingran, A.G. (1992). Inland Fishes of India and Adjacent Countries, vol. 1. A.A. Balkema, Rotterdam the reproductive performance of Atlantic salmon. Behav Evol 8: 470-480.

Theodorou, K., Couvet, D. (2004). Introduction of captive breeders to the wild: harmful or beneficial. Consery Gene 5: 1-12.

Trumbo, S.T. (2007). Can the 'challenge hypothesis' be applied to insects?. Hormones and Behavior 51: 281-285.

Truscott, B., Walsh, J. M., Burton, M. P., Payne, J. F., and Idler, D. R. (1983). Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. Comp Biochem Physiol 75: 121-130.

Turnbull, J., Bell, A., Adams, C., Bron, J., Huntingford, F. (2005). Stocking density and welfare in cage farmed Atlantic salmon: application of a multivariate analysis. Aqua 243: 121–132

Zuberi, A., Ali, S., Brown, C. (2011). A non-invasive assay for monitoring stress responses following simulated predator attack: A comparison between wild and captive-reared rainbow fish *(Melanoteania duboulayi).* Aqua 32: 267-272

Zuberi, A., Ali, S., Brown, C. (2014). Effect of confinement on water-borne and whole body cortisol in wild and captive-reared Rainbow fish *(Melanoteania duboulayi).* Int J Agric Biol 16: 183-188.

Zupanc, G. (2006). Neurogenesis and neuronal regeneration in the adult fish brain. J. Comp. Physiol A Neuroethol Sens Neural Behav Physiol 192: 649-670.

Zuberi, A., Ali, S., Brown, C. (2011). A non-invasive assay for monitoring stress responses following simulated predator attack: A comparison between wild and captive-reared rainbow fish *(Melanoteania duboulayi).* Aqua 32: 267-272

Zuberi, A., Ali, S., Brown, C. (2014). Effect of confinement on water-borne and whole body cortisol in wild and captive-reared Rainbow fish *(Melanoteania duboulayi).* Int J Agric Biol 16: 183-188.

Zupanc, G. (2006). Neurogenesis and neuronal regeneration in the adult fish brain. J. Comp. Physiol A Neuroethol Sens Neural Behav Physiol 192: 649-670.