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Seasonal changes in the estradiol level in different age groups of Amur common carp in Terai region of Uttarakhand

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Abstract
Fish reproduction is a synchronous of physiological elements along with environmental factors influenced by seasonal changes, and both this factors plays a key role in triggering the hormonal secretion leading to successful production of offspring's. A study on the estradiol (E2) level from gonads and plasma samples of two different age groups (1+ and 2+ years) of amur common carp for both male and female was conducted seasonally (spring, summer, autumn and winter) using RP-HPLC method. The study showed that both the age groups have similar seasonal pattern in gonadal and plasma E2 levels, with spring season showing highest level followed by summer and autumn respectively. A slight increase in E2 level was observed post autumn i.e. winter season. As compared to male counterpart, the peak gonadal E2 levels were observed during spring as 6.75±0.02 ng/mg and 4.69±0.09ng/mg for 2+ and 1+ old respectively. Similarly the peak plasma E2 levels of 7.55±0.01 ng/mg and 4.17±0.03ng/mg were recorded for 2+ and 1+ old during peak spring season respectively. Gonadal and plasma E2 showed significant differences (p<0.05) in relation to age, seasons and interaction (age & seasons) in both age groups. Using Pearson's correlations (p<0.01) significant positive results were observed between GSI with gonadal and plasma E2. The present study indicates the role of E2 and seasonal influences in regulating hormonal changes thus effecting reproductive success of Amur common carp, *Cyprinus carpio haematopterus*, during peak seasons of spring followed by summer and age factor playing a key role in reproduction. Therefore, fully matured 2 years and above age amur common carp are more feasible for breeding programme and spring season will be the most conducive season for reproduction.

Keywords: Amur common carp, estradiol, seasonal reproduction, Tarai region

Introduction
Physiological changes need to be restructured according to the seasonal based changes in order to accomplish particular biological functions, like reproduction (Tripathi and Verma, 2004) [29] and fish respond well to seasonal changes related to reproduction and environmental conditions (Dygert, 1990) [8]. Water quality parameters affect the reproductive process in most of the sub-tropical species (Cornish, 1998) [6]. Factors like temperature, food accessibility etc have also been reported to influence reproduction (Migaud *et al.*, 2010) [21]. Others like photoperiod in case of temperate fishes (Jobling, 1995) [16], daylight and temperature for non-seasonal spawners in tropical fishes like catfish (Manosroi *et al.*, 2003) [20] and tilapia (Campos-Mendoza *et al.*, 2004) [4] plays a major role for gonadal maturity. Hilder and Pankhurst (2003) [12] reported seasonal temperature as the major parameter for tropical fishes while photoperiod has less importance in tropical zones. Seasonal changes triggered some coordinated spawning in fishes like *Salmo trutta* (Katarzyna and Jozef, 2005) [17] and *Oncorhynchus kisutch* (Estay *et al.*, 1998) [9] in late winter while American shad (Hoffman *et al.*, 2008) [13] in early spring. Ovulation in common carp showed perennial spawning behavior and spawned multiple times per year with retention of more than 20% matured egg (Alikunhi, 1966) [1] observed extended seasonal breeding behavior in Cyprinus carpio ranging from August (late winter) to April (mid-autumn). Timing the release of steroidal hormones with the reproductive process favours the success of reproduction (Crews, 1984) [7]. Reproductive activities in female fish generally start with a low level of sex steroids especially 17β-estradiol during previtellogenesis, which gradually increases reaching to the peak level at the time of vitellogenesis. Among the estrogens groups, 17β-estradiol (E2) controls ovulation in female fish. The expression of vitellogenin (VTG) genes is regulated by circulating E2 in the hepatocytes, which result in the synthesis of several closely related vitellogenin proteins. The source of proteins for chorion synthesis in the liver, follows a similar pattern under the

stimulation of estradiol and are transported into the oocyte for the formation of the egg chorion (Hamazaki *et al.*, 1989; Oppen-Bemsten *et al.*, 1991) [10, 22]. Limited literatures are available on seasonal study based on different age groups on E2 for common carp.

Materials and Methods

Amur common carp (*C. c haematopterus*) with two age groups (1+ and 2+ year's old) were culture in designated pond A and B depending on the age groups at the College of Fisheries, G. B. Pant University of Agri. & Tech., Pantnagar (Uttarakhand). Regular feeding (containing 25% protein) was carried out once daily @ 3% body weight. The experimental pond site is located at about latitude of 29.01 °N, long 79.3 °E, 344 m above MSL which comes under the Tarai region of the Great Himalayas. Seasonal collection of fish samples were carried out from pond A and B. Prior to regular sample collection of blood and tissues the fish were anaesthetized using clove oil @ 30 mg/l (Velisek *et al.*, 2005) [30]. Blood samples are normally collected within 8-10 mins (as far as possible). Using a lithium heparin coated plasma tubes the blood samples were drawn and dispensed for hormonal estimation. The supernatant was collected in 2 ml micro centrifuge tubes after centrifuging the heparinized blood for 12 mins at 10,000 rpm (11200 x g) at temperature of 4 °C and analyzed immediately. From the gonads, approximately 5-10 gm of tissue samples was collected for hormonal estimation. Observations on water quality for both the experimental ponds including water temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS) and free carbon dioxide (CO_2) were recorded periodically. Using a digital meter, temperature, TDS (accuracy ±2%) and pH (accuracy 0.01 pH) were recorded whereas titrimetric method (APHA, 1992) [2] was used for measuring DO and CO_2. Pure hormones were purchased from Sigma India for standardization of the experimental protocol.

Hormonal Estimation

Steroidal hormones – Estradiol (E2) estimation from blood samples (plasma) were analysed using Reversed Phased High-Performance Liquid Chromatography (RP-HPLC) using Dionex Ultimate 3000 operated by Chromeleon software (version 6.8). Chromatographic conditions were performed according to set protocol of Soranganba and Singh, (2018) [26] including validation, quantitation, linearity, accuracy, stability, repeatability and precision of the assay. 100 mg volume of 1 ml standard PP-tubes [119855] of SPE (Solid Phase Extraction) LiChrolut RP-18 were used for each aliquot and pre-treated as per Budzinski *et al.*, (2006) [3] and Chen-Hao Zhai *et al.*, (2009) [5] with little modifications.

Statistical Analysis

Analysis of variance (ANOVA) as used for data analysis. Significance level at $p<0.05$ was considered for data differences. All data were expressed in mean ± SEM.

Results and Discussion

Plasma and gonadal E2 levels of both the age groups are shown in Tables 1 and 2. Both the 1+ and 2+ years old carps showed parallel gonadal and plasma E2 levels seasonally with maximum E2 level observed during spring season, which decreases' in summer and further continued to decrease to the minimum level in autumn followed by slender boost in winter season (Figures 1 & 2). Statistically significant differences ($p<0.05$) were observed in both age groups for gonadal and plasma E2 levels in relation to age, seasons and interaction (age & seasons). Female showed higher gonadal and plasma E2 levels than their male counterpart in both the age groups. Significantly positive correlation was observed between gonadosomatic index with gonadal E2 and plasma E2 as observed using Pearson's correlations ($p<0.01$).

Table 1: Gonadal E2 (ng/mg) Levels of Amur Common Carp in Different Seasons

Age groups	Summer season	Autumn season	Winter season	Spring season
Male 2+	0.64±0.01	0.29±0.01	0.55±0.01	0.84±0.01
Female2+	4.88±0.04	3.15±0.01	4.64±0.11	6.75±0.02
Male 1+	0.26±0.01	0.16±0.02	0.25±0.02	0.35±0.01
Female 1+	2.37±0.04	1.80±0.05	3.26±0.01	4.69±0.09

Data as mean ± SEM (n=5)

Table 2: Plasma E2 (ng/ml) Levels of Amur Common Carp in Different Seasons

Age Groups	Summer season	Autumn season	Winter season	Spring season
Male 2+	1.85±0.01	1.25±0.01	1.62±0.02	2.35±0.05
Female 2+	4.68±0.11	2.62±0.02	3.90±0.04	7.55±0.01
Male 1+	1.13±0.01	0.85±0.01	1.24±0.01	1.84±0.02
Female 1+	2.94±0.01	1.84±0.02	2.15±0.04	4.17±0.03

Data as mean ± SEM (n=5)

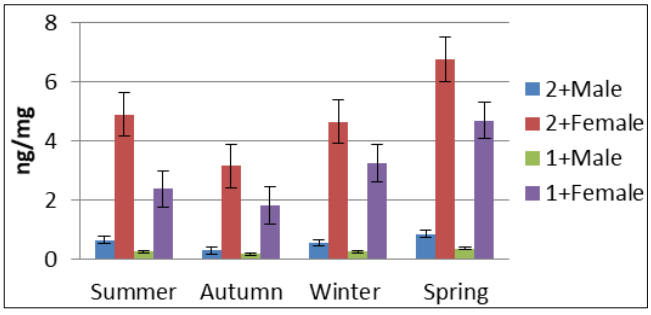

Fig 1: Gonadal E2

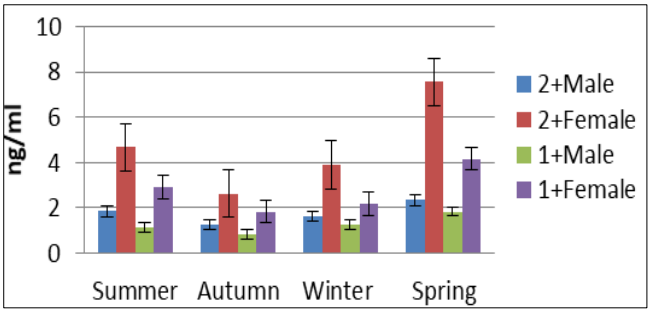

Fig 2: Plasma E2

The gonadal and plasma E2 levels showed significant seasonal and age related differences, showing peak during spring followed by summer season with higher levels in female than male. Variable seasonal and age related plasma E2 levels were reported in *Labeo rohita* (Suresh *et al.*, 2008) [27] similar pattern under the, *Rutilus frisii* Kutum (Heidari *et al.*, 2010) [11], *Xiphophorus nigrensis* (Ramsey *et al.*, 2011) [23], *Acipenser persicus* (Hosseinzade *et al.*, 2012) [14] and *Cyprinus carpio* (Taghizadeh *et al.*, 2013) [28]. Higher levels of E2 in female fish confirm the importance of this hormone in gonadal development and positive correlation with GSI further shows its role in gonadal development. Increase in ovarian GSI and development of oocyte associated with changes in E2 levels have been reported in *Lateolabrax maculatus* by Lee and Yang (2002) [18]. Slightly lower peak during summer corresponding with GSI is indicative of another spawning readiness in female. Higher level in older age groups (2+ years) might be due to higher hormonal demand correlated to higher reproductive performance as indicated by GSI levels. Roy *et al.*, (2001) [24] showed diurnal rhythm of E2 level only during preparatory and pre-spawning phases in the tropical freshwater catfish, *Clarias batrachus*. Significant positive correlation showed in the gonadal and plasma E2 with GSI in the present study is indicative of importance of this hormone in female gonadal maturation. The accumulation of yolk in oocyte cytoplasm with response to increase in plasma E2 levels were the main reason for increase of GSI during breeding season (Lubzens *et al.*, 2010[19]). Ismail *et al.*, (2011) [15] investigated the annual gonadal hormonal level of wild matured mahseer and observed a correlative change in reproductive hormones (E2) with the ovarian maturation. The rise and fall of plasma estrogen levels in freshwater catfish, *Clarias batrachus* towards regulating the gonadotropin level during its reproductive cycle have been clearly mentioned by Singh and Singh (1983) [25].

Conclusion

Based on the above study, Amur common carp has the most potent reproductive behavior in the spring season. Observations on changes in hormonal levels related to reproductive activity would be helpful in understand the age for onset of maturity in young Amur common carp. Higher levels of steroidal hormones correlating with peak seasons of spring and summer seems to favours enhance gonadal maturity and better prospect of spawning provided with suitable agro-climatic conditions. The present investigation might be helpful in mitigating any potential breeding programmes for achieving off-season seed production programme. Based on the present research, matured age group of 2+ years old may be considered as a competent candidate for exercising in any breeding programme than the younger age groups and showed better reproductive potential.

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Conflict of interest: None

References

1 Alikunhi KH. Synopsis of biological data on common carp, *Cyprinus carpio* (Linnaeus), 1758 (Asia and the Far East). Food and Agriculture Organization of the United Nations; c1966.
2 APHA. Standard method for water and wastewater examination, 17th edn. American Public Health Association, Washington, D.C; c1992.
3 Budzinski H, Devier MH, Labadie P, Togola A. Analysis of hormonal steroids in fish plasma and bile by coupling solid-phase extraction to GC/MS. Analytical and bioanalytical chemistry. 2006;386(5):1429-1439.
4 Campos-Mendoza A, Mc Andrew BJ, Coward K, Bromage N. Reproductive response of Nile tilapia (*Oreochromis niloticus*) to photoperiodic manipulation; effects on spawning periodicity, fecundity and egg size. Aquaculture. 2004;231:299-314.
5 Chen-Hao Zhai, Yun Zou, Rou-Nan Jin. Determination of Hormones in Fish (*Carassius carassius*) by Sampli QOPT Solid Phase Extraction with High Performance Liquid Chromatography. Application Note. Food safety. Agilent Technologies Co. Ltd; c2009.
6 Cornish AD. Seasonal steroid hormone profiles in plasma and gonads of the tilapia, *Oreochromis mossambicus*. Water SA, 1998, 24(3).
7 Crews, D. Gamete production, sex hormone secretion, and mating behavior uncoupled, Horm. Behav. 1984;18: 22-28.
8 Dygert P. Seasonal changes in energy content and proximate composition associated with somatic growth and reproduction in a representative age-class of female English sole. Trans. Am. Fish. Soc. 1990;119:791-801.
9 Estay F, Roberto N, Nelson FD, Luis V, Alfredo T. Gametogenesis and sex steroid profiles in cultured coho salmon (*Oncorhynchus kisutch*, Walbaum). The Journal of Experimental Zoology. 1998;280:429-438.
10 Hamazaki TS, Nagahama Y, Iuchi I, Yamagami K. A glycoprotein from the liver constitutes the inner layer of the egg envelope (*Zona pellucida* Intema) of the fish, Oryzias latipes. Dev. Biol. 1989;133:101-l 10
11 Heidari B, Roozati SA, Yavari L. Changes in plasma levels of steroid hormones during oocyte development of Caspian Kutum (*Rutilus frisii* Kutum, Kamensky, 1901). Anim. Reprod. 2010;7(4):373-381
12 Hilder ML, Pankhurst NW. Evidence that temperature change cues reproductive development in the spiny damselfish, *Acanthochromis polyacanthus*. Environmental biology of fishes. 2003;66(2):187-196.
13 Hoffman JC, Karin EL. Deborah AB. John EO. Overwintering habitats of migratory juvenile American shad in Chesapeake Bay. Environmental Biology of Fishes. 2008;81:329-345.
14 Hosseinzade M, Imanpoor MR. Aghilinejhad SM. Changes in Serum Steroid Hormones by Ovarian Development of Persian Sturgeon (*Acipenser Persicus*) during Final Maturation Induced by Hormonal Treatment. Scientific Reports. 2012;1:532.
15 Ismail MFS, Siraj SS, Daud SK, Harmin SA. Association of annual hormonal profile with gonad maturity of mahseer (*Tor tambroides*) in captivity. General and comparative endocrinology. 2011;170(1):125-130.
16 Jobling M. Environmental Biology of Fishes. Chapman and Hall, London, c1995, p.435.
17 Katarzyna D, Jozef D. Differentiation of gonad maturation in sibling precocious males of the sea trout (*Salmo trutta* L.) in their first year of life. Aquaculture 2005;250:713-725.
18 Lee WK, Yang SW. Relationship between ovarian

development and serum levels of gonadal steroid hormones and induction of oocyte maturation and ovulation in the cultured female Korean spotted sea bass *Lateolabrax maculatus* (Jeom-nongoeo). Aquaculture 2002;207:169-183
19 Lubzens E, Young G, Bobe J, Cerda J. Oogenesis in teleosts: how fish eggs are formed. Gen Comp Endocrinol. 2010;165:367-389
20 Manosroi A, Meng-Umphan A, Manosroi J. Annual sex hormonal profiles, gonad development and age determination of the Mekong giant catfish (*Pangasianodon gigas*, Chevey). Aquaculture Research. 2003;34: 1379–1385
21 Migaud H, Davie A, Taylor JF. Current knowledge on the photo neuroendocrine regulation of reproduction in temperate fish species. Journal of Fish Biology. 2010;76(1):27-68.
22 Oppen-Bemsten DO, Gram-Jensen E, Walther B. Origin of teleost eggshell ZR-proteins and their significance during oogenesis: *in vitro* liver synthesis of eggshell proteins induced by estradiol-17β In: AP Scott, JP. Sumpter, DE. Kime, MS. Rolfe (Editors), Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, Norwich, U.K. 7-12 July 1991, FishSymp91, Sheffield; c1991, p. 306-308.
23 Ramsey ME, Wong RY, Cummings ME. Estradiol, reproductive cycle and preference behavior in a northern swordtail. General and comparative endocrinology. 2011;170(2):381-390.
24 Roy SM, Lal B, Singh TP. Circadian variation in circulating levels of melatonin, testosterone and estradiol-17β in a tropical freshwater catfish, *Clarias batrachus*, during different phases of the annual reproductive cycle. Biological rhythm research. 2001;32(5):569-578.
25 Singh IJ, Singh TP. Annual changes in the total gonadotropic potency in relation to gonadal activity in the freshwater catfish (*Clarias batrachus* L.). J Interdiscipl. Cycle Res. 1983;14(3):227-239.
26 Soranganba N, Singh IJ. Simultaneous Determination of Fish Steroidal Hormones using RP-HPLC with UV Detection by Multi-step Gradient Elution Technique. Int. J Curr. Microbiol. App. Sci. 2018;7(2):1397-1404.
27 Suresh DVNS, Baile VV, Rao PP. Annual reproductive phase-related profile of sex steroids and their carrier, SHBG, in the Indian major carp, Labeo rohita. General and comparative endocrinology. 2008;159(2):143-149
28 Study the seasonal steroid hormones of common carp Taghizadeh V, Imanpoor MR, Mehdinejad N. in Caspian Sea, Iran. Springer Plus. 2013;2(1):193.
29 Tripathi G, Verma P. Sex-specific metabolic changes in the annual reproductive cycle of a freshwater catfish. Comp. Biochem. Physiol. Part B.2004;137:101-106.
30 Velisek J, Svobodova Z, Piackova V, Groch L, Nepejchalova L. Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). Vet Med. 2005;50(6):269-275.