www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(2): 21-27

www.thepharmajournal.com
Received: 10-12-2021
Accepted: 12-01-2022

GB Wani
Division of Fishery Engineering, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Jammu and Kashmir, India

AM Najar
Division of Fisheries Resource Management, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Jammu and Kashmir, India

SI Darve
Division of Fisheries Resource Management, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Jammu and Kashmir, India

FA Shah
Division of Aquatic Animal Health Management, Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Jammu and Kashmir, India

SA Dar
Division of Aquatic Animal Health Management, Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Jammu and Kashmir, India

Corresponding Author
SI Darve
Division of Fisheries Resource Management, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Jammu and Kashmir, India

Endocrine profiling of *Schizothorax esocinus* by radioimmunoassay: A model for induced breeding of *Schizothoracine*

GB Wani, AM Najar, SI Darve, FA Shah and SA Dar

Abstract
Sex steroid hormones play an important role in many physiological processes, particularly in the reproduction of vertebrates including fish. The complete knowledge of reproductive hormones and their actions at different stages of maturity of fishes is essential in the implementation of breeding and conservation management programmes of fishes. Sex steroid hormone can be estimated through blood or from tissue. As blood collection is not possible in dead fishes, a detailed procedure was adopted for the estimation of hormone from tissue. *Schizothorax esocinus* ovaries form the good source of reproductive steroid hormones *viz*. estradiol-17 β (E_2) and progesterone (P_4). These hormones were studied and quantified in the ovaries of *S. esocinus* extracts throughout the year. On the basis of this study, it was recommended that spawning season of *S. esocinus* extends from May to June. However, spawning may occur in April and May extending upto July. This represent the extremes of spawning and were governed by prevailing weather conditions. Therefore, for the scientific breeding programmes of this species these months are considered best.

Keywords: *Schizothorax esocinus*, reproduction, breeding, hormones and spawning

Introduction
The role of hormones in the regulation of various aspects of reproduction is well established in teleosts. The association of changes in gonadal development with gonadal steroids has proven to be a valuable tool for understanding the hormonal control of reproduction in teleosts Taghizadeh *et al.* (2013) [26]. Hormone delivery system through live food in fishes is also established Darve *et al.* (2013) [5]. The fish gonads respond by secretion of sex steroids hormone such as E_2 in female which promotes the vitellogenesis. The development of germ cell and maturation of oocytes are regulated by gonadotropic hormones. The occurrence of steroid production in different cells of the ovary may be related to different phases of oocytes development Lubzens *et al.* (2009) [15]. Physiologically, the most active gonadal hormones in fishes are estrogens and progesterone (P_4) from the follicles and corpora lutea of the ovaries respectively. The estrogens are in the form of estradiol-17 β (E_2) with its several derivatives. E_2 is the most physiologically active type of estrogen produced by granolosa cells of pre-ovulatory follicles and is also involved in controlling the various important physiological events in female reproductive cycle in all vertebrates including fish. This indicates a strong relationship between the ovarian development and the production of ovarian steroid hormones. P_4 is highly effective in inducing oocyte maturation Sabet *et al.* (2009) [22] and Wallace *et al.* (1978) [30] which includes migration of nucleus or germinal vesicle (GV) from central position to the surface of the oocyte, disappearance of the wall of GV (germinal vesicle breakdown–GVBD), chromosome condensation, resumption of meiotic activity and extrusion of first polar body.
Radioimmunoassay (RIA) method permits the precise and rapid measurement of large number of samples containing low concentrations of hormones upto pico-grams Heidari *et al.* (2010) [9]. It is basically a competitive binding assay in which fixed amounts of antibody and labeled antigen react in the presence of known (standard) or unknown (samples) amounts of the antigen. The amounts by which the binding of labeled antigen to its antibody are competitively inhibited by increasing amounts of standard antigen preparation are recorded (dose response curve or standard curve). The amount of antigen present in an unknown sample can then be calculated from the standard curve by comparing the binding of labeled antigen.

It is important to understand the ovarian hormonal milieu to develop techniques of induced breeding. The present study is proposed to investigate the annual reproductive steroid hormonal profile. Although in Kashmir region of Indian carp culture is based on exotic carps Wani *et al.* (2016) [31] and trout culture is dominant by *Oncorhynchus mykiss* (Rainbow trout) but in capture fisheries Snow trout is one of the dominant species. This study could be of immense help in restoring the cold-water fishery resources and its management of Kashmir valley with *S. esocinus* as a model for devising breeding techniques for cold water Schizothroracine group of fishes.

Materials and Methods

The female brooders of *S. esocinus* were collected from Jhelum river of Kashmir over a period of one year from January 2013 to December 2013. Gonadosomatic Index (GSI) was calculated by the formula Qasim *et al.* (1973) [21] GSI (%) = (Weight of ovaries / Weight of Fish) x 100. Ten selected fishes (having almost similar length and weight) were utilized for hormonal study every month. For hormonal estimation, the ovaries were crushed with the help of pestle and mortar. The crushed ovaries were homogenized with twice the volume of diethyl ether. The ovary homogenates were subjected to centrifugation at 3000 rpm for 15 min. The supernatant was decanted into assay tubes and the residue was discarded. The ether extract was evaporated on water bath maintained at 30ºC to reduce the volume to minimum. The extracts were further evaporated and dried before the assay was started. The concentration of E_2 and P_4 were measured from each sample in duplicate using 100 µl aliquots of ovarian extract by using RIA kits. RIA for estimation of E_2 and P_4 for *S. esocinus* were carried out at Sher-e-Kashmir Institute of Medical Sciences (SKIMS), Srinagar Kashmir, India by using hormone assay kits and protocols procured from Beckman Coulter (Immunotech, s.r.o.), Prague 10-Czech Republic. The steroid assay buffer was made from the reagents (Table-1) in 1000 ml of double distilled water. The RIA kit for E_2 and P_4 estimation comprised of reagents shown in Table-2 and estimated as per protocol of the KIT. E_2 was estimated by adding successively 100 µL of calibrator, control or sample in coated tubes (number marked in these tubes accordingly) followed by 500 µL of tracer and the tubes were vortex mixed. Incubation was carried out for 3 hours at 18-25 °C. The contents of the tubes were aspirated carefully by shaking at 350 shakes per min. For estimation of P_4, 50 µL of calibrator, control or sample and 500 µL of tracer were added sequentially in coated tubes numbered and marked for this purpose. The tubes were vortex mixed. Incubation was carried out for 1 hour at 18-25 °C with shaking (350 shakes per minute). The contents of the tubes were aspirated carefully. Estimation of E_2 and P_4 was made by using gamma scintillation counter.

Results

The concentrations of E_2 and P_4 in ovarian tissues of *S. esocinus* were measured throughout the annual reproductive cycle on monthly basis, in an attempt to determine whether different stages of ovarian development were related with various levels of steroid concentrations in this fish. On the basis of morphology and histological observations, six different stages have been identified in the present study *viz*. immature stage, early maturing phase, late maturing phase, mature/ pre-spawning phase, spawning phase and spent phase. Both E_2 and P_4 were found to be present in the ovaries throughout the year. In order to maintain uniformity, the values of E_2 and P_4 are presented in terms of pico-grams per gram ovarian tissue (pg/g ovarian tissue). E_2, P_4 and GSI were worked out on monthly basis for these fishes and presented in Table 3 and Fig 1.

During the recovery phase (July - August), the recorded value of E_2 were 32.69 and 19.59 pg/g ovarian tissue, respectively. The E_2 values in September was 3.31 pg/g ovarian tissue and concentration of the hormone showed slight increase in its level in October to a value of 8.39 pg/g ovarian tissue. Gradual increase in the concentration of E_2 was recorded during early maturing phase. The values recorded were 10.49, 16.68 and 18.66 pg/g ovarian tissue during November, December and January respectively. The values of E_2 increased enormously during February and estimated to be 198.78 pg/g ovarian tissue. The highest concentration of 423.29 pg/g ovarian tissue was recorded during March. These months which coinsided with late maturing phase, represented the period of active vitellogenesis and yolk deposition as also revealed by histological observations. During April the values of E_2 showed gradual decline in concentration to a value of 298.22 pg/g ovarian tissue. During May the value declined further and the observed concentration was144.69 pg/g ovarian tissue. The concentration of E_2 in June declined significantly to 94.32 pg/g ovarian tissue.

During the two-month period of spent/recovery phase, the values of P_4 recorded were 269.30 pg/g ovarian tissue in July and 120.31 pg/g ovarian tissue in August. The concentration recorded during July was the highest of all in the annual reproductive cycle. There was steep fall in the levels of P_4 in August (120.31). During September and October, the gradual decrease in P_4 concentration was recorded. The values recorded for these months were 86.56 pg/g ovarian tissue and 59.21 pg/g ovarian tissue respectively. In November and December, the P_4 levels dropped further, reaching a concentration of 29.15 pg/g ovarian tissue in November followed by 21.44 pg/g ovarian tissue in December. Further decrease in the P_4 concentrations to a value of 9.67 pg/g ovarian tissue was recorded during January. During February, P_4 concentration recorded was 7.05 pg/g ovarian tissue. During April which formed mature phase, P_4 concentration increased enormously to a value of 68.31 pg/g ovarian tissue. During spawning period in the months of May and June, P_4 concentrations were very high with values of 130.88 and 180.69 pg/g ovarian tissue respectively. This could be attributed to presence of corpora lutea which form the source of secretion of P_4. Fig. 1 depicts interaction of E_2, P_4 and GSI during different months of the year. While as peak E_2 concentration with a value of 423.29 pg/g ovarian tissue was observed in March during the period of vitellogenesis. Highest concentration of P_4 was recorded in July when corpora lutea was present in abundance. However, the maximum GSI value (18.92) was recorded during May just before spawning and lowest P_4 value of 2.75 pg/g ovarian tissue was recorded in March. These months indicated the early maturing phase. The Scatter plot (Fig. 2) depicts negative correlation between E_2 and P_4 ($r = -0.265$). However, E_2 showed significant positive correlation ($r = 0.677$) with GSI values (Fig. 3). Non-significant negative correlation was found between P_4 and GSI ($r = -0.035$) and is graphically depicted in Fig. 4.

Table 1: Contents of steroid assay buffer

S. No.	Contents	Quantity
1.	Sodium dihydrogen phosphate (hydrated) $NaH_2PO_4.2H_2O$ (Mw 156)	3.05 g
2.	Disodium hydrogen phosphate (hydrated) $Na_2HPO_4.2H_2O$ (Mw 178)	18.3 g
3.	Sodium chloride (NaCl)	8.8 g
4.	Thiomersal (merthiolate)	0.1 g
5.	Galatin	1.0 g
6.	Double distilled water	1000 ml

Table 2: Reagents for E_2 and P_4 estimation

Reagents for E_2 estimation		Reagents for P_4 estimation	
Sr. no.	**Reagents**	**Sr. no.**	**Reagents**
1	Anti-estradiol antibody coated tubes : 02x50 tubes (ready to use).	1	Anti-progesterone antibody-coated tubes: 02 x 50 tubes (ready to use).
2	^{125}I-labelled estradiol (55X) : one 01 ml vial	2	^{125}I-labbeled progesterone : One 55 ml vial (ready to use).
3	Tracer buffer: one 54 ml vial.	3	Calibrators : Six 0.5ml vials (ready to use).
4	Calibrator : Seven 01 ml vials. (ready to use).	4	Control : One 0.5 ml vial (Ready to use).
5	Control serum : One 01 ml vial (ready to use).		

Table 3: Annual profile of estradiol-17β (E_2), progesterone (P_4) in term of picogram per gram ovarian tissue (pg/g ovarian tissue) and gonadosomatic index (GSI) for *S. esocinus*

Months	E_2	P_4	GSI
January-2013	18.66	9.67	6.98
February-2013	198.78	7.05	8.32
March-2013	423.29	2.75	12.89
April-2013	298.22	68.31	14.23
May-2013	144.69	130.88	18.92
June-2013	94.32	180.67	12.35
July-2013	32.69	269.30	3.85
August-2013	19.59	120.31	1.23
September-2013	3.31	86.56	2.83
October-2013	8.39	59.21	2.96
November-2013	10.49	29.15	5.69
December-2013	16.68	21.44	6.12

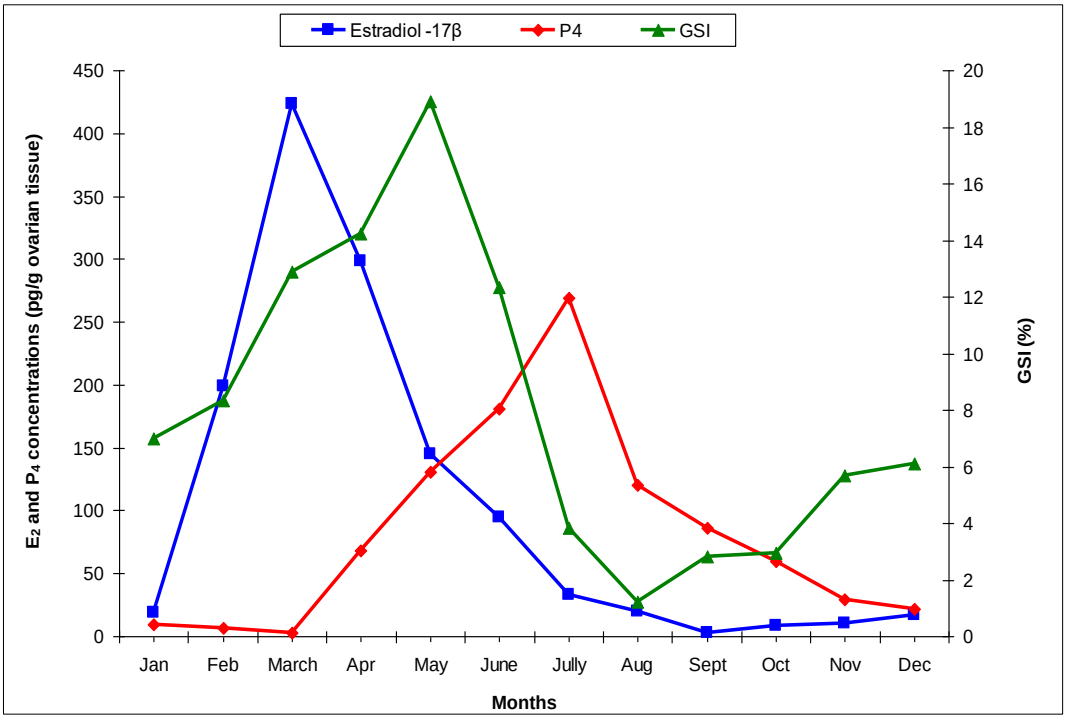

Fig 1: Interaction of estrodial-17β (E_2), progesteron (P_4) concentration and gonadosomatic index (GSI) for *S. esocinus* in different months of a year

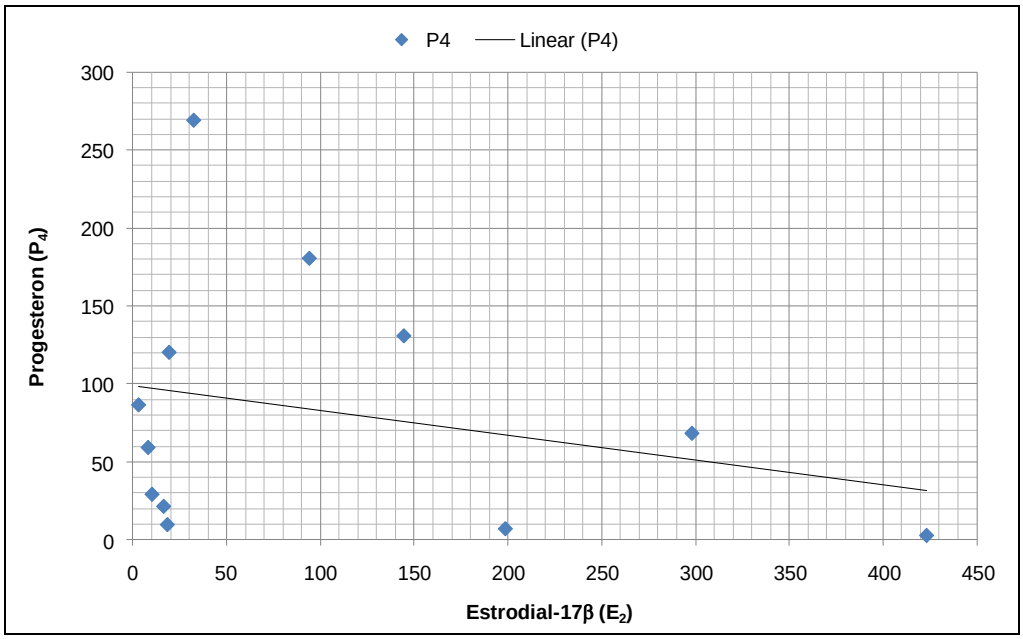

Fig 2: Scatter plot showing negative correlation between estrodial-17β (E_2) and progesteron (P_4) of *S. esocinus* in different months of a year

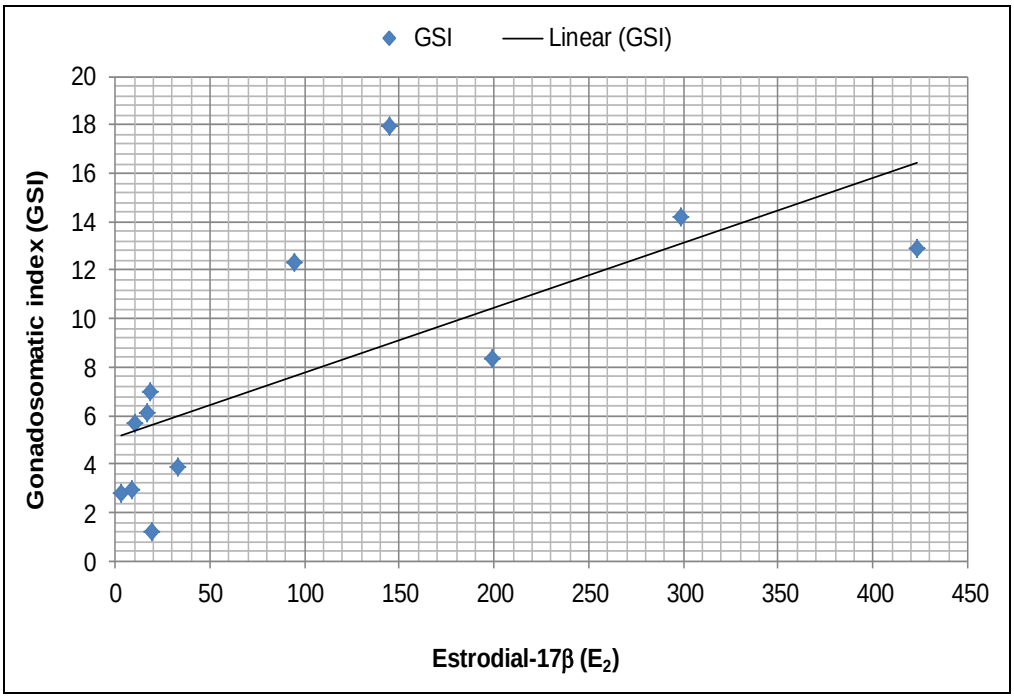

Fig 3: Scatter plot showing positive correlation between Estrodial-17β (E_2) and gonadosomatic index (GSI) of *S. esocinus* in different months of a year

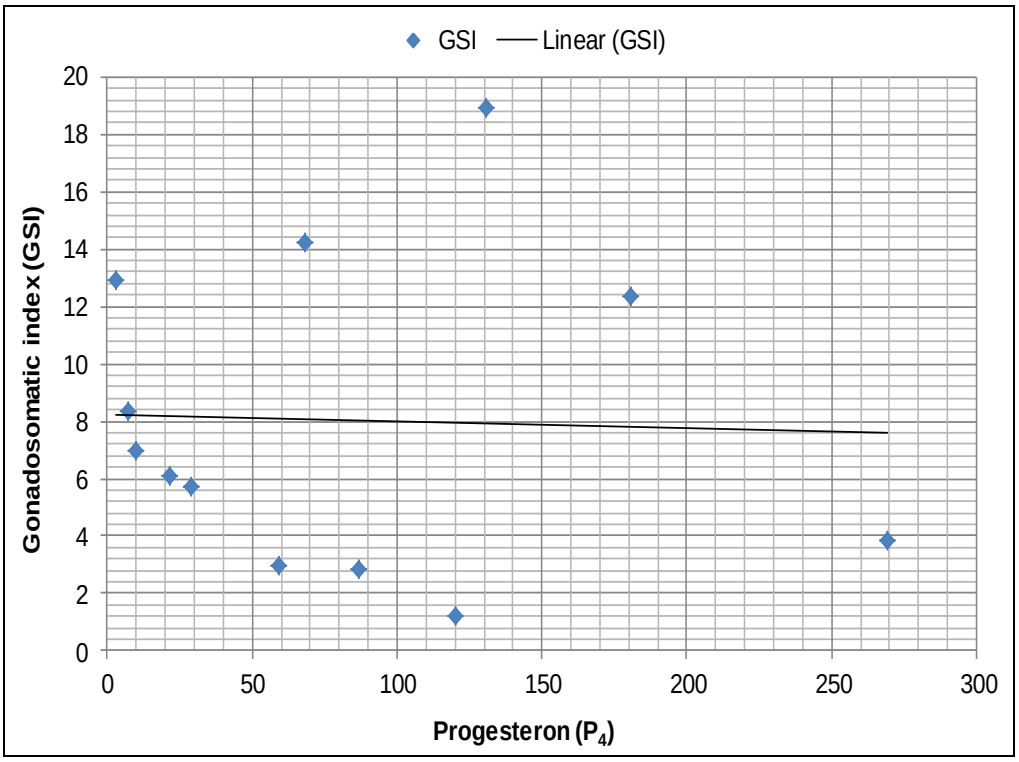

Fig 4: Scatter plot showing negative non significant correlation between progesteron (P_4) and gonadosomatic index (GSI) of *S. esocinus* in different months of a year

Discussion

The ovarian development and associated changes in the steroid hormone levels *viz.* E_2 and P_4 of *S. esocinus* are important for fishery management of this species. In order to precisely determine the spawning period the ovarian development in *S. esonicus* was studied by using different criteria *viz.* gonadosomatic index (GSI) and steroid ovarian hormone profiling. In case of *S. esocinus* of the present study the developmental stages of ovary comprised of six phases. These are in accordance with universal scale of six stages of ovarian development for temperate fishes Nikolsky (1963) [20] and reported Seena *et al.* (2012) [24] Sex steroids have long been recognized as key hormones regulating sexual differentiation, physiological aspects of reproduction and development of primary and secondary sexual characteristics Nelson RJ, (2005) [19]. In many teleosts, it has been shown that E_2 induces the synthesis and secretion of vitellogenic proteins by the liver Kagawa *et al.* (1981) [11] and Shimizu *et al.* (1985) [25]. The plasma E_2 at very high concentration during vitellogenic phase while very low level of E_2 was reported during oocyte maturation and ovulation Kagawa *et al.* (1983) [12] and Scott *et al.* (1983) [23]. Similarly, E_2 level was found to be high during vitellogenesis and then decreased slightly and maintained during oocyte maturation Unal *et al.* (2005) [27] E_2 plays an important and significant role in oocyte development in Japenese eel Kazeto *et al.* (2011) [13]. In *C. tarichi*, ovarian E_2 level showed high values before and after spawning, but decreased dramatically after spawning. E_2 induces hepatocytes of the liver to synthesize the female specific plasma vitellogin (VTG). VTG is actively sequestered from blood stream by developing oocytes in the ovary where it is deposited as yolk. Through accumulation of yolk, the oocyte increases enormously in size and cause the ovary as a whole to grow Meunpol *et al.* (2007) [16]. In the present study, the highest concentration of E_2 was recorded during February to April with maximum concentration of E_2 in March. These months formed the period of active vitellogenesis which is also confirmed by histological studies. The present findings were in agreement with the observations of other scientist Unal *et al.* (2006) [28], who reported that repeated injections of E_2 evoked an amplification of vitellogenin synthesis in fishes.

It is generally accepted that estrogens are not active in inducing oocyte maturation in fish Van *et al.* (1991) [29], in the present study where E_2 showed a decreasing trend from April showed inverse relation with maturation of oocytes. In contrast E_2 was found to be ineffective in inducing oocyte maturation in *Labeo rohita* and *Cirrhinus mrigala* Haider *et al.* (1989) [8]. The early workers believed that in the majority of oviparous elasmobranchs and teleosts, the discharged follicles do not become reorganised to form corpora lutea but instead collapsed and are readily resorbed Guraya *et al.* (1979) [7]. Beside this, the hypertrophy of follicular epithelium to form post ovulatory corpora lutea which were sites of synthesis of lipids and cholesterol have also been reported by others. There is immediate transformation of follicle cells into lutein cells during ovulation Chieffi *et al.* (1970) [3] P_4 has shown to be a potent maturation inducing steroid in several species of teleosts Goswami *et al.* (1974) [6] in many teleosts it has been reported that P_4 plays a major role in oocyte maturation and ovulation Lee *et al.* (2002) [14]. Thus P_4 increases significantly at the time of maturation and ovulation. Before spawning P_4 level was significantly low and after spawning the level increased rapidly[26], which is in accordance with the present study. It was observed that after spawning phase (July) the P_4 value was at its peak which may be due to the formation of atretic follicles (atresia) which form an important source of P_4 secretion. In the present study, E_2 showed significant positive correlation (r=0.677) with GSI value. Similar results were found by in case of Persian sturgeon, *Acipenser percicus*, and observed positive correlation between E_2 and GSI Unal *et al.* (2006) [28]. In

support to our findings significant positive relationship between E_2 and GSI (r=0.67), has been reported in tilapia (*Oreochromis mossambicus*) Cornish DA, (1998) [4]. In the present study no correlation was found between P_4 and GSI (-0.034).

Teleost have been reported to have different spawning periodicity and are seasonal breeders. In genus schizothoracids, The spawning season varies from species to species and their exist periodicity because of varied ecological environment. *S. richaordsoni spawns* in waters of kumon, Himachal Pradesh between March to June Jhingran VG. (1982) [10], July to December Bisht JS, (1974) [2] July to September in Garhwal Himalaya Misra M, (1982) [17] In case of Kashmir snow trout, *S. niger exhibits* spawning period from March and April Najar AM, (2002) [18] *S. plagiostomus* was reported to be biannual breeder Bhatnager GK, (1964) [1]. In the present study *S. esocinus* showed the highest concentration of P_4 in the month of July. This might be due to the presence of atretic follicles, which form the major source of P_4 secretions during the post spawning phase (spent phase) of the fish. Spawning phase of *S. esocinus* extends from May to June. The present findings on the spawning *S. esocinus* have been reconfirmed by histological studies. During this period ova were seen lying freely in the lumen of ovary.

References

1. Bhatnager GK. Observations on the spawning frequency of certain Bhakra reservoir fishes. Indian Journal of Fisheries. 1964;1:485-502.
2. Bisht JS. Seasonal histological changes in the hill stream teleost, *Schizothorax richardsonii* (Gray). Acta Anatomy. 1974;93:512-525.
3. Chieffi G, Botte V. The problem of "Luteogenesis" in non mammalian vertebrates. Bull. Zool. 1964;37:85.
4. Cornish DA. Seasonal steroid hormone profiles in plasma and gonads of the tilapia, *Oreochromis mossambicus.* ISSN 0378-4738 = Water SA. 1998;24(3):257-263.
5. Darve SI, Wani GB, Dhakar HS. Gonadotropin hormone delivery system through live food in Neon tetra (*Paracheirodon innesi*). Bioinfolet. 2013;10(2B):762-763.
6. Goswami SV, Sundararaj BI. Effects of C_{18}, C_{19}, C_{21} steroids on *in vitro* maturation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch). General and Comparative Endocrinology. 1974;23:282-285.
7. Guraya SS, Kaur S. Morphology of the postovulatory follicle (or *Corpus luteum*) of teleost (*Cyprinus carpio* L.). Ovary. Zool. Beitr. 1979;25:381-390.
8. Haider S, Inbaraj RM. Relative *in vitro* effectiveness of estradiol 17β, androgens, Corticosteroids, progesterone and other pregnene derivatives on germinal vesicle breakdown in oocytes of Indian major carps, *Labeo rohita, Cirrhinus mrigala* and *Catla catla*. Fish Physiology and Biochemistry. 1989;6(5):289-295.
9. Heidari B, Roozati SA, Yavari L. Changes in plasma levels of steroid hormones during oocyte development of caspian kutum (*Rutilus frisii kutum*, Kamensky, 1901). Animal Reproduction. 2020;7(4):373-381.
10. Jhingran VG. Fish and fisheries of India *(*Hindustan Publishing Corporation, Delhi, India), 1982, 954.
11. Kagawa H, Takano K, Nagahama Y. Correlation of plasma estrodial 17-β and progesterone levels with ultrastructure and histochemistry of ovarian follicles in the white- spotted char, *Salvelinus leucomaenis.* Cell and Tissue Research. 1981;218:315-329.
12. Kagawa H, Young G, Nagahama Y. Relationship between season plasma estradiol-17β and testosterone levels and *in vitro* production by *Ovarian follicles* of Amago salmon (*Oncorhynchus rhodurus*). Biology of Reproduction. 1983;29:301-309.
13. Kazeto Y, Tosaka R, Matsubara H, Ijiri S, Adachi S. Ovarian steroidogenesis and the role of sex steroid hormones on ovarian growth and maturation of the Japanese eel. The Journal of steroid biochemistry and molecular biology. 2011;127(3):149-154.
14. 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 maculates* (Jeom-nong-eo). Aquaculture. 2002;207:196-183.
15. Lubzens E, Young G, Bobe J, Cerda J. Oogenesis in teleosts: how fish eggs formed. Eneral and Comparative Endocrinoloy. 2009;165:367-389.
16. Meunpol O, Iam-Pai S, Suthikrai W, Piyatiratitivorakul S. Identification of progesterone and 17α-hydroxyprogesterone in polychaetes (*Perinereis* sp.) and the effects of hormone extracts on penaeid oocyte development *in vitro*. Aquaculture. 2007;270:485-492.
17. Misra M. Studies on fishery biology of Schizothorax richardsonii (Gray)-an economically important food fish of Garhwal Himalaya, Ph.D. thesis, Garhwal University, Srinagar, 1982.
18. Najar AM. Ovarian development and steroid production in snowtorut, *Schizothorax niger* Heckel. Applied Fisheries and Aquaculture. 2002;11(1):39-46.
19. Nelson RJ. An introduction to behavioral endocrinology (Sinauer Associates), 2005.
20. Nikolsky GV. The ecology of fishes (Academic Press London and New York), 1963, 52.
21. Qasim SZ. An appraisal of the studies on maturation and spawning in marine teleosts from the Indian waters. Indian J Fish. 1973;20(1):166-181.
22. Sabet SS, Reza IM, Bagher AF, Saeed G. Study on sexual maturity and levels of gonad steroid hormones in female kutum *Rutilus frisiikutum* kutum (Kamenskii, 1901) during spawning season from river Sefid-Rood of the southern Caspian sea. Journal of Cell and Animal Biology. 2009;3(11):208-215.
23. Scott AP, Sumpter JP. A comparison of the female reproductive cycles of autumn-spawning and winter-spawning streams of rainbow trout (*Salmo gairdneri*). General and Comparative Endocrinology. 1983;52(1):79-85.
24. Seena PA, Jose TM, AnnaMercy TV. Reproductive biology of the endemic ornamental barb, *Puntius pookodensis* Anna Mercy and Eapen Jacob 2007, from the Western Ghats of India. Indian Journal of Fisheries. 2012;59(2):49-55.
25. Shimizu A, Aida K, Hanyu I. Endocrine profiles during the short reproductive cycle of an autumn-spawning bitterling, *Acheilongnathus rhombea*. General and Comparative Endocrinology. 1985;60(3):361-371.
26. Taghizadeh V, Imanpoor MR, Mehdinejad N. Study the seasonal steroid hormones of common carp in Caspian sea. Iran. Springer Plus. 2013;2:193.
27. Unal G, Karakasi H, Elp H. Ovarian follicle ultrastructure and changes in levels of ovarian steroids during

oogenesis in *Chalcalburnus tarichi* Pallas, 1811. Turkish Journal of Veterinary and Animal Sciences. 2005;29:645-653.
28. Unal G, Karakisi H, Mahmut EL. Levels of some ovarian hormones in the pre- and post-spawning periods of *Chalcalburnus tarichi* Pallas, 1881, and the postovulatory structure of follicles. Turkish Journal of Veterinary Animal Science. 2006;30:427-434.
29. Van Weerd JH, Richter CJ. Sex pheromones and ovarian development in Teleost fish. Comparative Biochemistry Physiology. 1991;100(3):517-527.
30. Wallace RA, Selman K. Ooogenesis in *Fundulus heteroclitus* preliminary observations on oocyte maturation *in vivo* and *in vitro*. Developmental Biology. 1978;62:354-369.
31. Wani GB, Balkhi MH, Darve SI, Bhat TH, Hussain N. Knowledge and adoption of fish farming practices by fish farmers of India Himalayas: District Ganderbal (Kashmir). Annals of Biology. 2016;32(2):238-241.