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Gonadal Development and Maturity in Previous year bred and Fresh Brooders of *Labeo rohita (ham.)*

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ABSTRACT
This study was conducted in the College of Fisheries, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand to observe the gonadal development histologically along with correlated factors in the previous year bred and fresh brooders of Labeo rohita. The Specimens of Labeo rohita were reared and collected from the Instructional Fish Farm of the College of Fisheries, Pantnagar, Gonadal development and production of gametes are critically reviewed and discussed in relation to maturation of gonads, oogenesis, spermatogenesis during the pre spawning and spawning phase. The observations on previous year bred fish did suggest that possibility for them attaining early ovarian and testicular development in subsequent breeding season. This study indicated that the stimulation of changes with regards to activities in testis, ovary and liver was comparatively more in previous year bred group.
Keyword:*Labeo rohita, Gonadal development, GSI, HSI, oogenesis, spermatogensis, breeding*

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INTRODUCTION

Fishes, being an important natural source of protein, have great significance in the life of mankind and provide certain other useful products as well as economic substance to many nations. It is well established fact that the knowledge on certain aspects of fish biology during different stages of reproduction and associated process with it such as biochemical constituents like lipid and water during gonadal maturation will have great impact on proper management for quality seed production.

Labeo rohita (Rohu) is the most important amongst the Indian major carps and highly demanded in the marked. Gonadal development in *L.rohita* normally commences from February and gonadal maturity is attended only by June-July in northern India [14, 19]. Advancement in gonadal maturity for spawning earlier than normal breeding season i.e. pre-spawningphase can be achieved through hormonal manipulations. Biological indices i.e. Gonado somatic Index (GSI) and Hepato somatic index (HSI) are generally used as a reliable criterion for expression of gonadal recrudescence and maturation of fishes [3, 13]. The gonadal development process and maturation are associated with the increase in gonadal lipid accumulation and significant changes in lipid classes in developing gonads. [22, 18]. Kumar *et al.* [14] studies the in Tarai region of Uttarakhand and stated four spermagenic phages of testes. Singh *et al.* [19] have been observed seasonal ovarian cycle in freshwater teleost *L. rohita* (Ham.) in the Tarai region of Uttarakhand.

Spawning season of fish can be determined through monthly histological examination of gonads (testis and ovary) and that could categorically define the size and age of fish at first maturity in natural and controlled system. [1]
The present study embodies the physical and physiological changes associated with the ovarian and testicular development in previous year bred and unbred *L. rohita* during its pre-spawning and spawning phase of reproductive cycle. This study may also help in exploring the possibilities of improving the fertility and in developing techniques for quality seed production for prolonged period.

MATERIAL AND METHODS

The adult male and female specimens were collected from the Instructional Fish Farm of the College of Fisheries, Pantnagar, Uttarakhand. These specimens of *L. rohita* having 500-1200g in weight and 32-40cm in length were stocked in the brood stock pond 2500kg/hg and routine package of practices for rearing of brood stock was followed.
After recording weight and length of every specimen those were sacrificed for the collection of gonads (Testis and ovary) and liver samples. Different stages of reproductive cycle were distinguished and the basis of descriptions of Singh and Singh [21], Kumar *et al.* [14] and Singh *et al.* [19]. GSI and HSI indices are expressed as gonad and liver as weight X100/total body weight.
For the estimation of water content in the gonad as and liver, the samples were weighed to the nearest 0.1mg and dried in oven at 60±2^{0}C. After 72 hours dry weight of samples was recorded and calculated the water content as % water = {wet weight (g)-dry weight(g)}x100/wet weight. Estimation of lipid content in the gonad and liver were followed as per method described by Folch *et al.* [6]. ANOVA was used to evaluate the significance of variation in GSI, HSI water and lipid content in the gonad as (Ovary and testis) and liver in relation to gonadal development during annual reproductive cycle in *L. rohita.*
For histological study, the microscopic slides were prepared by the following procedure as followed by Agarwal [1]. The development stages of germ cells in the testis ad oocytes in the ovary of previous year bred and unbred *L. rohita* were observed by the mounted slides with different stages.

Results and Discussions

The changes in GSI, HSI water and lipid content in the liver and gonads of both sexes of *L.rohita* in previous year bred fish and fresh unbred fish groups have been summarized in Table 01.
The GSI in both sexes of male and female specimens of previous year bred *L. rohita* was slightly more than those of unbred *L. rohita* during the period of experiment and it was slightly significant in the pre-spawning and highly significant during spawning season. This exhibited the positive correlation of GSI with the advancing of maturity of gonads.
Gondal development and maturity in both sexes of *C. mrigala* [21] and *C. batrachus* [20] was shown to be positively correlated with increasing temperature and day length *L. rohita* also might have been positively correlated with the increasing day length and temperature [13,18]. Rae and Calvo [17] reported that the GSI values for both sexual cycle of *Patagonotothen tessellata*were high during peak maturity of fish and low during resting phase.
The HSI in both sexes of male and female specimens of previous year bred *L. rohita* was slightly decreased significant by with that of the bred fish. In contrast to GSI, the HSI decreased significantly during pre-spawning phase and lowest level was recorded in June during spawning phase and negative correlation existed between GSI and HSI during annual reproductive cycle of *L. rohita* [13, 18]. Singh and Singh [20] have reported that the HSI in *C. batrachus* had increased during pre-spawning and spawning phase with increasing GSI similar observations have also been made in *C. mrigala* [21]. HSI is often used as an estimation of energy status of the fish [23, 4].
The water content in testes and ovary of unbred specimens of *L. rohita* was slightly significantly less than in the previous year bred *L. rohita*. This was positively correlated with the advancing of maturity of *L. rohita* during pre-spawning and spawning season.

Table 01: Changes in GSI, HSI water and lipid content in gonadas (Testis and ovary) of unbred and previous year bred specimens of *L. rohita*

Month	Sex	Group	GSI	HSI	Water content %		Lipid content %	
					Gonad	Liver	Gonad	Liver
March	Male	Unbred	0.305±0.006	0.811±0.012	77.80±1.67	80.96±1.53	26.75±2.06	47.00±3.16
		Previous year bred	0.329±0.01	0.793±0.05	78.71±1.21	79.89±2.20	25.78±2.50	46.75±3.21
	Female	Unbred	1.22±0.07	0.86±0.16	72.97±1.89	73.48±1.63	25.60±2.30	49.58±1.15
		Previous year bred	2.11±0.83	0.85±0.16	73.98±0.89	71.52±2.07	27.20±1.91	47.69±3.41
April	Male	Unbred	0.366±0.02	0.77±0.074	79.07±1.58	78.01±2.0	24.00±2.94	40.75±3.30
		Previous year bred	0.398±0.018	0.751±0.036	79.89±1.35	77.57±1.42	23.12±2.08	39.25±4.03
	Female	Unbred	2.33±0.83	0.81±0.17	75.87±1.58	72.46±2.53	27.65±1.0	46.75±1.25
		Previous year bred	3.09±0.03	0.76±0.075	76.92±1.16	70.05±2.02	28.92±0.05	44.50±0.50
May	Male	Unbred	0.41±0.05	0.73±0.08	80.51±1.20	77.20±1.89	22.47±2.01	37.50±2.64
		Previous year bred	0.56±0.02	0.69±0.10	81.68±1.58	76.42±1.52	22.02±1.91	36.18±2.23
	Female	Unbred	2.98±1.42	0.78±0.16	78.81±1.20	71.56±1.50	27.98±1.20	44.22±0.80
		Previous year bred	3.75±0.80	0.70±0.30	80.86±1.72	68.23±1.22	29.26±1.52	43.46±1.10
June	Male	Unbred	0.48±0.06	0.70±0.12	81.90±1.65	75.27±1.57	20.96±2.02	35.00±3.8
		Previous year bred	0.59±0.05	0.68±0.08	82.73±1.26	74.23±1.71	20.05±1.67	34.22±2.78
	Female	Unbred	4.05±0.52	0.63±0.50	82.0±0.88	70.22±1.11	29.58±0.60	42.23±1.50
		Previous year bred	4.68±0.48	0.61±0.78	84.16±0.56	66.56±1.25	30.93±1.10	40.52±1.23

The water content in the liver of both male and female unbred fish was compared to previous year bred specimens in the pre-spawning season and more in the spawning season and this was negatively correlated with the gonadal development of fish. The water content in testis and ovary of *L. rohita* varied throughout the reproductive cycle and was significantly different between spawning and non-spawning phases which seemed to be positively correlated with gonadal development [13, 19]. The hydration of testis had been considered important for spermiation in *H.fossilis* [18]. Krivobok [15] had observed that the weight of liver in male *Clupea harengus* increased prior to maturation and after spawning but decreased during maturation and spawning. Decrease in size of liver has been reported to occur before spawning in *Esox Lucius* [16].

The lipid concentration in the testis of unbred *L. rohita* was slightly more than the previous year bred fish and this was slightly significantly decreased from pre-spawning to spawning season. This trend was also found in the ovary of unbred and previous year bred*L. rohita*. The total lipid content in the ovary of previous years bred fish was higher than the unbred fish during the experiment.The lipid concentration in the liver of both sex of unbred *L. rohita* was slightly greater than previous year bred fish. The decreasing trend of lipid concertation in both male and female of unbred and previous year bred *L. rohita* from pre-spawning to spawning season.

In the subtropical fishes *C. batrachus* [21],*C. mrigala* [20] and *L. rohita* [14, 19] were formed to experience increase in testicular and ovarian lipid and decrease in HSI and liver lipid during spawning season when GSI values were on rise. In the Arctic charr (*Salvelinus alpinus*), it has been found that the final stages of gonadal growth dependent upon the mobilization and re-allocation of endogenous reserves [12].

Decreased HSI and liver lipid with concomitant increase in GSI and gonadal lipid content during pre-spawning and spawning phase of *L. rohita* may be due to mobilization of lipid from liver to gonad [14, 18].

Decrease in liver lipid of *O. nerka* was associated with increased gonadal lipid [10]. They have suggested that 0.57% of total body energy was transferred to gonad and rest part of it was utilized for long spawning migration. Similar inverse relationship has also been shown between body fat reserves and gonadal development and maturation in *G. morhua* [11] and in *Esox Lucius* by Diana and Mackey [5].

Histologically observations for both the sexes (male and female) of unbred and previous year bred *L. rohita* are presented in tubular form (Table 02). On the basis of morphological

observations for the pre-spawning (March, April and May) and spawning season (June) of unbred and previous year bred *L. rohita* revealed that the testis of the previous year bred fish significantly activation of spermatogonia and spermatogenic processes during pre-spawning phase. It was evidenced by the presence of all types of spermatogenic cells with dominance of secondary spermatocytes and spermatids as well as sperms,while in the unbred fish exhibited the pre dominance of only spermatids with moderate sperm bundles.
The interstitial cells in the testes of previous years bred fish group were showing more activity as characterized by their increased nuclear size.
The histological observations of testis of previous year bred fish revealed advancement of spermatogenic, spermeogenic activity with fully matured spermatozoa during spawning season. While in the unbred fish dominated sperm mass primary and secondary spermatocytes and newly spermatozoa were observed.
In the pre-spawning season of the unbred *L. rohita*, follicular layer, oocytes and early yolk stages had been observed. Differentiation of thecal, granulosa layer in the oocyte with cytoplasmic content and yolk globules has also been examined in the unbred fish during pre-spawning season. The eggs were fully grown and completely packed with yolk mass with cortical alveoli and nucleus were observed during spawning season of unbred *L. rohita.*
While in the previous year bred *L. rohita* extensive development of yolk globules in the oocyte with follicular epithelium, noncellular zona pellucida, increased size of oocytes with ooplasm and yolk globules were observed during pre-spawning phase of *L. rohita*. This also exhibited pronounced number of mature oocytes with heavily loaded ooplasm. Fully grown eggs completely packed with yolk mass with transparent egg envelop and indistinct nucleus with well developed zona pellucida, follicular layer and theca surrounded the egg were observed in the previous year bred fish *L. rohita.*
This histologically study of the gonad of both female and male indicated that the previous year bred *L. rohita* was having the better gonadal development cyclicity than the fresh unbred fish.
Indian major carps have been reported to have second time gametogenesis leading to double spawning [2]. These observations indicated the second time brooder of fish was capable of more activating the gonadal maturity either pre-spawning season. The existence, origin, structure and functions of interstitial cells in teleost have been described extensively by Hyder [9]; Gresik *et al*. [7] and Guraya [8].
The signs of activation of spermatogenesis and oogenesis characterized by increased in their size and nuclear size during experimental period noticed in previously bred group. These observations are suggested that the possibility of variability in gonadal development in previous bred and unbred groups of fish in next breeding season. Efforts of working out on similar lines are not found in the literatures available till now hence any interference present observations may not be possible.

Table 02: Histological changes and gonadal cyclicity (Testis and ovary unbred and Previous year bred *Labeo rohita* during pre-spawning and spawning season.

Gonadal cycle	**Historical changes in Unbred *L. rohita***	**Historical changes in Previous year bred *L. rohita***
Male- **Pre-spawning season**		
March	Testis showing increased in lobular size and spermatogenic cells were present.	Revealed significant activation of spermatogenic activity and formed primary spermatogonia.
April	Increased lobular size filled with spermatogenic and started formation of spermatozoa.	Inducement of spermatogenic and spermeogenic process Most of lobules were filled with spermatids and sperm mass.
May	Intra lobular space decreased and abundance of spermatocytes, spermatids.	Lobules were completely packed with spermatozoa. The testicular and lobular wall became thinner.
Spawning season		
June	Dominated sperm mass, primary and secondary spermatocytes and spermatozoa. Lobular wall became thin.	Thin lobular wall and fully matured spermatozoa presented with liquefied milt.

Female- **Pre-spawning season**		
March	Follicular layer was now better developed. The oocytes and early yolk stage are more in number.	Extensive development of yolk globus has been observed with the presence of oocytes, follicular epithelium and non-cellular zona pellucida.
April	Differentiated follicular had been observed with differentiation of thecal and granulosa layer.	Size of oocytes had been increased and occupied whole of the ooplasm around the nucleus. Rapid accumulation of yolk globules resulted growth of oocytes.
May	Cortical alveoli randomly distributed throughout the cytoplasm, yolk globules were observed in the oocytes. Follicular epithelial layer increased in thickness.	Exhibited pronounced number of matured oocytes. The ovary is filled up with ripe eggs. Germinal vesicle migrated and heavily loaded ooplasm with yolk material.
Spawning season		
June	The eggs were fully grown and completely packed with yolk mass. Cortical alveoli became clearly apparent adjacent to yolk envelop. districted nuclear was presented.	Fully grown eggs completely packed with yolk mass with transparent egg envelop. The nucleus became indistinct with well developed zona pellucida, follicular layer and theca surround the egg.

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