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ORIGINAL ARTICLE

Steroid Level in Breeding Stages of Freshwater Fish, *Hetropneustes fossilis* (Bloch) under Laboratory Conditions

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ABSTRACT

The cholestral level in matured fishes ensures all reproductive process including mating chances, sperm release and egg maturity and so its quantity is variable during different stages of reproductive cycle in fishes. This paper was aimed to assess cholestral quantity in gonadal cycle and their role as steroid production in both male and female fishes to ensure reproductive success in freshwater air-breather Hetropneustes fossilis during the study period.

Keywords: *Cholestral, Gonad, steroid production, seasonal variation*

INTRODUCTION

Like all other vertebrates, the fish also reproduce sexually and their reproductive cycles are governed by both extrinsic (climatic) and intrinsic (endocrine) factors. Majority of the fish are dioecious as eggs and sperms are formed in separate individuals, which are released in surrounding water where fertilization takes place generally during spawning phase of their gonadal cycle. Siddiqui (1966) studied seasonal variation in the contents of total cholesterol in serum, muscle, liver & gonads & stated that serum cholesterol level showed no appreciable variation throughout the year except during peak spawning period when its level was recorded low. Tandan & Chandra (1976) in *C. batrachus* observed lowest & highest values of blood cholesterol in the month of August (spawning) & November (winter) respectively. Sex related cyclic changes in hepatic & serum cholesterol levels in *H. fossilis* have also been reported by Jaiswal et al (1978). Kumar (1999) reported that serum cholesterol level declined during spawning, but increased during post spawning periods.

METHODS AND MATERIALS

Healthy & living specimens of *Hetropneustes fossilis* (Bloch), in between 10-13 cm. length and 35±5 gm weight groups were procured from local market and brought to the laboratory in large buckets containing ground water & covered with net cloths. In the laboratory, they were treated with terramycin solution (15 mg/l) in ground water for 48 hrs, washed by several changes of water & then treated with Aquous Solution. of Pot. Permanganate (2mg/l) for 15 minutes followed by proper washing with ground water, so that the fish should be free from any dermal infection by ectoparsites, fungi & other infections. Thereafter, the male & female fishes were placed in large aquaria/ plastic tubs/steel tubs separately in ground water & acclimatized for 10 days. Care was taken to discard the population if mortality exceeds 10% during the treatment of tetramycin & potassium permanganate solution upto 48 hours. The fish were provided chopped goat's liver/earthworm pieces/ tubifex daily at least three hours prior to change of ground water.

100 mg or 50 mg tissue was weighed and placed in a mixture of methanol and aceton in 1:1 ratio and after alternative boiling and cooling, centrifuged and the supernatant was taken in separate test tube. It was placed in a water bath and evaporated to dryness. It was then cooled to room temperature. The residue was then dissolved in 0.1 m of distilled water. To this, 8.0 ml of working Killiani's reagent was added and processed as for blood.

Total Cholestral= O.D. of 1.0 ml of xK^{-1} protein x4x10 tissue homogenate in tissue (mg/gm)

Where K^{-1} Cholestral = 48.61 mg/ml (constant) and 4 is the total amount of homogenate of 100mg tissue.

RESULT AND OBSERVATIONS

The total cholesterol content in serum of normal male & female fish varied in between 205.42±2.20 to 298.00±2.29 and 228.54±2.15 to 320.69±3.02 mg/dl respectively. The lowest value in both the sexes has been recorded in August while highest value in male & female has been recorded in February & March respectively. A gradual increase has been observed from September to February/March followed by a decline upto August. The seasonal variations showed that the lowest value in both sexes has been seen in spawning and highest value in male during resting & female during maturing phases. When compared with the value of male & female of same gonadal cycle, a significant increase ($P<0.01$) in females has been observed in maturing, pre-spawning & spawning phases & ($P<0.05$) in post-spawning phase. However, when compared with the value of male & female of resting phase with that of the respective sexes of other gonadal phases, significant decrease ($P<0.01$) has been observed in males of all phases & in female, a significant increase ($P<0.01$) in maturing & decline in post-spawning & ($P<0.01$) in pre-spawning & spawning phases (Table 1; Figure 1).

Table 1: Total cholestral in Liver and Gonads of *Hetropneustes fossilis*

Breeding Stage	Liver		Gonad	
	Male	Female	Testis	Ovary
Resting (Dec-Jan)	6.04±0.19	6.15±0.18	5.13±0.91	5.26±0.12
Maturing (Feb-Apr)	5.76±0.16	5.45±0.22	5.50±0.14	5.67±0.16
Pre-spawning (May-June)	4.51±0.20	4.16±0.35	6.66±0.14	7.09±0.20
Spawning (Jul-Aug)	3.74±0.21	3.21±0.13	8.19±0.24	8.48±0.27
Post-breeding(Oct- Nov)	4.97±0.16	4.77±0.20	5.96±0.14	6.16±0.27

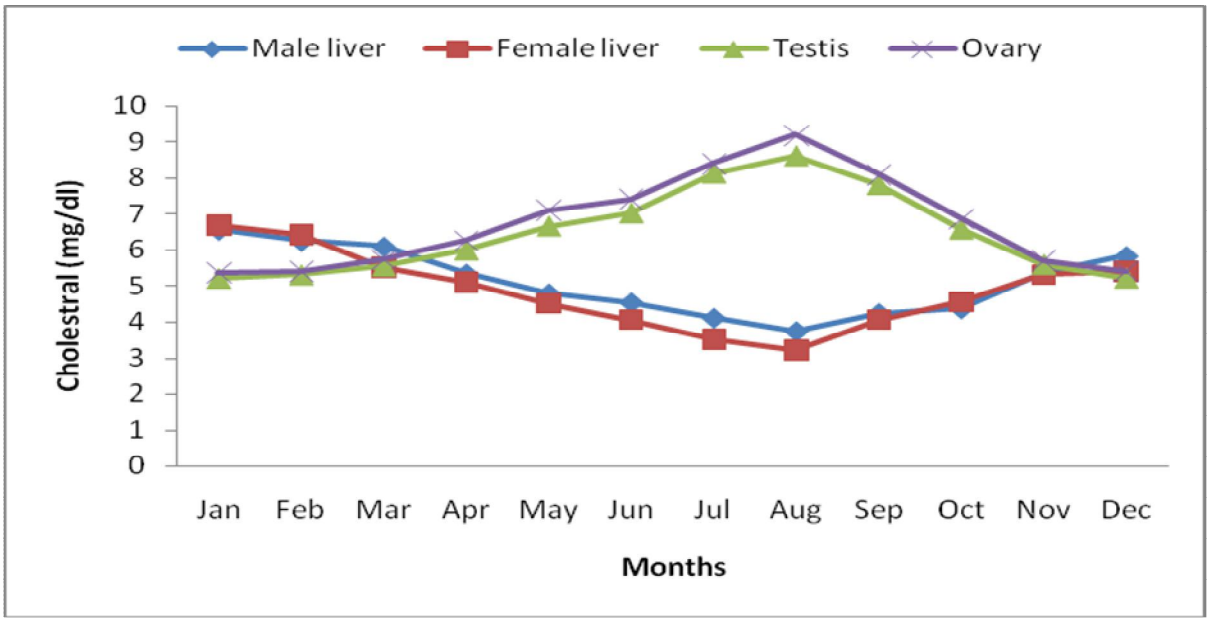

Fig. 1: Monthly variation of Cholestral in Liver and Gonads of *Hetropneustes fossilis*

The liver cholesterol content in male & female fish varied in between 3.60± 0.19 to 6.36±0.20 & 3.02±0.15 to 6.45±0.25 mg/gm respectively. The lowest & highest values in both the sexes have been recorded in August & January respectively. The seasonal variations showed that the highest values in both male & female are during resting phase and lowest during spawning phase. However, the difference in liver cholesterol level in between male & female has not been found significant in any gonadal cycle, but when compared with the value of male & female of resting

phase with that of the respective sexes of other gonadal phases, a significant (P<0.05) decrease has been observed in the males of maturing & post-spawning phase and (P<0.01) in both sexes during pre-spawning & spawning phases and in females only during post spawning phase (Table 1).

The cholesterol content in the testes & ovaries varied in between 5.10± 0.12 to 8.40±0.26 and 5.21±0.14 to 8.86±0.30 mg/gm respectively. The lowest & highest values in both sexes have been observed in January & August respectively. A gradual increase has been observed from February to August followed by decline from September to January. The seasonal variations showed that lowest cholesterol content in both sexes has been recorded during resting phase and highest during spawning phase. However, the cholesterol content in male & female gonads during same gonadal cycle have no significant differences but when compared with the value of male & female gonads with that of the respective sexes of other gonadal cycles, a significant increase (P<0.01) has been recorded in both sexes during pre-spawning & spawning phases & (P<0.05) during post-spawning phase (Table 1).

DISCUSSIONS

There are several reports on the seasonal variations in serum & tissue cholesterol level in fish. A correlation of serum cholesterol level in fish has been shown to exist between one or more factors i.e. age, temperature, tolerance, activity, sexual maturity & spawning period. Cholesterol is a steroid and is a well known precursor of gonadal harmones. Steroidogenesis (a process of conversion of cholesterol into sex hormones) is an enzyme depended metabolic activity and the enzyme held responsible for synthesis and/or metabolism of such hormones are hydroxysteroids dihydrogenases (Schreibmas, *et al.*, 1982).

In the present study, the serum & liver cholesterol contents has been recorded minimum during August and maximum during January & March respectively while the cholesterol contents in the gonads (testes & ovary) have been recorded lowest during January & highest during August in both male & female fish. Further the seasonal variations showed that the blood cholesterol level was significantly more in females than the males in all the phases of the gonadal cycle except in resting phase, while liver cholesterol content has been recorded more in males than the females but not significant, whereas, ovary contained more cholesterol content than testes. Thus, the serum cholesterol showed a positive correlation with liver but an inverse relationship with gonads. The increase/decrease in blood cholesterol content either enhance cholesterol synthesis by liver or inhibits its excretion to the bile ducts, whereas, the decreased level during breeding period might be due to increased breakdown of cholesterol into free fatty acids. Similarly the decreased level in liver cholesterol content might be due to overall hypermetabolic state of the fish caused by persistent stress & may be linked to the general stress syndrome.

Bano & Hameed (1979) & Singh (1990) in *C. fasciatus* observed almost an inverse relationship between ovary & blood with liver & muscle and suggested the diversities of muscle cholesterol to the development of gonads in the form of sex hormones. Shalini (1989) in *H. fossilis* exposed to cadmium & nickel & Kumari (1990) in the same fish exposed to sevin observed a significant increase in blood cholesterol level but a decline in liver & muscle of the fish. But Gupta (2003) in *H. fossilis* exposed to different concentrations of fenvalerate observed an initial increase followed by decrease in blood cholesterol and a gradual decline in gills, liver, Kidney & gonads cholesterol levels. Mishra, *et al.*, (2004) in *C. punctatus* exposed to sublethal carbaryl & Cartap observed a significant decline in lipid & cholesterol contents in liver, kidney & gills throughout the exposure period & suggested that hypocholestesterimic conditions may probably be due to either increased breakdown of cholesterol into free fatty acids or more utilization of cholesterol during corticosteriodogenesis is under pesticide impact. It is also been given by Jyoti & Narayan (2001) in *H. fossilis* & *C. batrachus* exposed to different pesticides respectively.

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