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# Some biochemical changes in the reproductive cycle of a hill stream teleost *Puntius chilinoides* (McClelland)

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**Abstract.** The protein content was highest in the ovaries of *Puntius chilinoides* during the maturing stage and in the testes during the mature stage. The activity of the acid phosphatase and the number of isozymes decreased in the testes during maturation, whereas in the ovaries the activity increased during the maturation and spent stages. The alkaline phosphatase activity in the testes increased during maturation phase, while in the ovaries the highest activity of the enzyme was recorded at the maturing stage and the lowest during the mature stage. Cholesterol level in the ovaries was highest during the maturing stage, while in the testes it was noticed during the immature stage. The sugar contents in the gonads were highest at the mature stage. The results are discussed in relation to the reproductive cycle in *P. chilinoides*.

**Keywords.** Biochemical changes; protein; acid phosphatase; alkaline phosphatase; cholesterol; sugars.

## 1. Introduction

It is well known that several metabolic changes occur during the development of gonads and in fact all the metabolic activities inside a developing tissue are ultimately under some biochemical control. The metabolic activities are controlled by the enzymes. Now it is clear that lysosomes are the main organelles where the acid hydrolases like the acid phosphatase are localised. The alkaline phosphatase is also much important in animal tissues. Phosphatases in general play a very important role in phosphate ($P_i$) availability in the tissues. Inorganic phosphate ($P_i$) is required in the synthesis of several metabolites during developmental stages. On the other hand, carbohydrates, fats and cholesterol also play a significant physiological role during the developmental stages in gonads.

There are several reports on the biochemical changes that occur during growth and development in fish. Lal (1963) reported decline in protein contents in the ovaries in *Cirrhina mrigala* during maturation. Contrary to this, Ehlebracht (1973) reported an increase in the protein content during maturation. Wegmann and Goetting (1971) studied a distribution of protein, polysaccharides, nucleic acids and fats in *Xiphophorous helleri*. Shaffi *et al* (1974) have reported higher alkaline phosphatase activity in the ovaries of *Clarias batrachus* during maturation. Siddiqui (1966) in *Channa punctatus*, Singh and Singh (1979) in *Heteropneustes fossilis* and Sen and Bhattacharya (1981) in *Anabas testudineus* reported the cholesterol level in different stages of maturity in the gonads.

Studies on the biochemical changes during the development of the gonads in hillstream fishes are scanty. Therefore, in addition to the seasonal morphohistological

studies of the gonads and pituitary gland, the biochemical changes in protein, acid phosphatase, alkaline phosphatase, cholesterol and sugar contents of the gonads of a hillstream minor carp *Puntius chilinoides* of Garhwal Himalaya during different stages of development were studied.

## 2. Material and methods

On the basis of detailed seasonal morphohistological changes in the gonads and pituitary gland of *P. chilinoides*, the following stages of development *viz* the immature, maturation, mature, spent and resting stage have been studied. The important morphohistological changes have been observed during immature, maturation, mature and spent stages, therefore, the present biochemical study has been conducted during these stages of maturity. Sexually mature *P. chilinoides* of each sex were collected regularly from the Khandagaad, a tributary of Alaknanda. The soluble protein contents of the gonads were determined by the method of Lowry *et al* (1951). The soluble sugar contents were estimated by the Anthrone method (Mac Cready *et al* 1950). The total cholesterol was determined by the modified method of Zlatkis *et al* (1953). For the determination of phosphatases, the homogenates were prepared in cold grinding medium consisting of 0·1 M Tris-HCl buffer (pH 7·5) and centrifuged at 2000 × g. The acid phosphatase activity was determined by the method of Baijal *et al* (1972). The assay system comprised 1 ml of 0·2 M acetate buffer (pH 5·5) 0·1 ml 0·2 M $MgSO_4$ and enzyme preparation and water, making the volume 1·9 ml. The alkaline phosphatase activity was determined following the method described by Bodansky (1932), using 1 ml 0·2 M barbitone buffer (pH 9). For both the phosphatases 0·1 ml of 0·1 M $\beta$-glycerophosphate was added as a substrate. Phosphate was determined following the method described by Fiske and Subbarow (1925). The unit of the enzyme activity was expressed as the amount which librates one $\mu$ mole of pi per minute at 37°C.

Change in protein profile and isozymes of acid phosphatase in the gonads of *P. chilinoides* were analysed for qualitative changes in their protein and acid phosphatase composition by means of disc electrophoresis. A 20% (*W/V*) homogenate was prepared by grinding the tissue in pre-chilled tris-HCL buffer (pH 7·5) at 0·5°C and centrifuged at 2000 × g. The supernatant was used for electrophoritic separation of protein and acid phosphatase using three gels for each sample. The method of disc electrophoresis in polyacrylamide gel as described by Davis (1964) was followed. 0·15 ml extract with 0·05 ml of 1 M sucrose was layered on 7·5% acrylamide gel using bromophenol blue as a tracking dye. The samples were run in cold at pH 8·3 using Tris-glycine buffer with a current of 3 mA per tube. The process was carried out till the tracking dye reached the lower end of the gel. The gels were then taken out. For proteins, the gels were stained in 0·25% Coomassie brilliant blue for 15 hr and destained in 7% acetic acid at 5 mA current per tube.

For isozymes of acid phosphatase the gels were incubated in proper incubation mixture as described by Brewbaker *et al* (1968). The zymograms of the stained gels were prepared and the transmittance of bands was measured with the help of a densitometer (Toshniwal, type CM 11).

## 3. Observations

### 3.1 *Protein*

The protein contents were higher in the ovaries in comparison with the testes during all the four stages (immature, maturing, mature and spent) (figure 1A). In the testes, the protein contents increased during the maturing stage and the highest value of protein was observed at the mature stage; and a sharp decline in the spent stage was observed. In the ovaries, the protein contents increased only during maturing stage (stage II). The protein contents then showed a slight decline in the mature period and finally a sharp decline at the spent stage.

### 3.2 *Acid phosphatase*

The activity of the acid phosphatase showed a marked decrease during maturation in the testes. The activity was highest at the immature stage and a significant decrease in

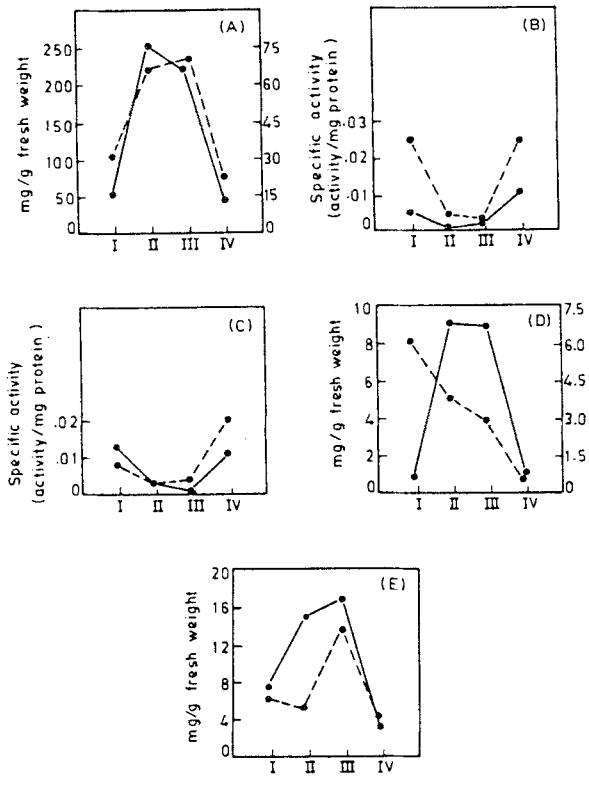


**Figure 1.** Seasonal biochemical changes in the testes (●—●) and ovary (●---●) of *P. chilinoides*. **A.** Protein; **B.** Acid phosphatase; **C.** Alkaline phosphatase; **D.** Cholesterol; **E.** Sugar. (I, II, III and IV represent immature, maturation, mature and spent phases respectively).

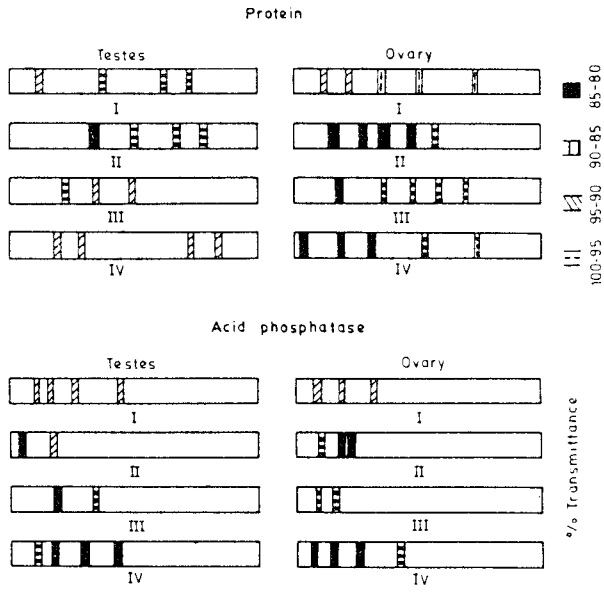


**Figure 2.** Zymograms of protein and acid phosphatase by polyacrylamide gel electrophoresis of the gonads of *P. chilinoides*. (I, II, III and IV represent immature, maturation, mature and spent phases respectively).

the activity was observed at maturing and mature stages. During the spent stage the activity increased significantly. The activity of this enzyme showed a different pattern in the ovaries. The acid phosphatase activity in the ovaries was found highest at the immature stage. At the maturing stage a significant decrease was noticed after which there was a continuous increase at the mature and spent stages (figure 1B). The activity of the acid phosphatase and the number of the isozymes decreased in the testes during maturation, whereas in the ovaries the activity increased during maturation and spent stages (figure 2).

### 3.3 *Alkaline phosphatase*

In the ovaries the highest alkaline phosphatase activity was recorded at the maturing stage which decreased sharply up to the mature stage. At the spent stage the activity again increased. In the testes it showed a different pattern. The activity was lowest at the maturing stage and then continuously increased up to the spent stage (figure 1C).

### 3.4 *Cholesterol*

The cholesterol content in the testes was highest in the immature stage. The highest cholesterol level in the ovaries was found during the maturing and the lowest during the immature stage (figure 1D).

### 3.5 *Sugars*

The amount of soluble sugars in both the gonads was highest at the mature stage as compared to the other three stages (figure 1E).

### 3.6 *Changes in protein profile and isozymes of acid phosphatase*

Five protein bands were observed at all the four stages of development in the ovary. However, in the mature stage the first band had a very low *Rf* value, whereas the band having the highest *Rf* value in the immature, mature and spent stages was not detected in the maturing stage. In the testes four protein bands were detected at all the stages except at the mature stage where only three bands were detected and the fourth band (the band of highest *Rf* value) was absent. The *Rf* value of each individual band was different at different stages.

In the ovary four bands of acid phosphatase were detected at the spent stage, only two bands at the mature stage and three bands at the immature and maturing stages. The first and the second band (I and II from origin) had the same *Rf* values at all the four stages. In the testes acid phosphatase activity appeared as four bands at the immature and spent stages with the same *Rf* values, although the *Rf* values of the third and fourth band in the spent stage was slightly less. The maturation and mature stages showed only two bands, one band in common (figure 2).

## 4. Discussion

The protein contents in the ovaries was much higher than in the testes during the annual cycle which is in consonance with previous studies. The low protein content at the spent stage in *P. chilinoides* is indicative that rapid protein synthesis is necessary only during maturation for the developing oocytes and sperms. The protein profile of the ovaries and testes also indicated that the original bands disappear during the developmental stages, indicating that the new proteins are synthesized.

The activity of the acid phosphatase and the number of isozymes decreased in the testes during maturation and spent stages. These results indicate that in the ovaries the acid phosphatase plays a significant role in the synthesis of essential metabolites by liberating Pi. However, in the testes the decline in the acid phosphatase activity is probably indicative of the fact that the enzyme apparently plays a less significant role during maturation (stage II and III), but seems to play a significant role during the spent stage as the level of the enzyme activity increases during the spent stage.

Shaffi *et al* (1974) have reported higher alkaline phosphatase activity in the ovaries of *Clarias batrachus* during maturation. *In P. chilinoides* the alkaline phosphatase activity increased in the ovaries during the maturing phase (stage II) indicating that during this period the synthesis of new proteins takes place as alkaline phosphatase has been reported to be involved in protein synthesis (Shaffi *et al* 1974). At the mature stage the activity of alkaline phosphatase was lower, showing a decline during the process of maturation. In the testes the alkaline phosphatase activity showed a different pattern. The activity was lowest at the maturing stage (stage II) and then continuously increased

up to the spent stage, suggesting that in the testes the alkaline phosphatase plays an important role during the development of the sperms.

Siddiqui (1966) recorded maximum ovarian cholesterol level in the gonads of *Channa punctatus* at the end of the maturing phase, while in *Heteropneustes fossilis* Singh and Singh (1979) observed a decline in the cholesterol level of the ovaries during the pre-spawning phase, but an increase during the spawning phase. In *Anabas testudineus* Sen and Bhattacharya (1981) reported low ovarian cholesterol level during the pre-spawning phase and high cholesterol level during the post-spawning phase.

In *P. chilinoides*, the high cholesterol level in the ovaries was found during the maturing stage and the lowest during the immature stage, while in the testes the high level of cholesterol was noticed during the immature stage and lowest during the spent stage. It is considered that the high cholesterol level in the gonads acts as a reservoir to meet the cholesterol demand of the maturing gonads and the decreased level might be due to the increase in the rate of steroidogenesis.

The sugar contents in the gonads of *P. chilinoides* were highest at the mature stage, suggesting that during maturation the accumulation of sugars takes place in the ovaries and testes. The sharp decline in the sugar contents during the spent stage, confirms the previous studies (Lal 1963).

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