

Ontogenic variation of Alkaline Phosphatase Activity in Different Tissues During the Development of *Labeo rohita* (Ham.)

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Abstract: Activity of the enzyme alkaline phosphatase is demonstrated histochemically in the ontogenic stages of *Labeo rohita* 24 hrs after hatching (AH) upto 60 mm (fingerling stage) in different tissues such as yolk sac, liver, intestine and mesonephric tubules. Alkaline phosphatase activity is moderate upto 60 hrs but when the yolk sac is absorbed and mouth opens at 72 hrs there is moderate activity in the yolk sac boundary. Food is located in the alimentary canal from third day after ah and from this time, intestinal epithelium and liver show intense activity upto fingerling stage. Mesonephric tubules do not exhibit any activity up to 60 hrs AH, in 72 hrs AH, moderate activity is noticed which intensifies as the development proceeds. The expression of this enzyme is implicated in a variety of embryological and developmental processes and it works at alkaline pH 9 and above.

Key words: Alkaline phosphatase • *Labeo rohita* • Developmental stages

INTRODUCTION

Alkaline phosphatase is an important regulative enzyme in bio-metabolic processes [1] and plays a vital role in digestion, absorption and transition of nutrients [2]. As the stability of enzyme activity can influence the body's biological metabolism and adaptive capacity [3] it is essential to quantify its activity during the early developmental stages of fish.

Labeo rohita is one of the Indian major carps that occurs in both lentic and lotic ecosystems of India. It is an economically important, fast growing fish that attains the sexual maturity at the age of two years. The ontogenic development of digestive and absorptive enzymes during the post hatch period has been described in many fish species [4-10] and has yielded information applicable to understanding their pattern of food utilization.

Alkaline Phosphatase is a group of enzymes found primarily in the liver. Acid and alkaline phosphatases are some of the main enzymes along with other digestive enzymes whose activity was detected during larval development of White Sea bream, *Diplodus sargus* [11]. Intestinal alkaline phosphatase is considered to be involved in absorption of nutrients such as lipid, glucose, calcium and inorganic phosphate [12-16].

The Golgi apparatus has been shown to be the source of this enzyme production in the enterocytes in rat [17] from where the enzymes then move to the intestinal brush border which is its active site. Moreover, the appearance and localization of this absorptive enzyme correlates well with those of other digestive key enzymes including maltase, lipase and peptidases.

In view of the above findings, present work aimed to study the role of alkaline phosphatase in liver and intestine during the development of *L. rohita* to testify its role in digestion and absorption of food material. Danielli [18] declare that apart from the nuclei and brush border of kidney tubules, this enzyme seldom occurred in the cytoplasm but such studies are not reported in any of the fresh water teleosts. We are the first to report the distribution of alkaline phosphatase during developmental stages of an Indian major carp *L. rohita*, in the above mentioned tissue and also the yolk sac and kidney tubules and the correlation of its appearance in different tissues.

MATERIALS AND METHODS

All the developmental stages of *L. rohita* were collected in monsoon season from Government of

Maharashtra Fish Seed Centre, Pench located 45km from Nagpur. Live fish seeds were carried to the laboratory in oxygen filled polythene bags. To study enzyme alkaline phosphatase activity, the developmental stages were fixed in cold buffered neutral formalin for 24-72 hrs. After fixation, material was transferred to 10% sucrose for 1 hr, 20% sucrose for 1 hr and kept in 30% sucrose overnight. The stages were cut in transverse and saggital planes at 8-10µm on a Leica cryocut.

The fingerlings whose length ranged 30-35mm and which had ossified skeleton were decalcified as follows:

The material stored in 70% alcohol was transferred to a decalcifying solution containing 10 ml of formic acid, 5 ml of formalin and 85 ml of distilled water. The material was kept in the above solution for 5 days for decalcification. In order to test whether the tissue is completely decalcified or not, 1ml of 5 % sodium or ammonium oxalate was added to 5 ml of decalcifying fluid containing the tissue. This was allowed for 5 minutes. Lack of precipitate indicates that decalcification is complete.

Alkaline phosphatase activity was demonstrated by Gomori method in 1939 [19]. Tissue was fixed in cold buffered formalin for 24-72 hrs. 10-12µm sections were cut on a cryocut at -18 to -20°C. After washing with distilled water, the sections were incubated in Gomori medium for 3 hrs at 37°C. Sections were washed with distilled water and then treated with 2% solution of cobalt

nitrate for 2-3 min, washed with distilled water and treated with yellow ammonium sulphide for 1 min, washed in distilled water and mounted in glycerin jelly. Black deposits in the sections confirmed alkaline phosphatase activity in the tissues.

RESULT

In 24 hrs after hatching (AH) diffuse staining of the enzyme alkaline phosphatase is observed in the peripheral wall of yolk sac which remains weaker in 36hrs, 48 hrs and 60 hrs. In 72 hrs stage the yolk sac is completely absorbed and larva starts feeding activity. At this stage moderate staining is observed in the boundary wall of the yolk sac. Intestine also exhibits weak staining upto 36 hrs which becomes moderate in 48 hrs and 60 hrs AH in the epithelium. It shows moderate to intense diffuse staining in 10mm stage right upto 60mm stage.

Liver exhibits very weak staining in 24 hrs. Weak to moderate staining is noticed from 36 to 72 hrs. Moderate to intense staining is observed from 10mm to 60mm stage. Mesonephric tubules remain unstained upto 60 hrs stage. In 72 hrs stage, moderate staining is noticed. From 10mm to 60mm stage, kidney shows moderate to intense staining. In yolk granules, no staining for alkaline phosphatase is observed from 24 to 72 hrs.

Figure Alkaline phosphatase activity in different tissues in developmental stages of *Labeo rohita*.

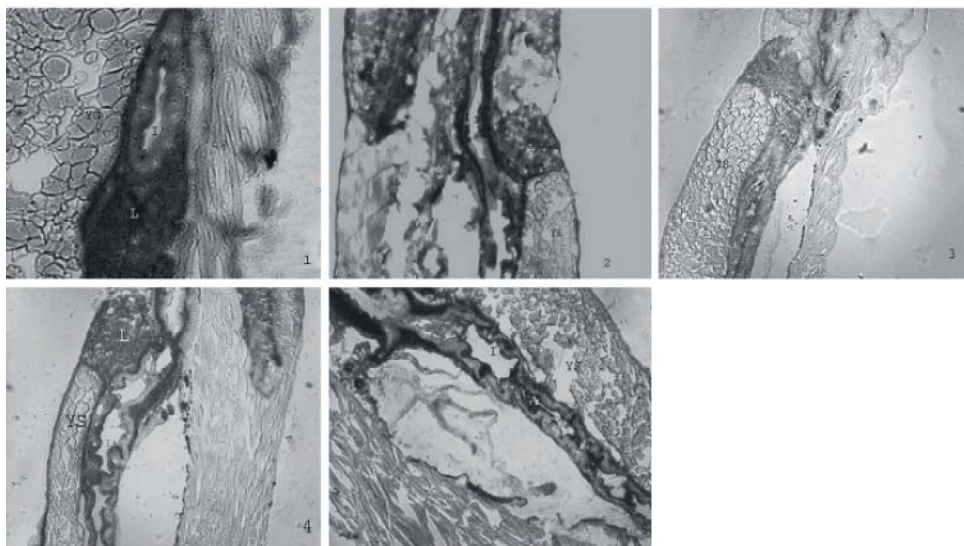


Fig. 1. Sagittal section of 24 hrs hatching showing alkaline phosphatase activity in intestine (I), liver (L) and peripheral wall of yolk sac (YS) X200.
Fig. 2. Sagittal section of 36 hrs hatching showing alkaline phosphatase activity in intestine (I), liver (L) and yolk sac (YS) X200.
Fig. 3. Sagittal section of 48 hrs hatching showing alkaline phosphatase activity in intestine (I), liver (L) and yolk sac (YS) X200.
Fig. 4. Sagittal section of 60 hrs hatching showing alkaline phosphatase activity in intestine (I), liver (L) and yolk sac (YS) X200.
Fig. 5. Sagittal section of 72 hrs hatching showing alkaline phosphatase activity in intestine (I), liver (L) and yolk sac (YS) X200.

Photo plate 1:

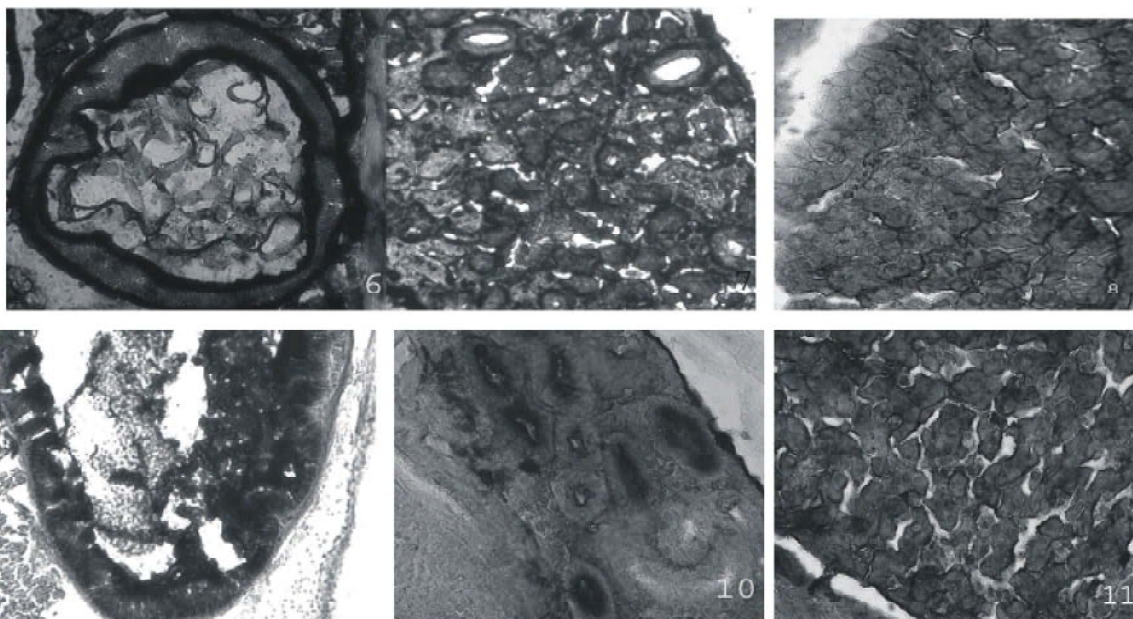


Fig. 6. Sagittal section of 10 mm stage showing alkaline phosphatase activity in intestine (I) X200
 Fig. 7. Sagittal section of 10 mm stage showing alkaline phosphatase activity in kidney (K) X100.
 Fig. 8. Sagittal section of 10 mm stage showing alkaline phosphatase activity in liver (L) X200.
 Fig. 9. Sagittal section of 20 mm stage showing alkaline phosphatase activity in intestine (I) X200
 Fig. 10. Sagittal section of 20 mm stage showing alkaline phosphatase activity in kidney (K) X200.
 Fig. 11. Sagittal section of 20 mm stage showing alkaline phosphatase activity in liver (L) X200.

Photo plate 2:

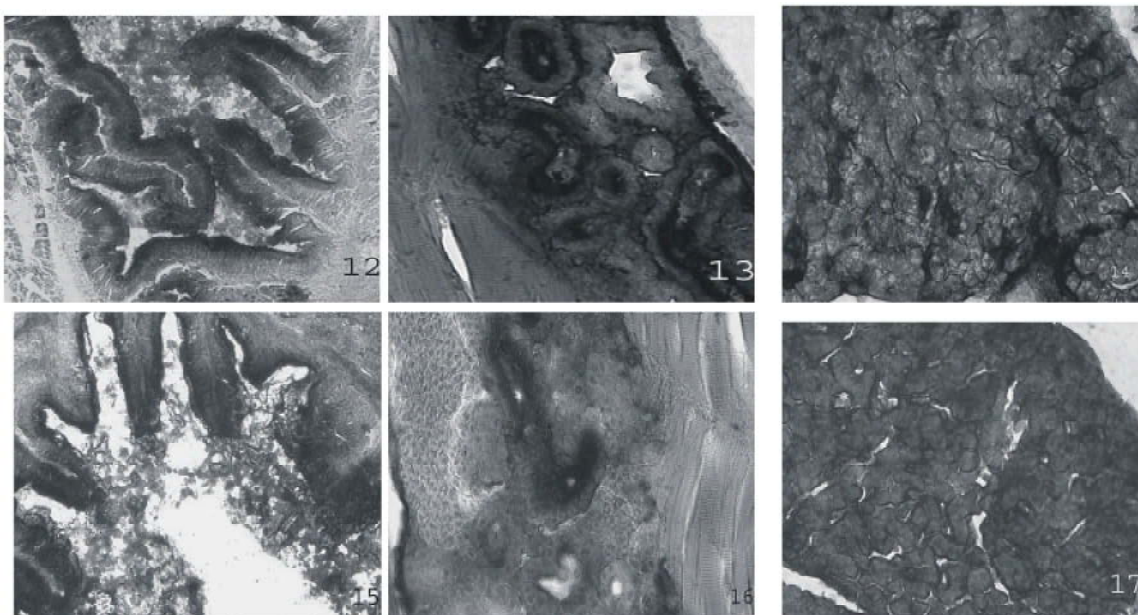


Fig. 12. Sagittal section of 30 mm stage showing alkaline phosphatase activity in intestine (I) X200
 Fig. 13. Sagittal section of 30 mm stage showing alkaline phosphatase activity in kidney (K) X200.
 Fig. 14. Sagittal section of 30 mm stage showing alkaline phosphatase activity in liver (L) X200.
 Fig. 15. Sagittal section of 40 mm stage showing alkaline phosphatase activity in intestine (I) X200
 Fig. 16. Sagittal section of 40 mm stage showing alkaline phosphatase activity in kidney (K) X200.
 Fig. 17. Sagittal section of 40 mm stage showing alkaline phosphatase activity in liver (L) X200.

Photo plate 3:

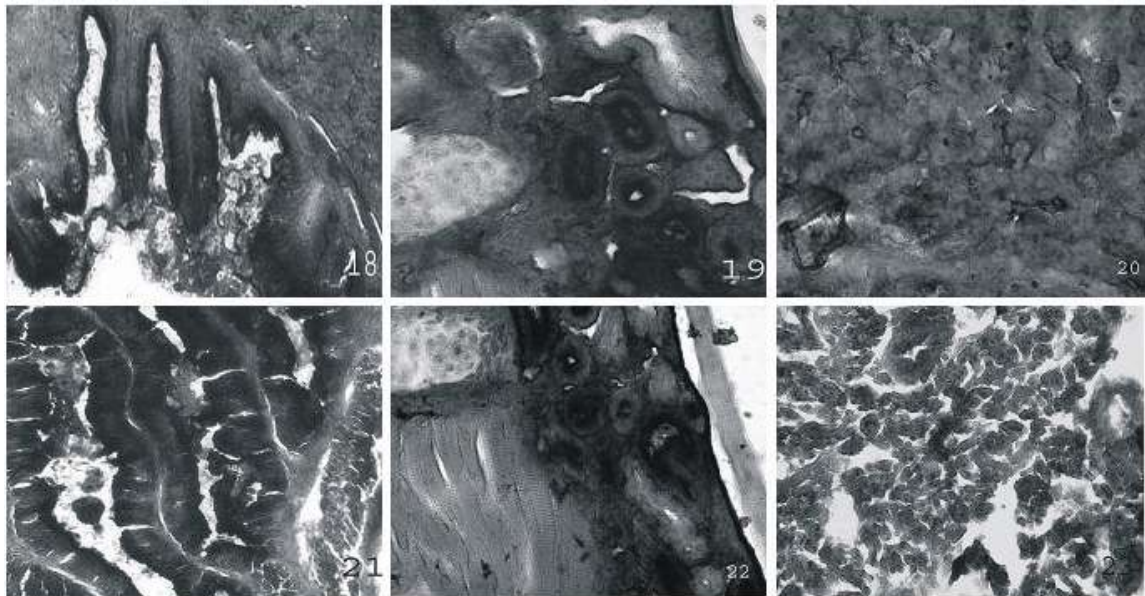


Fig. 18. Sagittal section of 50 mm stage showing alkaline phosphatase activity in intestine (I) X200.
 Fig. 19. Sagittal section of 50 mm stage showing alkaline phosphatase activity in kidney (K) X200.
 Fig. 20. Sagittal section of 50 mm stage showing alkaline phosphatase activity in liver (L) X200.
 Fig. 21. Sagittal section of 60 mm stage showing alkaline phosphatase activity in intestine (I) X200.
 Fig. 22. Sagittal section of 60 mm stage showing alkaline phosphatase activity in kidney (K) X200.
 Fig. 23. Sagittal section of 60 mm stage showing alkaline phosphatase activity in liver (L) X200.

Photo plate 4:

DISCUSSION

Localization of alkaline and acid phosphatase in normal and neoplastic tissue and cells continues to be an extremely important endeavor [20]. Alkaline activity in yolk sac is comparatively weaker in *L. rohita* upto 72 hrs during which mouth is not opened and nutrient supply is from the yolk sac.

Intestine upto 72 hrs exhibits weak to moderate activity for the enzyme. The role of incomplete alimentary tract seems to be minimal till the absorption of yolk sac and 72 hrsAH mouth opens and intestinal epithelium exhibits intense activity which persists as the development proceeds upto 60mm stage. Food was first found in the anterior part of the alimentary tract of carp, *Cyprinus carpio* larvae on the third day after AH [21]. Same is observed in *L. rohita* also. Liver initially upto 72 hrs exhibits weak to moderate activity for alkaline phosphatase, but in later stages, its staining intensifies for the enzyme. The significance of such an activity in the intestine and liver may be connected to various types of food to be broken down by the larva and its switching over to varied foods as the development proceeds. In rat intestine, the close proximity of this enzyme with various

digestive enzymes is documented by Alpers *et al.* [17] facilitating efficient absorption of smaller particles. Liver function becomes important for various metabolic activities. The variation in the activity profiles of digestive enzymes is correlated either to the developmental events such as functional start of the stomach (22 day after AH) or to changes in the nature of the diet in White Sea bream [11]. Mesonephric tubules are unstained upto 72 hrs, moderate staining is found from 10mm onwards around the tubules and later there is intense staining upto 60mm stage.

Osmotic and ionic regulation during early development of teleosts poses certain questions like what are the mechanisms that provide regulative capacity in the egg, embryo and larva and when or at what development stages do these regulatory mechanisms become functional? In teleosts, the principal effector organs of osmotic and ionic regulation are renal complex, gut, branchial epithelium [22] and integument but according to [23] rather than the guts and chloride cells of gills, renal complex may be more involved in embryonic osmotic regulation and remained neglected. From the present studies, it can be concluded that alkaline phosphatase in kidney may indicate that after 10mm

onwards kidney plays a role in osmotic regulation because stability of enzyme activity can influence the body's biological metabolism and adaptive capacity [3] and alkaline phosphatase is found in sites which are those in which it occurs under prevalent physiological conditions [18].

The relationship between metabolic rate and temperature in early ontogenesis was studied in *Esox lucius* by [24] and in *Clupea harengus* by [25]. The activities of almost all the enzymes in general, depend upon number of external factors such as temperature, pH etc. [26]. In culture practices of major craps in general and *L. rohita* in particular, if the environmental parameters are simulated and regulated and suitable planktons are provided as food, the enzyme alkaline phosphatase can act as a indicator for the healthy development of *L. rohita*.

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