Activity Of Alkaline Phosphatase From *Lithobates catesbeianus* During Metamorphosis.

Gonçalves, A. M.¹, Santos, L.F.J.¹, Casanova, D.C¹., Pizauro, J.M¹.

Depto. Tecnologia; FCAVJ-UNESP.

During the anurans' metamorphosis the main function of the tail is to support the morphophysiological changes of aquatic larva to terrestrial adults, as the animals do not eat during this period. Alkaline phosphatase is an important enzyme in the phosphate's mobilization, which is required for the anabolism of various essential biomolecules. The increase of activity may indicate the moments of greatest degeneration of the tail. The objective of this study was to observe the activity of alkaline phosphatase during the metamorphosis of bull frog (Lithobates catesbeianus) tadpoles. The tadpoles were kept in aquaria, at 27 °C and separated by stages of development. Samples were taken from tails of 3 animals in each stage, frozen in liquid nitrogen and stored at -70 °C. The tails were homogenized in Tris-HCl 5 mM buffer, pH 7.5, containing MgCl₂ 2 mM and ZnCl₂ μM, centrifuged at 10,000 rpm for 10 minutes, at 4°C. The apparent optimum pH for the hydrolysis of PNPP by the enzyme was maximal at pH 10.5, and the enzyme was assayed at 37 °C in 2-amino-2-methyl-1-propanol (AMPOL) 100 mM buffer contained 1 mM p-nitrophenylphosphate. The activity at the beginning of metamorphosis (stage 42) was 9.80 U.mg⁻¹, reaching the value of 102.66 U.mg⁻¹ at the end of metamorphosis (stage 45). The increase in alkaline phosphatase activity had shown that the absorption of the tail is more intense at the end of 44° stage and 45° stage, where the tail completely disappears. It is in this period that the most notable changes occur in the organs during larval development and it is necessary to release phosphate to support further morphophysiological changes.

Keywords: Alkaline phosphatase; Tadpole; *Lithobates catesbeianus*, Metamorphosis.