Osmoregulation and ontogeny: kinetic characterization of Na⁺/K⁺-ATPase activity of zoea IX from the freshwater shrimp *Macrobrachium amazonicum*.

Masui, D.C.; Augusto, A.²; McNamara, J.C¹.; Furriel, R.P.M.; Valenti, W.C.² and Leone, F.A.

Deptos. de Química and ¹Biologia - FFCLRP/USP. 14040-901. Ribeirão Preto, SP;

²Depto de Biologia Aplicada à Agropecuária – FCAV/UNESP. 14884-900. Jaboticabal, SP.

Na⁺/K⁺-ATPase has a crucial involvement in the osmoregulatory mechanisms responsible for crustaceans adaptation to different salinities. In some species, activity levels follow ontogenetic changes in osmoregulatory ability, but little has been investigated among palaemonid shrimps. Here we characterize Na⁺/K⁺-ATPase activity from *M. amazonicum* zoea IX. Larvae were supplied by FCAV/UNESP prawn hatchery, Jaboticabal, São Paulo State, Brazil. Activity stimulation by ATP (V= 266.8±12.6 Umg⁻¹; $K_{0.5}$ = 0.09±0.004 mM; n_{H} = 0.7), Mg²⁺ $(V= 245.0 \pm 12.0 \text{ Umg}^{-1}; \text{ K}_{0.5}= 0.56 \pm 0.03 \text{ mM}; \text{ n}_{H}= 1.8)$ and Na⁺ $(V= 249.1 \pm 11.9 \text{ m})$ Umg^{-1} ; K_{0.5}= 4.2±0.2 mM; n_H= 2.2) occurred with cooperative kinetics, while K⁺ stimulation (V= 247.0±12.3 Umg⁻¹; K_{M} = 0.91±0.04 mM) followed Michaelis-Menten behavior. Ouabain inhibited 72% of total ATPase activity with K= 92.0±4.5 µM. The specific activity estimated for zoea K was 1.6-fold higher than that estimated for zoea I, and 1.4 and 2-fold higher, respectively, than those estimated for the gill tissue of juveniles and adults. The apparent affinity of zoea IX enzyme for ATP was 2-fold higher than those estimated for zoea I and adult and juvenile shrimp gills. Whereas the apparent affinity of zoea IX enzyme for K⁺ was 2.2 to 3-fold higher than those estimated for zoea I and adults and juveniles gill tissue, affinity for Na⁺ was 2.7-fold higher than that estimated for zoea I. Comparative kinetic characterization of Na⁺/K⁺-ATPase in different ontogenetic phases may contribute to unravel the strategies involved in the invasion of freshwater biotopes by the palaemonids.

Keywords: *Macrobrachium amazonicum*, Na⁺/K⁺-ATPase; Ontogeny Supported by: FAPESP and CNPq