

REGULATION OF RENAL Na, K-ATPASE ACTIVITY IN THE CLIMBING PERCH, *Anabas testudineus* (BLOCH) EXPOSED TO WATER-BORNE AMMONIA**A.S. VIJAYASREE^{a1}, L. DIVYA^b AND OOMMEN V. OOMMEN^c**^aDepartment of Zoology, Fatima Mata National College, Kollam, Kerala, India^bDepartment of Animal Sciences, Central University of Kerala, Kasaragod, India^cDepartment of Computational Biology & Bioinformatics, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India**ABSTRACT**

The osmoregulation in teleosts is regulated by the gills, kidney, and gut. Na⁺/K⁺-ATPase (NKA) is an index of osmoregulation expressed in animal cells to create an electrochemical gradient providing the driving force for the transport of ions in fish gills and kidneys. The present study evaluated the Na⁺, K⁺-ATPase activity (NKA) and expression in the kidney of the climbing perch, *Anabas testudineus*, exposed to different concentrations of water-borne ammonia (10 and 100 μM ammonium sulphate) for 48 hrs and during recovery in clean freshwater for another 96 hrs. There was a significant change in Na⁺, K⁺-ATPase activity and expression in control and ammonia exposed freshwater adapted fish. The western blot demonstrated the immunoreactive bands at ~100 kDa in the kidney of freshwater adapted perch and ammonia exposed groups. NKA protein expression as well as specific activity in kidneys significantly increased in the FW anabas. The primary function of the kidney in fresh water, is to excrete excess water, while reabsorbing most of the filtered solutes. The low levels of ions in freshwater with higher Na⁺, K⁺-ATPase activity in kidneys of the euryhaline teleosts provides an enhancement of reabsorbing tubular NaCl destined for large volumes of urinary output. To the best of our knowledge, however, the positive correlation between distribution of NKA activity and protein abundance in kidneys of *Anabas* in response to water borne ammonia stress has been demonstrated here for the first time. The present study concludes that the climbing perch can tolerate fairly high levels of water-borne ammonia, resulting in compensatory modifications of both hydro mineral and metabolic processes.

KEYWORDS: Na⁺K⁺ATPase, Kidney, Osmoregulation, *Anabas*, Western Blot

The aquatic environment is particularly sensitive to the toxic effects of contaminants since a considerable amount of the chemicals used in industry, urbanization and in agriculture enters marine and other aquatic ecosystem (Osman, et al., 2009). Ammonia is the major nitrogenous excretory product that is lost from the body across the gills to the aqueous environment, in teleost fish. Fishes tolerate ammonia and are able to maintain their plasma ammonia levels within a range (Mommensen and Walsh, 1992). Freshwater fishes excrete ammonia whereas ammonia is converted into urea in sea water fish (Frick and Wright, 2002). Maintaining an internal environment with stable conditions is essential for animals to survive in a variety of habitats. The osmoregulation of teleosts is regulated by the gills, kidney and gut. Na⁺K⁺ATPase (NKA) is a crucial primary enzyme expressed in animal cells to create an electrochemical gradient providing the driving force for ion transport in osmoregulatory organs, including fish gills and kidneys (Yang et al., 2016). Therefore, the present study focused on the kidney NKA expression of the freshwater fish, when exposed to water borne ammonia environment.

MATERIALS AND METHODS**Experimental Animals and Environments**

The experimental animal used in the present study is the Climbing perch, *Anabas testudineus*, Bloch

(1792). Perch of both sexes weighing 45-50g were maintained in large tanks and fed once a day with 1.5% body weight commercial fish feed. The specimens were given prophylactic treatment by bathing twice in 0.05% potassium permanganate solution for two minutes to avoid any dermal infections. Before commencement of the experiment, the fish were transferred to glass aquaria (20L) and kept for two weeks at water temperature 28±1°C and photoperiod 12hL:12h D cycle. Twenty-four fish were divided into 4 groups of six each and placed in separate glass aquaria. The fish of group I were freshwater control; group II and III were treated with 10 and 100 μM ammonium sulphate for 48 hr respectively. The group IV fish were first kept at 100 μM ammonium sulphate for 48h and later kept for recovery in clean freshwater for another 96 h. Feeding was stopped 24h prior to sampling.

Sampling and Analyses

After the treatment, the experimental fish were anaesthetized in 2-phenoxyethanol (SRL, Mumbai) and blood was collected from the caudal vein. The fish were then sacrificed by spinal transection and kidney tissues were excised and washed in ice-cold 0.25 M SEI buffer (pH 7.1) and kept at -20°C.

Electrophoretic Analyses**Polyacrylamide Gel Electrophoresis (SDS-PAGE)**