

**THE SLEEPER *BOSTRICHTHYES SINENSIS* (FAMILY ELEOTRIDAE)
PRODUCES AND STORES GLUTAMINE
DURING AERIAL EXPOSURE**

Y. K. Ip¹

¹Department of Biological Sciences, National University of Singapore,
10 Kent Ridge Road, Singapore 117543, Republic of Singapore.
Tel: (65) 874-2702. Fax: (65) 779-2486.

S. F. Chew², R. S. S. Wu³, and I. A. W. Leong¹
²Biology Division, Nanyang Technological University,
National Institute of Education,

469 Bukit Timah Road, Singapore 259756, Republic of Singapore,

³Department of Biology and Chemistry, City University of Hong Kong,
Tat Chee Avenue, Kowloon, Hong Kong, Republic of China

EXTENDED ABSTRACT ONLY - DO NOT CITE

Bostrichthyes sinensis is reputed as a good food fish rich in proteins, and is served as a tonic. It inhabits brackish water and finds its niche in the crevices of the river mouths of Shang Xi and Guangdong, China. In its natural habitat, it may encounter aerial exposure frequently during low tides, as it usually remains quiescent in the absence of water. Upon aerial exposure in the laboratory, the ammonia excretion rate decreased to one-fourth that of the submerged control. Different from the loach *Misgurnus anguillicaudatus* (Chew and Ip, unpublished results), *B. sinensis* was incapable of eliminating ammonia in gaseous form. The ammonia excretion rate of *B. sinensis* exposed to the terrestrial condition was ¼ of the submerged control, and ammonia was accumulated in the body of the fish. Although all the enzymes of the ornithine-urea cycle were present in the liver of this fish, the activity of hepatic carbamoyl phosphate synthetase using glutamine as a substrate was too low for the cycle to be functioning. Indeed, ammonia accumulated in the tissues was not converted to urea.

There are three other strategies which seem commonly adopted, singly or in combination, by fish to survive in the terrestrial condition:

reduction in proteolysis and/or amino acid catabolism to slow down the build up of ammonia.

partial catabolism of amino acid to alanine without releasing ammonia, and detoxification of internally produced ammonia to glutamine.

Strategies (1) and (2) are not detoxification mechanisms. Mudskippers (*Boleophthalmus boddarti* and *Periophthalmodon schlosseri*) adopt strategy (1) during aerial exposure in constant darkness (Lim et al, submitted for publication). However, *P. schlosseri* adopts strategies (1) and (2) when it is exposed to the terrestrial condition in a dark:light regime. Under such experimental conditions, it undergoes a reduction in amino acid catabolism in general with partial catabolism of certain amino acids to alanine to sustain activity on land (Ip et al, submitted for publication).

B. sinensis, did not produce and accumulate alanine when exposed to the terrestrial condition. Instead, it detoxified internally produced ammonia to glutamine (strategy 3 above) during the first 24 h of aerial exposure. During this period, the reduction in equivalent ammonia excreted amounts to 370 mmol, which could be solely answered for by the accumulation of 441 mmol (ammonia equivalent) as glutamine in the muscle. To our knowledge, glutamine has only been reported to play a role in ammonia detoxification in fish in response to high environmental ammonia concentrations (Mommensen and Walsh, 1991; Peng et al., 1998), with only one exception--the marble goby *Oxyeleotris marmoratus* (Jow et al, 1999).

Contrary to the production of alanine, as observed in the mudskipper *P. schlosseri* (Ip et al., submitted for publication) and the snakehead *Chana asiatica* (Chew and Ip, unpublished results), formation of glutamine is energetically expensive. *B. sinensis* and *O. marmoratus* are relatively inactive on land, and the reduced energy demand for muscular activity may provide them with the opportunity to exploit glutamine formation as a means to detoxify ammonia.

The accumulation of glutamine in *B. sinensis* leveled off after 48 h of aerial exposure. By the 72nd h of aerial exposure, there was a very great discrepancy between the reduction in excretion of ammonia equivalent (1109 mmol) and the retention of ammonia equivalent (458 mmol) as ammonia and urea. These results indicate that there might have been a reduction in proteolysis and amino acid catabolism when *B. sinensis* was exposed to the terrestrial condition for a long period (Strategy 1 above). Reduction in proteolysis and amino acid

catabolism is an effective strategy to slow down the internal build up of ammonia. If the rate of amino acid catabolism decreases and the rate of proteolysis remains unchanged, the steady state concentrations of free amino acids will increase. However, if the rate of proteolysis decreases to a greater extent than the rate of amino acid catabolism, the steady state levels of free amino acids will decrease. In this regard, *B. sinensis* is different from *O. marmoratus*, which is incapable of reducing proteolysis or amino acid catabolism in response to aerial exposure. *O. marmoratus* adopts strategy (3) for the whole period (72 h) of aerial exposure (Jow et al., 1999).

References

- Ip, Y. K., C. B. Lim, S. F. Chew, J. M. Wilson, and D. J. Randall. Production and accumulation of alanine through partial amino acid catabolism facilitate *Periophthalmodon schlosseri* (mudskipper) to use amino acid as an energy source while active on land. J. Exp. Biol. (submitted for publication).
- Jow, L. Y., S. F. Chew, C. B. Lim, P. M. Anderson and Y. K. Ip. 1999. The marble goby *Oxyeleotris marmoratus* activates hepatic glutamine synthetase and detoxifies ammonia to glutamine during terrestrial exposure. J. Exp. Biol. 202:237-245.
- Lim C. B., P. M. Anderson, S. F. Chew and Y. K. Ip. *Mudskippers (Periophthalmodon schlosseri and Boleophthalmus boddaerti)* reduce the rate of protein and amino acid catabolism to slow down internal ammonia build up during aerial exposure in constant darkness. J. Exp. Biol. (submitted for publication).
- Mommsen, T. P. and P. J. Walsh. 1991. Urea synthesis in fishes: Evolutionary and Biochemical Perspectives. In "Biochemistry and molecular biology of fishes, 1. Phylogenetic and biochemical perspectives". (P. W. Hochachka and T. P. Mommsen, eds). pp 137-163. Elsevier.
- Peng, K. W., S. F. Chew, C. B. Lim, S. S. L. Kuah, W. K. Kok and Y. K. Ip. 1998. The mudskippers *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti* can tolerate environmental NH₃ concentrations of 446 and 36 μM, respectively. Fish Physiol. Biochem. 19: 59-69.

