BOTH *PERIOPHTHALMODON SCHLOSSERI* (MUDSKIPPER) AND *CHANNA ASIATIA* (SNAKEHEAD) ACCUMULATE ALANINE DURING AERIAL EXPOSURE, BUT ONLY *P. SHLOSSERI* CAN SUSTAIN LOCOMOTORY ACTIVITY ON LAND THROUGH PARTIAL AMINO ACID CATABOLISM

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EXTENDED ABSTRACT ONLY-DO NOT CITE

Periophthalmodon schlosseri is a mudskipper, which can be suffocated when submerged in water and is very active on land. When it was exposed to terrestrial conditions under a 12 h:12 h light:dark regime, the fish could be very active, and levels of total free amino acids increased significantly in the muscle and plasma. Alanine level increased three-fold in the muscle, four-fold in the liver, and two-fold in the plasma. From these results, we concluded that *P. schlosseri* was capable of partial catabolism of certain amino acids to support activity on land. The amino groups of these amino acids were transferred directly or indirectly to pyruvate to form alanine. The resulting carbon chain was fed into the Krebs cycle and partially oxidized to malate, which could replenish pyruvate through the function of malic enzyme. This favourable ATP yield from partial amino acid catabolism was not accompanied by a net release

of ammonia. Such an adaptation would be advantageous to *P. schlosseri* confronted with the problem of ammonia excretion during aerial exposure.

Indeed, when *P. schlosseri* were forced to exercise on land after 24 h of aerial exposure, the alanine level in the muscle increased significantly, with no apparent change in glycogen content. In addition, there was no significant change in the ATP level and energy charge of the muscle. In contrast, when *Boleophtalmus. boddaerti,* another species of mudskipper, were exercised on land glycogen levels in the muscles decreased significantly, and lactate levels increased. In addition, muscle energy charge was not maintained and the ATP level decreased significantly. Hence, it can be concluded, that when *P. schlosseri* were active on land, they were capable of using certain amino acids as a metabolic fuel and avoided ammonia toxicity through partial amino acid catabolism. Such a strategy is the most cost-effective way of slowing down internal ammonia build-up without involving energy expensive ammonia detoxification pathways. Furthermore, an examination on the balance between nitrogenous excretion and accumulation in a 70 g *P. schlosseri* revealed that degradation of amino acids in general was likely to be suppressed to slow down the build-up of ammonia internally.

The fresh water snakehead, *Channa asiatica*, is an obligatory air-breather that resides in slow-flowing streams and in crevices near riverbanks. In its natural habitat, it may encounter bouts of aerial exposure during the dry seasons, but it cannot maintain activity on land like *P. schlosseri*. In the laboratory, the ammonia excretion rate of *C. asiatica* exposed to terrestrial conditions in a 12 h:12 h dark:light regime was ¼ that of the submerged control. Consequently, the ammonia contents in the muscle, liver and plasma increased significantly. Alanine increased 4-fold to 12.6 $\mu mol.g^{-1}$ in the muscle after 48 h of aerial exposure. The accumulated alanine could account for 70% of the deficit in ammonia excretion during this period, indicating that partial amino acid catabolism would have occurred. This would allow the utilization of certain amino acids as energy sources and, at the same time, minimize ammonia accumulation. There was a reduction in the aminating activity of glutamate dehydrogenase from the muscle and liver of specimens exposed to terrestrial conditions, presumably facilitating the entry of α-ketoglutarate into the Krebs cycle.

However, *C. asiatica* was unable to reduce the rates of proteolysis and amino acid catabolism as in mudskippers. The reduction in nitrogenous excretion during 48 h of aerial exposure was completely balanced by nitrogenous

accumulation in the tissues. Exercise on land led to a decrease in glycogen content with no significant effect on the ammonia and alanine contents in the muscle of *C. asiatica.* Hence, unlike the mudskipper *P. schlosseri, C. asiatica* was incapable of increasing the rate of partial amino acid catabolism to sustain locomotory activities on land. Taken altogether, it can be concluded that alanine accumulation through partial amino acid catabolism may be widely adopted by obligatory air-breathing fishes to avoid ammonia intoxication during aerial exposure, but not all of them can use it to fuel muscular activities.