PRODUCTION OF CO_2 INDEPENDENT OF O_2 UTILIZATION LEADING TO A CO_2:O_2 EXCHANGE RATIO OF >20: A NOVEL STRATEGY ADAPTED BY THE MUDSKIPPER *BOLEOPHTHALMUS BODDAERTI* TO LOWER THE PH OF, AND AMMONIA TOXICITY IN, THE WATER IN ITS BURROW

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Boleophthalmus boddaerti is a species of mudskippers found in mangrove swamps in estuaries of Singapore and Malaysia. The mud deposited there forms a suitable habitat in which it can thrive and build burrows. It stays in this confined volume of water in the burrow during high tides. In addition, it rears the developing embryos inside the burrow during the breeding season. Development of the embryos involves the mobilization of yolk proteins. Degradation of proteins and amino acids results in the production of ammonia, the toxicity of which is pH dependent. While the normal pH of seawater is around 8.0 to 8.3, the pH of the water in the mudskipper's burrow ranges from 6.8 to 7.3. How does this mudskipper manipulate the water pH in its burrow?

Here, we report that *B. boddaerti* responded to alkaline pH by increasing its production and excretion of carbon dioxide. The CO_2:O_2 exchange ratio for *B. boddaerti* exposed to pH 7 was approximately 1. However, when *B. boddaerti* was exposed to pH 9 the CO_2:O_2 exchange ratio increased to approximately 20 after 3 h. This is a unique observation, different from results reported for other fishes. The high respiratory exchange ratio obtained was resulted from a drastic increase in carbon dioxide excretion. Hydration of CO_2 would lead to the production of proton, lowering the pH of the external medium. Using various radiolabeled substrates, it was demonstrated that the increased production of carbon dioxide originated from the tricarboxylic acid cycle. The discrepancy between carbon dioxide production and oxygen consumption indicated that oxygen was not the terminal electron acceptor in mitochondrial redox balance. Indeed, mitochondria isolated from the muscle and liver of *B. boddaerti* were able to oxidize NADH in anoxia *in vitro*.

B. boddaerti also responded to the presence of NH_4Cl in the external medium at neutral pH by excreting more CO_2. After 3 h of exposure to15 mM NH_4Cl, the rate of carbon dioxide excretion was significantly higher than that of the control, with the oxygen consumption rate remained relatively unchanged. As a result, the CO_2:O_2 exchange ratio increased to approximately 10. This would suggest that *B. boddaerti* could increase the production and excretion of CO_2 when confronted with an ammonia-loading situation, possibly to reduce the toxicity of ammonia. The hydration of CO_2 in the external environment releases protons, which binds with NH_3 to form NH_4^+ which is the less toxic species. Since this mudskipper has the unique behavior of guarding and rearing the developing embryos in a limited volume of water in its burrow, we speculate that the increased production/excretion of CO_2 is a strategy developed to reduce the toxicity of ammonia in the burrow water during the breeding season. This can be regarded as a novel strategy of "environmental ammonia detoxification".