# ENVIRONMENTAL SULFIDE DETOXIFICATION: THE MUDSKIPPER *BOLEOPHTHALMUS BODDAERTI* REDUCES THE TOXICITY OF EXOGENOUS SULFIDE BY REGULATING THE DIRECTION AND/OR MAGNITUDE OF THE NET ACID ($H^+$) FLUX

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*Boleophthalmus boddaerti* is a species of mudskipper usually found along the intertidal zone of the mudflats in mangrove swamps in Singapore and Malaysia. Here, the water is saline or brackish, and is rich in algae that form its chief source of food. The mud deposited by the river at the estuary forms a suitable habitat in which these fishes can thrive and build their burrows. In the mud, organic decay leads to sulfide production, and the level of sulfide there can be high. Hence, the mudskipper would encounter sulfide whenever it stays inside the burrow, and indeed it exhibited a higher tolerance to sulfide than other fishes. The 24 h, 48 h and 96 h $LC_{50}$ values of sulfide for *B. boddaerti* with body weight ranging from 11.6-14.2 g were 0.786 mM, 0.567 mM and 0.467 mM, respectively.

The tolerance of *B. boddaerti* to sulfide was not due to the presence of a sulfide-insensitive cytochrome oxidase or effected by a shift to anaerobic fermentation. It possessed inducible mechanisms to detoxify sulfide in an ample supply or a lack of $O_2$. In normoxia, it detoxified sulfide to sulfate, sulfite and thiosulfate. However, in hypoxia, *B. boddaerti* detoxified sulfide mainly to sulfane sulfur. More importantly, it is capable of manipulating the pH of the external medium, which would affect the toxicity of sulfide in the environment, which is highly dependent on the ambient pH. It can adjust the pH of the external medium (8 vol. of 50% seawater), in the presence or absence of a buffer (2 mM MES, pH 6, or 2 mM Tris, pH 9), to close to 7 within several hours. It did so by varying the rate of titratable net acid efflux. After taking the ammonia flux into consideration, the rate of net acid efflux ($H^+$ excretion) was found to be pH-dependent, and increased significantly in the order pH 6< pH 7.2< pH 8< pH 9. At pH 6, the rate of $H^+$ excretion was the lowest, and the apparent alkalinization of the medium could be due to the excreted $NH_3$, which combined with proton in the external medium to form $NH_4^+$. At pH 8 or 9, *B. boddaerti* excreted much more $H^+$ into the medium so as to lower the ambient pH.

Net acid flux was inhibited partially by bafilomycin at pH 7 or 9, and was likely to be effected by a bafilomycin sensitive V-type $H^+$-pump in the gills and the opercular membrane. Sulfide inhibited ammonia efflux in *B. boddaerti*. However, more importantly, *B. boddaerti* responded to the presence of sulfide at pH 7.2 by absorbing $H^+$ from (or excreting $OH^-$ to) the ambient medium, with a net acid influx under such conditions, which led to a transient increase in the pH of the blood. Such a strategy would serve to lower the proportion of toxic hydrogen sulfide, which is the unionized and membrane-permeable form, in the environment; hence, effectively lowering the toxicity of sulfide. When exposed to sulfide at pH 8 or 9, the mudskipper decreased the rate of net acid efflux so as to avoid aggravating sulfide toxicity in the external environment.

This is the first report on the capability of a fish to manipulate the pH of the external environment in bulk by altering its rate of proton ($H^+$) excretion to lower the toxicity of sulfide in the external medium. It is likely that *B. boddaerti* acquired such a capability of "environmental sulfide detoxification" because of its unique behavior of building burrows and living in a limited volume of water in the sulfide-rich mudflats of mangrove swamps.