ALTERATIONS IN THE NITROGEN METABOLISM OF *Rivulus marmoratus* UNDER CONDITIONS OF PROLONGED AIR EXPOSURE -- ADAPTATIONS FOR SURVIVAL

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Abstract

Mangrove killifish (*R. marmoratus*) are capable of surviving prolonged periods out of water (>1 month) and can tolerate relatively high levels of external ammonia (>10 mM NH_4Cl). Under these extreme conditions alterations in nitrogen metabolism occur. During air exposure these fish continue to excrete both urea-N and ammonia, with a significant amount of the ammonia now being excreted through volatilization. Emersion also resulted in the accumulation of urea in the tissues after 4 days of exposure, while ammonia tissue levels did not change. Analysis of whole body tissue free amino acid levels revealed increases in both glutamine and alanine, whereas glutamate decreased significantly on days 1 and 4, but returned to control values by day 10. These findings demonstrate that *R. marmoratus* have evolved several strategies to prevent the toxic accumulation of ammonia, enabling them to survive under extreme environmental conditions.

Introduction and Objectives

The killifish *Rivulus marmoratus* is a small cyprinodontid, hermaphroditic fish which inhabits tropical mangrove forests. *R. marmoratus* can tolerate an incredible range of environmental conditions including relatively high levels of external ammonia (>10 mM NH_4Cl), and a range of salinities (0-68$^o/_{oo}$). They are also remarkable in their ability to survive out of water for extended periods of time (>1month). Thus, *R. marmoratus* may have evolved specialized mechanisms to deal with the problem of N-excretion under extreme conditions. Most teleosts are ammoniotelic (i.e. they excrete primarily ammonia), however ammonia is highly toxic and requires copious amounts of water for excretion. Thus, accumulation of ammonia under terrestrial conditions may occur and could be potentially poisonous. This study examined the mechanisms used by *R. marmoratus* to deal with the problem of nitrogen excretion on land.

Materials and Methods

Fish were held in separate containers at 25^{o}C in 17$^o/_{oo}$ artificial seawater, on a 12:12 hr photoperiod. Ammonia and urea excretion rates were measured in fish held in both water (control) and air (treatment), over a 10 day period. The amount of ammonia volatilized from fish held in air for 24 hours was quantified using the acid trap method described by Davenport and Sayer (1986). Ammonia and urea whole body tissue levels were measured after 1, 4, and 10 days of emersion according to the method of Wright *et al.* (1995). Whole body free amino acid levels (FAA) were also measured in air exposed fish over the same time period, using high performance liquid chromatography (HPLC) (Barton *et al.* 1995). The concentrations of ammonia and urea in water and tissue samples were determined using the methods of Ivancic and Degobbis (1984) and Rahmatullah and Boyde (1980), respectively.

Results

Emersed fish continued to excrete both ammonia and urea, but at decreased rates (~50% and 36% respectively, of submerged rates), throughout the 10 days of exposure. Ammonia volatilization was found to occur, with almost half (~46%) of the total ammonia excreted through this method. The level of urea found in whole body tissue extracts was not significantly different after one day of emersion (compared to control values) but did increase significantly on day 4 and 10. Tissue ammonia levels did not change under emersed conditions. Analysis of tissue FAAs showed a significant increase in both glutamine and alanine after one day of air exposure. A significant decrease in glutamate tissue levels was seen on days 1 and 4, with a return to control values occurring by day 10.

Conclusions

R. marmoratus utilize several different strategies to deal with the problem of nitrogen excretion while in the terrestrial environment. They are able to continuously excrete N-wastes during emersion. Toxic ammonia is converted to urea, some of which is stored within the tissues of the fish during emersion, indicating a shift towards ureotelism. Even more fascinating is the incredible ability of this fish to volatilize a relatively large percentage of ammonia. Changes in tissue FAA levels suggest an alternative method of storing nitrogenous wastes during air exposure. *R. marmoratus* have adapted several different strategies which allow them to survive out of water. Studying these adaptations can provide valuable insight in the evolution of nitrogen metabolism and excretion from the aquatic to terrestrial environment.

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