ACIDOPHILIC FISH OF THE AMAZON RIO NEGRO:

DO CALCIUM AND DOC AFFECT THEIR ACID TOLERANCE?

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Fish native to blackwater rivers such as the Rio Negro, Amazon, live in one of the most naturally acidic and dilute aquatic environments on earth. For example, Na^+ and Ca^{2+} ion concentrations are frequently < 10 μM due to the immense precipitation and ion-poor soils. In addition, pH's in the range 3.5-4.5 are common in the forest streams due to the poor buffering capacity and the vast input of organic acids from decaying plant material. The extreme pH alone would be extremely toxic to the majority of freshwater fish. This is due to ionoregulatory failure at the gills as a result of a) inhibition of active ion uptake, and b) acceleration of paracellular ion losses. The latter is also normally exacerbated in very soft water (low Ca^{2+}) due to the importance of external calcium in regulating the tight-junctional (i.e. paracellular) permeability of fish gills. It is possible that the ability of Rio Negro fishes to withstand these extreme conditions may be related to the presence of high levels of dissolved organic carbon (DOC). In addition, it is uncertain whether these fish have ionic effluxes that are dependent upon external calcium (Gonzalez et al., 1999). To examine these features of ion regulation we measured unidirectional ion fluxes (Na^+ and Cl^-) in adult Black piranha (*Pirhana preta*) resident to the blackwaters of the Rio Negro.

Materials and Methods

Experiments were conducted aboard the boat Amanai II during an expedition on the Rio Negro in December 1999. Animals were obtained from local fisherman and held in flowing Rio Negro water for several days prior to experiments. Fluxes were carried out with individual fish held in plastic containers holding 6 litres of aerated water. Fish were exposed to low pH (6.0-3.0) either in Rio Negro water with naturally high levels of dissolved organic carbon (DOC; >10 mg l^{-1}), or in well water containing negligible DOC but otherwise very similar ion contents. Unidirectional sodium influxes were measured by addition of ^{22}Na radioisotope to the external medium and monitoring its disappearance over time (usually 1 hour flux periods). Net sodium fluxes were calculated from changes in total [Na^+] measured by flame photometry, and unidirectional effluxes were calculated as the difference between net flux and influx (Gonzalez et al., 1997).

Results & Discussion

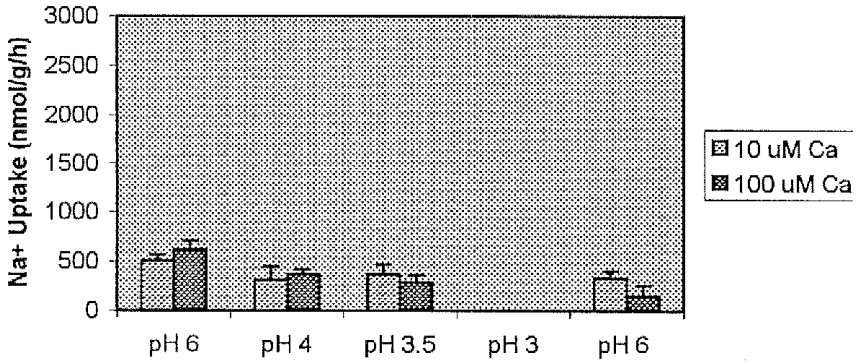
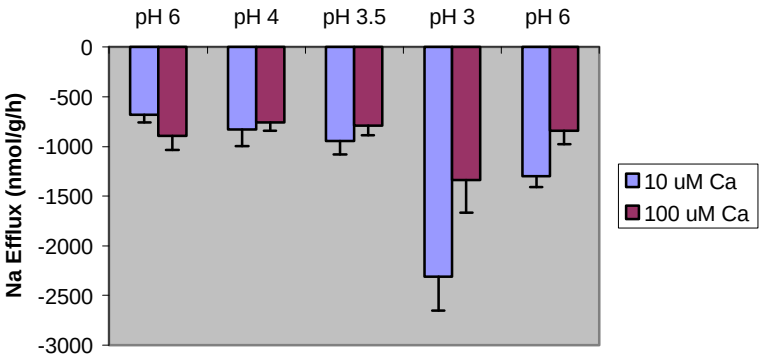


Figure 1. Effect of added calcium and pH on Na+ uptake in Pirhana pretain Rio Negro water

In Rio Negro black water, unidirectional and net Na^+ fluxes were unaffected at pH's $\geq$ 3.5, and calcium (10 or 100 μM) had no additional influence. However, at pH 3, Na^+ influx was abolished, Na^+ efflux was doubled, and 100 μM calcium significantly reduced the impact on efflux by about 40% (Fig.1 & 2). Thus the protective influence of calcium appears to be pH-

Figure 2. Effect of calcium and pH on Na+ effluxes in Piranha preta in Rio Negro water.



dependent in these Rio Negro fish. Further experiments showed that Na^+ efflux was insensitive to DOC at pH 6 and 3.5 (when fluxes were measured in DOC-free well-water). However, Na^+ influx was inhibited by ~30% upon transfer from Rio Negro blackwater (DOC > 10 mg l^{-1}) to water with low DOC (but with similar ionic content). This effect of DOC on the active Na^+ uptake mechanism suggests that the source of acidity (i.e. organic acids) may have some influence upon the acid tolerance of freshwater fish. The role of DOC in gill ion transport mechanisms clearly warrants further exploration if we are to understand the mechanisms behind the extremely high tolerance to acidity in these Amazonian fishes.

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