

**ION TRANSPORT IN RIO NEGRO FISH:  
DEALING WITH THE DOUBLE WHAMMY**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

The Rio Negro, a major tributary of the Amazon River, drains extremely mineral poor soils and is very dilute. Typical cation concentrations of the river in  $\mu\text{mol L}^{-1}$  are:  $\text{Na}^+ = 16.5 \pm 5.3$ ,  $\text{K}^+ = 8.2 \pm 2.7$ ,  $\text{Ca}^{2+} = 5.3 \pm 1.6$ ,  $\text{Mg}^{2+} = 4.7 \pm 1.4$  (Furch 1984); small forest streams that feed the Rio Negro can be even more dilute. These waters have a very low buffering capacity and the presence of organic acids from decaying vegetation (giving the water its tea color, and name), make it acidic ( $< \text{pH } 4.5$ ). Despite these seemingly harsh conditions, estimates indicate that almost twice as many species inhabit the Rio Negro as all the waters of North America.

Ion poor water of low pH such as that found in the Rio Negro pose a dual challenge for ion transport in fish. Both the scarcity of salts and low pH inhibit ion uptake (a double whammy for ion transport). Since the rate of ion uptake is concentration dependent, extremely low levels of  $\text{Na}^+$  and  $\text{Cl}^-$  in the water can reduced uptake simply because of the extreme scarcity of these salts. Alternatively, low pH can inhibit  $\text{Na}^+$  uptake since transport across the apical membrane of the gill epithelium requires the extrusion of  $\text{H}^+$ . The inhibition of  $\text{Na}^+$  transport can then lead to the inhibition of  $\text{Cl}^-$  uptake. (Perry and Randall 1982). The theoretical low pH limit for active ion uptake is believed to be about pH 4.0 (Gonzalez 1996). In San Diego and in Brasil colleagues and I, have been examining the ion transport capabilities of Rio Negro fishes to determine what specializations they possess that allow them to successfully ionoregulate and inhabit this river despite the harsh conditions.

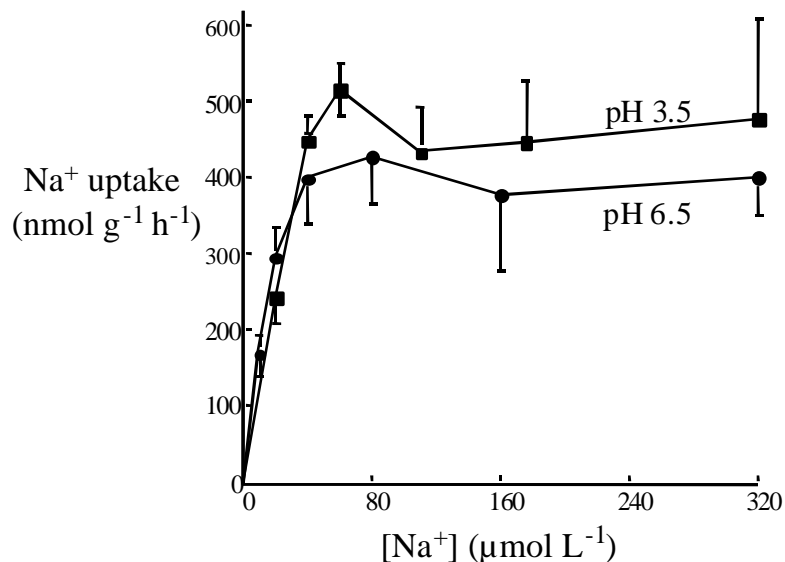
Our measurements of Na<sup>+</sup> transport in relation to water Na<sup>+</sup> concentration have shown that many Rio Negro species possess high affinity, high capacity transporters (Table 1). Among

Table 1. Kinetic parameters of Na<sup>+</sup> transport in some Rio Negro fishes.

<b>Species</b>	<b>K<sub>m</sub></b> <b>(<math>\mu\text{mol L}^{-1}</math>)</b>	<b>J<sub>max</sub></b> <b>(<math>\text{nmol g}^{-1} \text{h}^{-1}</math>)</b>
Neon tetras	12.9 ± 5.8	448.2 ± 43.5
Cardinal tetras	53.7 ± 7.8	773.0 ± 38.2
Blacksirt tetras	27.7 ± 2.7	691.3 ± 19.9

those tested, neon tetras have the lowest K<sub>m</sub> value recorded for freshwater fish (Gonzalez and Preest 1999). These characteristics are clearly adaptive for operation in ion-poor waters. They allow the species to take up salts at high rates even when water concentrations are very low (Gonzalez et al. 1997). Interestingly there appears to be a phylogenetic component to these characteristics. The three species listed above are characins, but several species of cichlids have much lower affinities.

Figure 1. The effect of water pH on the relationship between rate of sodium uptake and water  $\text{Na}^+$  concentration in neon tetras.



Our examination of pH effects on ion transport revealed that, in general, Rio Negro species possess a reduced sensitivity to low pH. Most interesting, however, is the finding that one species, neon tetras, possess an ion transport mechanism that is completely insensitive to pH (Fig. 1). Kinetic analysis of  $\text{Na}^+$  transport at pH 6.5 and 3.25 yielded identical  $K_m$  and  $J_{max}$  values ( $\text{Cl}^-$  transport was also found to be pH insensitive). In fact,  $\text{Na}^+$  uptake of neon tetras was still uninhibited at pH 3.25, an almost 2000-fold increase in  $\text{H}^+$  concentration, relative to pH 6.5, and the lowest pH at which uptake has ever been observed.

To probe the nature of the neon tetras' pH insensitive transport mechanism we measured the rate of  $\text{Na}^+$  uptake in the presence of various pharmacological agents that block different types of transporters. Amiloride analogues, such as DMA, HMA, MIA, and EIPA, that block  $\text{Na}^+/\text{H}^+$  antiporters had effects ranging from 0 to 40% inhibition.  $\text{Na}^+$  channel blockers like Benzamil and

Phenamil had no effect on Na<sup>+</sup> transport, H<sup>+</sup>-ATPase inhibitor vanadate lowered uptake by 25%. These results do not offer strong support for the presence of either Na<sup>+</sup>/H<sup>+</sup> antiporters or H<sup>+</sup>-ATPase/Na<sup>+</sup> channel arrangements in neon tetras.

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