Diverse Strategies for Ion Regulation in Fish Collected from the Ion-Poor, Acidic Rio Negro

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ABSTRACT

We measured unidirectional ion fluxes of fish collected directly from the Rio Negro, an extremely dilute, acidic blackwater tributary of the Amazon. Kinetic analysis of Na^+ uptake revealed that most species had fairly similar J_{max} values, ranging from 1,150 to 1,750 nmol g^{-1} h^{-1}, while K_m values varied to a greater extent. Three species had K_m values <33 μmol L^{-1}, while the rest had K_m values ≥ 110 μmol L^{-1}. Because of the extremely low Na^+ concentration of Rio Negro water, the differences in K_m values yield very different rates of Na^+ uptake. However, regardless of the rate of Na^+ uptake, measurements of Na^+ efflux show that Na^+ balance was maintained at very low Na^+ levels (<50 μmol L^{-1}) by most species. Unlike other species with high K_m values, the catfish *Corydoras julii* maintained high rates of Na^+ uptake in dilute waters by having a J_{max} value at least 100% higher than the other species. *Corydoras julii* also demonstrated the ability to modulate kinetic parameters in response to changes in water chemistry. After 2 wk in 2 mmol L^{-1} NaCl, J_{max} fell >50%, and K_m dropped about 70%. The unusual acclimatory drop in K_m may represent a mechanism to ensure high rates of Na^+ uptake on return to dilute water. As well as being tolerant of extremely dilute waters, Rio Negro fish generally were fairly tolerant of low pH. Still, there were significant differences in sensitivity to pH among the species on the basis of degree of stimulation of Na^+ efflux at low pH. There were also differences in sensitivity to low pH of Na^+ uptake, and two species maintained significant rates of uptake even at pH 3.5. When fish were exposed to low pH in Rio Negro water instead of deionized water (with the same concentrations of major ions), the effects of low pH were reduced. This suggests that high concentrations of dissolved organic molecules in the water, which give it its dark tea color, may interact with the branchial epithelium in some protective manner.

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Introduction

Recent studies of fish native to the ion-poor, acidic blackwaters of the Rio Negro reveal two basic strategies for maintenance of ion balance. In several characid species, ion regulation is characterized by high-affinity, high-capacity ion transport systems that are relatively insensitive to low pH (Gonzalez et al. 1997; Gonzalez and Preest 1999; Gonzalez and Wilson 2001). At the same time, diffusive ion loss is resistant to the stimulatory effects of low pH. The highest degree of specialization in this group is found in neon tetras (*Paracheirodon innesi*), which possess a very high affinity Na^+ transporter (K_m = 12 μmol L^{-1}) that is completely insensitive to water pH (Gonzalez and Preest 1999). Consequently, in waters as acidic as pH 3.5, they ion regulate much as they do in circumneutral waters. The alternate strategy is displayed by angelfish (*Pterophyllum scalare*; Cichlidae), which possess a low-affinity, low-capacity ion transporter (rates of Na^+ uptake are one-seventh that of neon tetras) that is pH sensitive and completely inhibited at around pH 4.0 (Gonzalez and Wilson 2001). To maintain ion balance, angelfish rely on equally low rates of diffusive ion loss that are resistant to stimulation by low pH. Their strategy is to limit net ion loss at low pH and wait out the exposure with minimal disturbance.

The studies describing these two ionoregulatory patterns were largely performed on fish acquired from tropical fish suppliers in North America or Europe. Some species were originally collected from the Rio Negro, but the chemistry of the water in which they were subsequently held before acquisition is unknown (Gonzalez and Wilson 2001). Other species were ac-

tually bred outside of the Amazon for multiple generations. Little is known about the effects these factors have on the physiology of the fish examined. Studies have shown that many species can readily acclimate to altered water chemistry (McDonald and Rogano 1986; Gonzalez et al. 1997) and that selection for unrelated traits can cause physiological differences to arise (Robinson et al. 1976). The question remains: how accurately do these studies reflect how fish in the wild are ion regulating in ion-poor, acidic waters?

Some studies on fish collected directly from the Rio Negro seem to confirm some aspects of the previous findings. For instance, fish collected directly from Rio Negro appear to be more tolerant of low pH than non–Rio Negro species (Gonzalez et al. 1998; Wood et al. 1998; Wilson et al. 1999). However, these studies were limited by inability to use radioisotopes to measure unidirectional ion fluxes. Without these measurements, we can say very little about the specific dynamics of ion regulation. Therefore, the primary goal of this study was to survey ion regulation in a variety of fish species collected directly from the Rio Negro shortly before testing. We used radioisotopes to measure unidirectional Na^+ fluxes in species from a range of families during exposure to ion-poor waters and low pH. In addition, we examined what effect dissolved organic carbon in Rio Negro water had on ion regulation during low pH exposure. Previous work suggested that organic compounds in the water might stabilize gill membranes and reduce the negative effects of low pH exposure (Gonzalez et al. 1998).

Material and Methods

Experimental Animals

Species from the Rio Negro examined in this study were acquired from either local tropical fish suppliers in Manaus, Brazil, or by seine nets or dip nets directly from the Rio Negro in the area of the Anavilhanas archipelago 100 km above Manaus. Species were identified at least down to genus and to species where possible. Five species were acquired from suppliers: an armored catfish (*Corydoras julii*; $N = 21$, wet mass $= 1.45 \pm 0.05$ g), a characid (*Hemigrammus* sp.; $N = 14$, wet mass $= 1.19 \pm 0.06$ g), a hatchet fish (family Gasteropelecidae, *Carnegiella strigata*; $N = 14$, wet mass $= 1.64 \pm 0.08$ g), and two cichlids (*Apistogramma* sp. A [$N = 21$, wet mass $= 1.19 \pm 0.06$ g] and *Satanoperca jurupari* [$N = 7$, wet mass $= 9.9 \pm 2.3$ g]). Three species were collected directly from the river: a catfish (*Pimelodes* sp.; $N = 21$, wet mass $= 1.67 \pm 0.05$ g) and two more cichlids (*Apistogramma* sp. B [$N = 7$, wet mass $= 0.77 \pm 0.09$ g] and *Geophagus* sp. [$N = 21$, wet mass $= 2.91 \pm 0.22$ g]). Fish from local suppliers were held in Rio Negro water until our acquisition and were then placed in INPA (Instituto Nacional de Pesquisas da Amazonia) well water, which is similar to the Rio Negro in concentrations of major ions and pH (Table 1), for a few days before testing. Fish collected by net from the Rio Negro were held in Rio Negro water before testing. Fish were not fed during the time they were held. The temperature of holding and test water was approximately 25°C, and they were under a natural photoperiod for Manaus (approximately 12L : 12D). Fish were generally held for only a few days and were not fed during this time period. If fish were held longer, they were fed flake food twice daily but were not fed for at least 24 h before the initiation of any tests.

Table 1: Water chemistry of Rio Negro and INPA well water

Water	pH	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	Total Carbon
Rio Negro	6.0	29.6	17.6	22.8	9.4	7.0	15.5
INPA well water	6.5	18.8	15.9	21.4	8.8	2.0	…

Note. Units for salts are micromoles per liter (μmol L^{-1}), and units for total carbon are milligrams per liter (mg L^{-1}) humic acid.

Water Chemistry Analysis

Water Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations were measured with a Perkin-Elmer model 3100 atomic absorption spectrophotometer (AAS) or a CELM model FC108 flame photometer. Water Cl^- concentration was measured with a colorimetric assay (Zall et al. 1956). Total dissolved organic carbon content was roughly estimated colorimetrically by measuring absorbance at 330 nm relative to humic acid standards.

Experimental Protocol

The net Na^+ flux (J_{net}^{Na}) and Na^+ influx (J_{in}^{Na}) were measured simultaneously, and Na^+ efflux (J_{out}^{Na}) was calculated for fish under a variety of conditions. Seven fish were weighed and placed in individual 40-mL chambers connected to a 50-L recirculating system filled with deionized water to which salts (NaCl, KCl, $CaNO_3$) had been added to approximately match the concentrations of the well water. Flow rate into each container was about 100 mL min^{-1}, and each container had an individual air line. At the beginning of a measurement period, flow was stopped to all containers, the radioisotope $^{22}NaCl$ was added to each chamber, and after a 5-min mixing period, a 6-mL water sample was removed from each. One hour later, a second water sample was removed from each chamber and flow was restored. One milliliter from each water sample was assayed for $^{22}Na^+$ with a Picker Cliniscaler gamma counter connected to a 4-inch NaI crystal with a well. The remainder of each sample was assayed for Na^+ concentration by flame photometry or AAS. J_{net}^{Na} was calculated from the change in the Na^+ con-

centration of the bath water during the flux period using the following equation:

$$J_{net}^{Na} = ([Na]_i - [Na]_f)V(M \times t)^{-1},$$

where $[Na]_i$ and $[Na]_f$ are the bath Na^+ concentrations at the beginning and end of the flux period, respectively, V is the bath volume in liters, M is the mass of the fish in grams, and t is the duration of the flux period in hours.

J_{in}^{Na} was calculated from the disappearance of isotope from the water and the average Na^+ concentration of the water during the flux period, employing back-flux correction as directed in Wood (1988), using the following equation:

$$J_{in}^{Na} = \frac{([R]_i - [R]_f)V_{ext} - SA_{int}([Na]_i - [Na]_f)}{(SA_{int} - SA_{ext})M},$$

where $[R]_i$ and $[R]_f$ are initial and final radioactivities (in counts min^{-1} mL^{-1}) and SA_{int} and SA_{ext} are, respectively, the mean internal and mean external specific activities (in counts min^{-1} μmol^{-1} Na) over the flux period. For calculation of SA_{int}, a value of 300 mL kg^{-1} was used for internal distribution volume, and 50 μmol g^{-1} was used for the total exchangeable internal pool of Na^+. $[Na]_i$ and $[Na]_f$ are the initial and final water Na^+ concentrations (in μmol mL^{-1}), V_{ext} is the volume of the flux chamber (in mL), and M is mass in kilograms. J_{out}^{Na} was calculated for each fish from the difference between J_{net}^{Na} and J_{in}^{Na}.

Experimental Series

Effect of Water Na^+ Concentration on Na^+ Fluxes. We examined the effects of water Na^+ concentration on Na^+ uptake (J_{in}^{Na}) and efflux (J_{out}^{Na}) during serial exposure to six Na^+ concentrations ranging from 10 to 320 μmol L^{-1} at pH 6.5. Seven fish were placed in individual chambers connected to the recirculating system and allowed to recover overnight in water identical to that in the holding tanks. To begin the experiment, the 50-L system was drained and refilled with deionized water. Salts were then added to reach the desired concentrations (all salts except NaCl were similar to INPA well water), and the pH was adjusted to 6.5. After a 15-min exposure to the first (lowest) Na^+ concentration, flow was stopped, isotope was added, and a 1-h flux period was initiated by taking a water sample. For the first four fluxes, 1.85 kBq of ^{22}Na was added to each chamber from a 37-kBq stock. For the fifth and sixth fluxes, 3.7 and 5.5 kBq ^{22}Na was added to each chamber, respectively. After the flux was completed, an aliquot of NaCl was added to the water to double roughly the Na^+ level. After a 15-min exposure to the new concentration, another flux period was started. This was repeated until fish had experienced all six Na^+ concentrations. From the data produced, the Michaelis-Menten constant (K_m), a measure of the transport mechanism's affinity for Na^+, and the maximum transport capacity (J_{max}) were estimated with nonlinear regression using the equation $J_{in}^{Na} = (J_{max}[Na^+])(K_m + [Na^+])^{-1}$ as the model. Measurements of J_{in}^{Na} of *S. jurupari* over the original range of Na^+ concentrations were very low, so the experiment was repeated over a larger range of Na^+ concentrations (up to 900 μmol L^{-1}).

In a follow-up test, we examined the ability of *C. julii* to acclimate to elevated Na^+ levels. The goal of this test was to see whether they could adjust the kinetic parameters of their uptake system in response to different Na^+ concentrations. We measured J_{in}^{Na} over a range of Na^+ concentrations in fish after 2 wk in INPA well water to which 2 mmol L^{-1} NaCl was added. Fish were placed in chambers connected to the recirculating system with the 2 mmol L^{-1} NaCl water overnight. To begin the flux measurements, the system was drained and flushed with deionized water to rinse out the extra NaCl. Then deionized water with the appropriate salts was added, and measurements were made as before.

Low pH Exposure. To describe the effects of acute low pH exposure on Na^+ regulation, J_{in}^{Na}, J_{out}^{Na}, and J_{net}^{Na} were measured at pH 6.5 (control) and during serial 1-h exposures, first to pH 4.0 and pH 3.5. Four species were examined in this way: *C. julii*, *Hemigrammus*, *C. strigata*, and *Apistogramma* A. Seven individuals were placed in individual flux chambers in the recirculating system containing 50-L deionized water with salts added to reach concentrations similar to the water in which the fish were held (pH 6.5), and the fish were allowed to recover overnight. A 1-h flux was performed at pH 6.5, and the pH of the water was lowered to 4.0 by addition of concentrated HNO_3. After a 30-min exposure, another flux was performed. This procedure was repeated for pH 3.5 exposure. Water pH was continuously monitored with an Orion model 250A pH meter and adjusted as needed. At the end of the fluxes, the pH of the water in the individual chambers was checked, and typically, it was within 0.1 units or less of the target pH.

Effects of Water Type on Na^+ Fluxes at Low pH. To evaluate the effects of Rio Negro water on ion regulation at low pH, J_{in}^{Na}, J_{out}^{Na}, and J_{net}^{Na} were measured in two species, *Geophagus* and *Pimelodes*, during exposure to pH 3.75 in Rio Negro water and in deionized water to which salts had been added. For each species, seven fish were placed in individual flux chambers connected to the system filled with either Rio Negro water or deionized waters with salts. The pH of both waters was adjusted to pH 6.5 with KOH. After an overnight recovery period, a 1-h flux measurement was performed at pH 6.5. The pH was lowered to pH 3.75 with HNO_3, and a flux measurement was made after a 15-min exposure. Finally, the pH was raised again to 6.5 by addition of KOH, and a third flux measurement was made.

Statistical Analyses

All data are reported as means ± 1 SE. Means were compared using paired *t*-tests or ANOVA (overall $P \leq 0.05$) with multiple comparisons (Scheffé test) if ANOVA proved significant. J_{max} and K_m values were compared by inspection of 95% confidence intervals. If they did not overlap, they were considered different.

Results

On the basis of the nonlinear regression analysis, sodium uptake in seven of the eight species studied displayed typical saturation kinetics (Fig. 1), including *Satanoperca jurupari*, which were tested over a greater Na^+ concentration range (data not shown). Na^+ uptake of *Apistogramma* B failed to saturate in the low concentration range, and the kinetic parameters could not be estimated. Among species for which kinetic analysis was performed, some significant differences were apparent (Table 2). Five of the seven species had fairly similar J_{max} values ranging from about 1,150 to 1,750 nmol g^{-1} h^{-1}. In contrast, *Corydoras julii* had a J_{max} value that was >3,500 nmol g^{-1} h^{-1}, and *S. jurupari* had a $J_{max} < 500$ nmol g^{-1} h^{-1}. Even greater variation was seen among the K_m values. *Pimelodes*, *Hemigrammus*, and *Carnegiella strigata* had similar very low K_m values (<33 μmol L^{-1}). In contrast, *C. julii* and *Geophagus* had K_m values about three to five times greater than the first group, and *Apistogramma* A and *S. jurupari* had K_m values almost 10 times higher. Obviously, *Apistogramma* B would have had an even higher K_m value if it had been possible to measure it.

When we plotted J_{out}^{Na} and J_{in}^{Na} versus water Na^+ concentration, species fell into two categories. *Hemigrammus*, *C. strigata*, and *Apistogramma* A were similar to *C. julii*, with J_{out}^{Na} and J_{in}^{Na} increasing with Na^+ concentration (Fig. 2*A*). In contrast, J_{out}^{Na} of *Pimelodes*, *Geophagus*, and *S. jurupari* remained constant over the whole range of water Na^+ concentrations (Fig. 2*B*). Regardless of whether or not J_{out}^{Na} rose with water Na^+ concentration, in all but two species, the Na^+ concentration where $J_{in}^{Na} = J_{out}^{Na}$ was ≤43 μmol L^{-1} (Table 2).

After *C. julii* was held for 2 wk in 2 mmol L^{-1} NaCl water, Na^+ uptake still displayed saturation kinetics (Fig. 3). However, there were significant changes in the kinetic parameters (Table 2). J_{max} dropped by more than half, and the K_m value fell by almost two-thirds (Table 2).

During exposure to low pH, the four species tested exhibited significant differences in ability to maintain ion balance. While *Apistogramma* A and *C. strigata* experienced relatively mild disturbances, as indicated by J_{net}^{Na}, *C. julii* and *Hemigrammus* had much larger disturbances (Fig. 4). The reason for the large disturbances in the latter two species was the greater stimulation of J_{out}^{Na}. *Corydoras julii* appeared to be the most pH sensitive because J_{out}^{Na} rose 2.5 times on transfer from pH 6.5 to 4.0 and 8.5 times on transfer to pH 3.5. J_{out}^{Na} of *Hemigrammus* did not increase on transfer to pH 4.0, but it rose 3.5-fold at pH 3.5. There were also significant differences in the sensitivity of J_{in}^{Na}

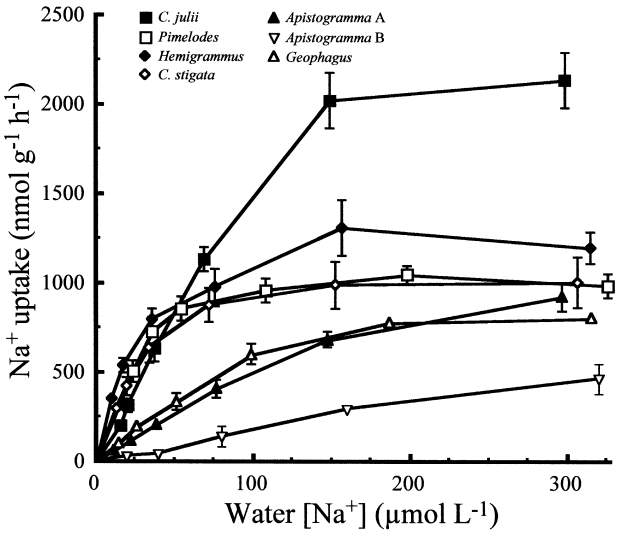

Figure 1. The relationship between Na^+ uptake and external Na^+ concentration for seven species of fish collected from the Rio Negro. Values are means ± SE ($N = 7$).

to pH among the four species (Fig. 4). J_{in}^{Na} of *Hemigrammus* was inhibited by 57% at pH 4.0 and completely inhibited at pH 3.5, while J_{in}^{Na} of *Apistogramma* A was completely inhibited at pH 4.0 and below. In contrast, J_{in}^{Na} of both *C. strigata* and *C. julii* was inhibited by only 20% at pH 4.0 and 45% at pH 3.5.

Exposure to low pH in Rio Negro water significantly reduced the inhibition of J_{in}^{Na} and stimulation of J_{out}^{Na} in the cichlid *Geophagus* relative to measurements in deionized water containing the same levels of major ions (Fig. 5*A*). At pH 6.5 (control), fish in both Rio Negro and deionized water had similar rates of Na^+ uptake, and J_{in}^{Na} of both was inhibited on transfer to pH 3.75. However, Na^+ uptake of fish in Rio Negro water was significantly less inhibited than in deionized water. On return to pH 6.5 water, J_{in}^{Na} of Rio Negro fish was significantly higher than J_{in}^{Na} of fish in deionized water. Differences were also observed in J_{out}^{Na}. Exposure to pH 3.75 in deionized water caused J_{out}^{Na} to rise 2.5-fold, while J_{out}^{Na} of fish in Rio Negro water was not changed (Fig. 5*B*).

Exposure to low pH in Rio Negro water also affected ion fluxes in the catfish *Pimelodes* (Fig. 6) but in very different ways than with *Geophagus*. At pH 3.75, J_{in}^{Na} of fish in Rio Negro water was only slightly inhibited, while uptake of fish in deionized water was unaffected (Fig. 6*A*). Surprisingly, on return to pH 6.5, both groups experienced a similar 80% inhibition of J_{in}^{Na}. J_{out}^{Na} of fish in Rio Negro water was unaffected by exposure to pH 3.75 or return to pH 6.5 (Fig. 6*B*). In contrast, J_{out}^{Na} of fish in deionized water rose about 55% at pH 3.75 and jumped 155% on return to pH 6.5.

Table 2: Kinetic parameters and r^2 values of nonlinear regression for Na^+ transport versus water Na^+ concentration for fish collected from the Rio Negro

Species	K_m (μmol L^{-1})	J_{max} (nmol g^{-1} h^{-1})	r^2	[Na^+] (μmol L^{-1})
Corydoras julii	147.8 ± 38.3	3,604.6 ± 449.9	.983	19
C. julii (2 mM NaCl)	56.1 ± 16.4	1,709.9 ± 173.0	.967	…
Pimelodes	29.7 ± 7.3	1,263.9 ± 82.2	.968	32
Hemigrammus	30.9 ± 5.5	1,440.0 ± 75.9	.983	22
Carnegiella strigata	32.5 ± 6.4	1,225.0 ± 74.5	.978	24
Apistogramma A	258.5 ± 46.1	1,752.5 ± 181.2	.995	80
Geophagus	111.8 ± 30.9	1,154.5 ± 135.5	.976	43
Satanoperca jurupari	276.7 ± 149.3	457.1 ± 102.9	.894	118
Paracheirodon innesi[a]	12.9 ± 5.8	448.2 ± 43.5	.944	…
Paracheirodon axelrodi[b]	53.7 ± 7.8	773.0 ± 38.2	.988	…
Gymnocorymbus ternetzi[c]	27.7 ± 2.7	691.3 ± 19.9	.932	…
Pterophyllum scalare[b]	136.1 ± 66.4	428.0 ± 96.7	.940	…

Note: Values are means ± SE ($N = 7$ for each species). Previously reported kinetic parameters for Rio Negro species acquired in North America or Europe are shown for comparison. Final column is water Na^+ concentration, where $J_{in}^{Na} = J_{out}^{Na}$ from Figure 2.

[a] Data from Gonzalez and Preest 1999.

[b] Data from Gonzalez and Wilson 2001.

[c] Data from Gonzalez et al. 1997.

Discussion

Kinetic analysis of Na^+ uptake in fish collected directly from the Rio Negro revealed significant differences among the species. Although J_{max} values were fairly similar, five of the eight species had maximum uptake capacities within the fairly narrow range of 1,150–1,750 nmol g^{-1} h^{-1}; K_m values varied to a much greater degree. While *Pimelodes*, *Hemigrammus*, and *Carnegiella strigata* had K_m values around 30 μmol L^{-1}, all other species had K_m values of 110 μmol L^{-1} or higher. Because of the extremely low Na^+ concentration of the waters in which the fish live, these differences in K_m significantly influence their rate of Na^+ uptake. For example, *Pimelodes* and *Geophagus* have similar J_{max} values, but *Geophagus* has a K_m more than five times higher than *Pimelodes* (Table 2), so its estimated rate of uptake (using the nonlinear equation) in water with a Na^+ concentration of 20 μmol L^{-1} is less than one-third as high.

While measuring J_{in}^{Na} across a range of Na^+ concentrations, we also measured J_{out}^{Na} (Fig. 2). The Na^+ concentration, where the J_{in}^{Na} and J_{out}^{Na} lines cross, indicates the minimum water Na^+ concentration at which the fish can maintain ion balance. For all but two species, the point of intersection was at a very low Na^+ concentration ($\lesssim$43 μmol L^{-1}), which matches fairly well with the Na^+ concentration of the water from which they were collected. Combining influx and efflux data, we can identify two basic ionoregulatory patterns. The presence of a high-affinity Na^+ transporter and high rates of uptake and loss in dilute waters characterizes one pattern (as seen in *Pimelodes* and *Hemigrammus*). A low-affinity transporter and much lower rates of uptake and loss in dilute waters distinguish the other, as with *Geophagus* or *Satanoperca jurupari*. These results appear to confirm previous findings for Rio Negro fish obtained in North America and Europe. Neon, cardinal, and blackskirt tetras have very high affinity transporters and high rates of Na^+ transport and diffusive loss (Gonzalez et al. 1997; Gonzalez and Preest 1999), but angelfish have a much lower affinity transporter and rates of uptake and loss (Gonzalez and Wilson 2001).

One species, the catfish *Corydoras julii*, does not display either ionoregulatory pattern described above. Despite having a fairly low affinity transporter, *C. julii* was able to maintain a rate of Na^+ uptake in 20 μmol L^{-1} NaCl water comparable to fish with much higher affinity transporters. Thus, there appears to be at least one additional pattern of ion regulation in Rio Negro fish. Given the 1,000-plus species of fish that inhabit the Rio Negro (Val and de Almeida-Val 1995), it seems likely that there may be many other species that also display this pattern of ion regulation.

The catfish *C. julii* is peculiar in another way. After 2 wk in Na^+-rich water, the affinity of their transporter for Na^+ rose markedly (K_m dropped by >50%; Fig. 3; Table 2). A more typical acclimatory response to Na^+-rich waters is to lower affinity (along with lowering uptake capacity) in order to return J_{in}^{Na} to levels comparable to rates in dilute waters (McDonald and Rogano 1986). It is even more curious because a rise in affinity would have little effect on J_{in}^{Na} since uptake is saturated in 2 mmol L^{-1} NaCl water. These changes could signal the mobilization of a separate population of transporters that are less dense but have a higher affinity. Regardless of the cause, a higher affinity may be of value on return to dilute water by ensuring that J_{in}^{Na} would remain high despite the lowered uptake capacity. Indeed, calculations using the nonlinear regression equation

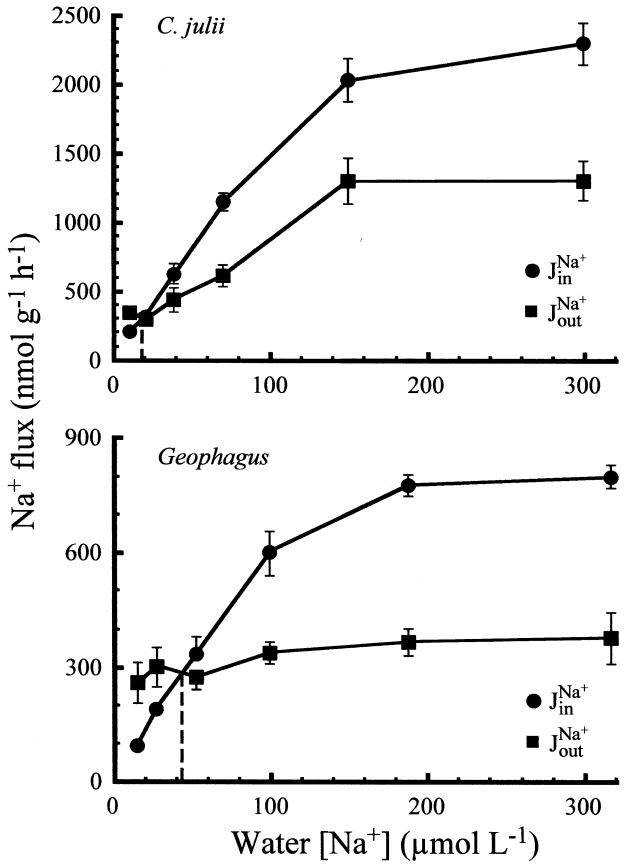

Figure 2. Na^+ influx (J_{in}^{Na}) and efflux (J_{out}^{Na}) in relation to water Na^+ concentration for two species collected from the Rio Negro displaying the two types of relationships observed. Values are means ± SE ($N = 7$). In each case, the vertical dashed line indicates the water Na^+ concentration, where $J_{in}^{Na} = J_{out}^{Na}$.

show that *C. julii* from low- and high-Na^+ waters would have virtually identical rates of Na^+ uptake in 20 μmol L^{-1} NaCl water. However, it is not clear why *C. julii* do not employ this high-affinity mechanism in dilute waters.

While the species examined in this study seem to match up well with those previously studied, there were some interesting differences. For instance, uptake capacities, as indicated by J_{max} values, for fish taken directly from the Rio Negro are at least 50% higher than J_{max} values for Rio Negro fish that had been acquired in North America or Europe (Table 2). These differences are not the result of temperature differences since all species were tested at around 25°C. Instead, they may reflect real species differences or genetic divergence during breeding outside the Rio Negro (for some species). Alternatively, the low J_{max} values in fish acquired in North America and Europe could indicate an acclimatory down-regulation of transport capacity in response to recent exposure to higher Na^+ concentrations. This is supported by the observation that *C. julii* lowered J_{max} by >50% during a 2-wk exposure to 2 mmol L^{-1} NaCl water (Fig. 3). Similarly, other species have demonstrated the ability to up-regulate transport during exposure to dilute waters (McDonald and Rogano 1986). Some data, however, appear inconsistent with this explanation. Despite being held in 50 μmol L^{-1} NaCl water for at least several weeks, species acquired in North America and Europe failed to raise J_{max} to levels comparable to those found in fish taken directly from the Rio Negro. A few weeks should be enough time to make adjustments since both neon and blackskirt tetras have demonstrated an ability to raise J_{in}^{Na} markedly in just a few hours (Gonzalez et al. 1997; Gonzalez and Preest 1999).

Given the great diversity in ionoregulatory characteristics among the species studied, one could ask why species in ion-poor water would have Na^+ uptake mechanisms with such different kinetic properties? Neither pattern is obviously superior since there seem to be significant advantages to both. A high-affinity, high-capacity system would ensure a high rate of uptake, while a low-affinity system would be more economical as long as efflux was equally low (Gonzalez and Dunson 1989). One possible explanation for the diversity of patterns may be found in the link between ion regulation and acid-base balance. Typically, Na^+ and Cl^- are exchanged across the gill epithelium for acid and base equivalents, respectively (Goss and Wood 1990). It is possible that species likely to incur acid-base disturbances due to activity or areas they inhabit (such as anoxic or acidic waters) could have greater ion transport capabilities to more quickly correct any disturbance. In contrast, less active fish or species that inhabit more moderate habitats can afford

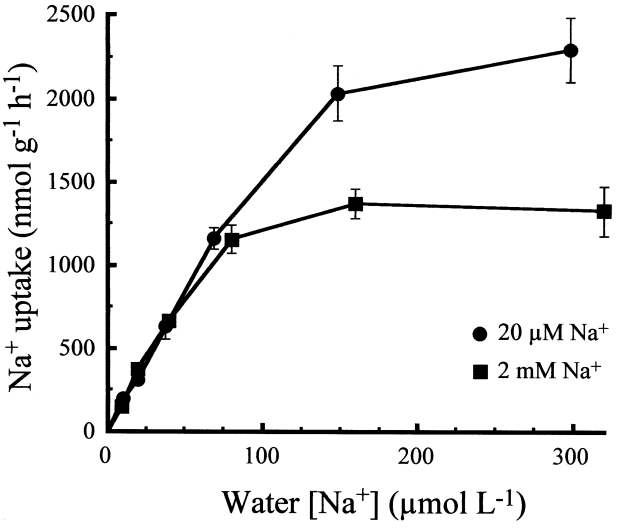

Figure 3. The relationship between Na^+ uptake and external Na^+ concentration *Corydoras julii* held in either 20 μmol L^{-1} or for 2 wk in 2 mmol L^{-1} NaCl water. Values are means ± SE ($N = 7$).

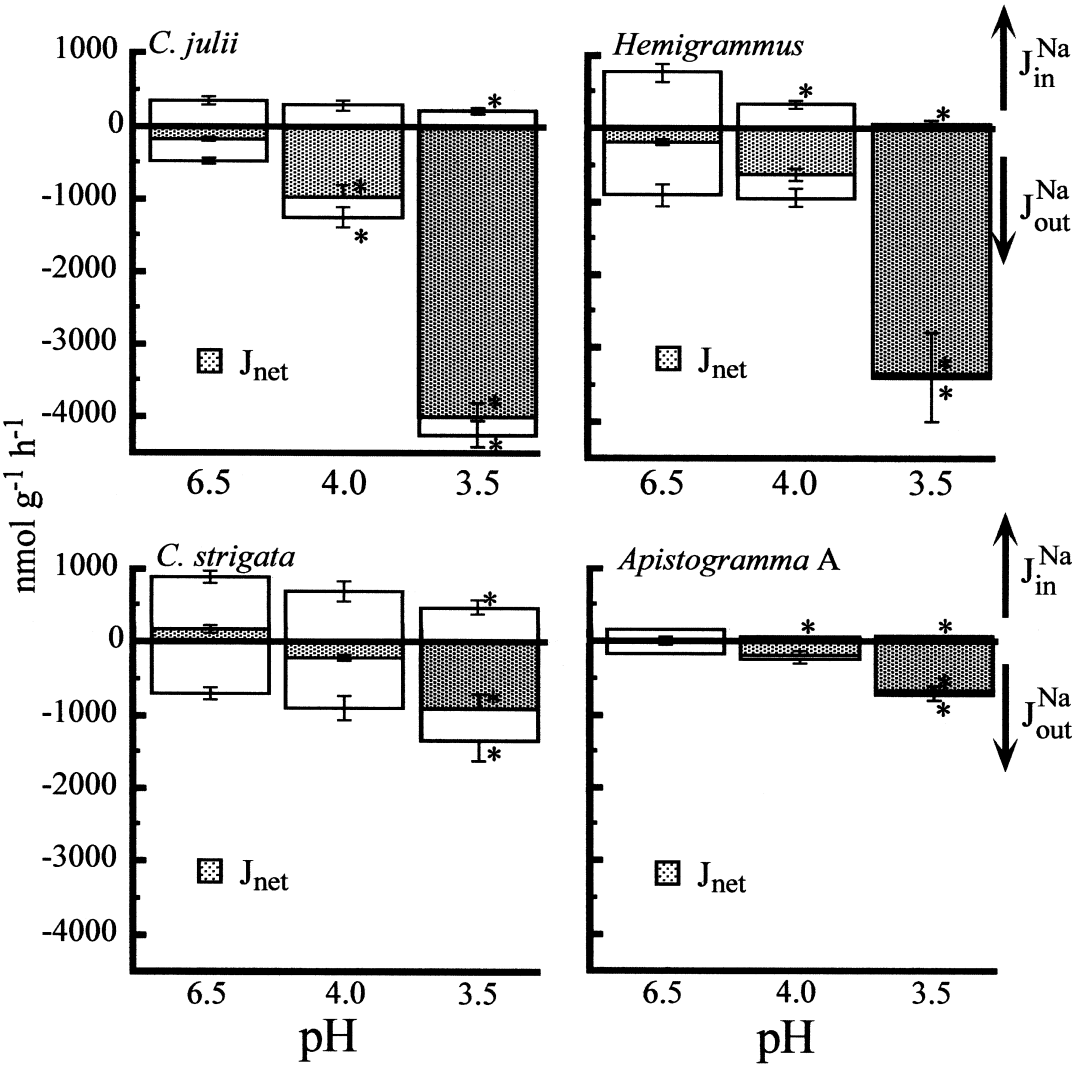

Figure 4. Effect of water pH on Na^+ influx (J_{in}^{Na}; *upward open bars*), efflux (J_{out}^{Na}; *downward open bars*), and net flux (J_{net}^{Na}; *shaded bars*) of four species of fish collected from the Rio Negro. Values are means ± SE ($N = 7$). Asterisks indicate significant differences from pH 6.5 group (control).

lower rates of ion flux because of less demand for rigorous defense of acid-base status.

Our data also suggest a plausible, phylogeny-based explanation for the differences in ion transport characteristics. The four species examined here, which belong to the family Cichlidae, all had high K_m values. The cichlid *Apistogramma* B could not be analyzed, but from its position in Figure 1, it seems likely to have a high K_m value. Further, angelfish (*Pterophyllum scalare*), examined previously, also have a high K_m value (Table 2). In contrast, four species belonging to the family Characidae (*Hemigrammus*, neon, cardinal, and blackskirt tetras) all possess very low K_m values. Thus, it appears, as suggested by Gonzalez and Wilson (2001), that a low-affinity Na^+ transport mechanism is characteristic of Amazonian cichlids, and conversely, a high-affinity transporter typifies characids. However, it should be noted that these two families are extremely species rich, and these physiological characters are very plastic. Any rigorous conclusion along these lines would require examination of many more species.

Despite the differences in ionoregulatory characteristics, all four species tested were fairly tolerant of low pH, relative to many North American or European species (McDonald 1983; Gonzalez 1996). Nonetheless, there were significant differences in their ability to maintain ion balance at low pH (Fig. 4). The less tolerant species *C. julii* and *Hemigrammus* exhibited large stimulations of J_{out}^{Na} at pH 4.0 and below. In contrast, J_{out}^{Na} of the

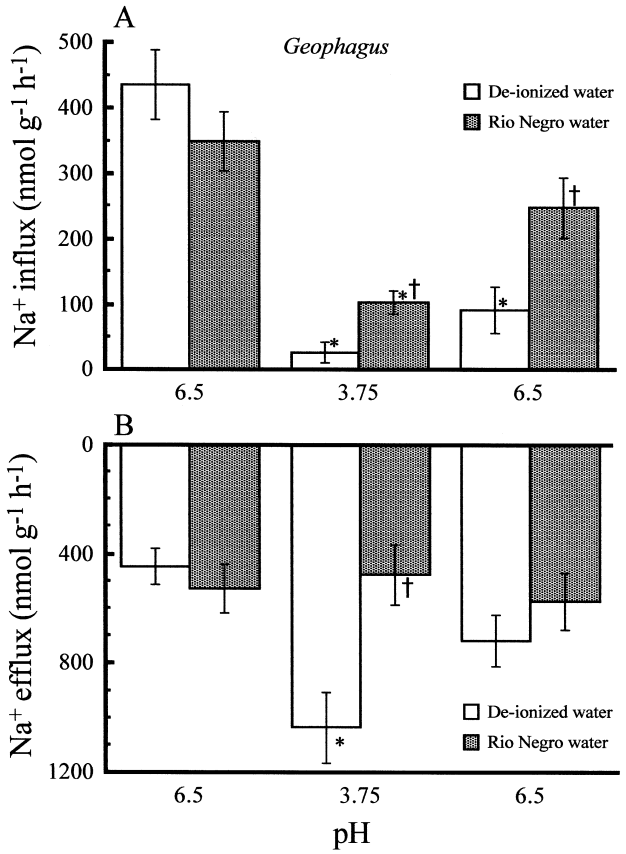

Figure 5. Sodium influx (*A*) and efflux (*B*) of *Geophagus* during exposure to pH 3.75 in deionized water or Rio Negro water. Values are means ± SE ($N = 7$). Asterisks indicate significant differences from initial pH 6.5 group (control), and crosses indicate significant differences between water types.

more tolerant *C. strigata* and *Apistogramma* A was unaffected by exposure to pH 4.0 and only mildly stimulated at pH 3.5. As shown many times before (Packer and Dunson 1970; McWilliams and Potts 1978; McDonald et al. 1983; Gonzalez and Dunson 1987, 1989; Freda and McDonald 1988; Gonzalez et al. 1997), the primary determinant of low pH tolerance is the degree to which J_{out}^{Na} is stimulated.

There were also differences among the species in sensitivity of J_{in}^{Na} to low pH, and two species were particularly noteworthy. J_{in}^{Na} of *C. julii* and *C. strigata* were unaffected at pH 4.0 and only moderately inhibited at pH 3.5, which makes their Na^+ uptake mechanisms among the most pH tolerant of any species tested (Gonzalez 1996). For most species examined, the lower limit for Na^+ transport is around pH 4.0 or higher (Gonzalez 1996). In comparison, neon and cardinal tetras possess Na^+ uptake mechanisms that are completely insensitive to pH, down to pH 3.25 (Gonzalez and Preest 1999; Gonzalez and Wilson 2001). While pH sensitivity varies among species, it appears that reduced sensitivity of Na^+ transport to low pH may be a fairly common characteristic of Rio Negro species.

Although the four species examined proved to be fairly tolerant of low pH, work with two additional species indicated that these species may be even more tolerant if they were tested in Rio Negro water instead of deionized water (plus salts). When *Geophagus* were exposed to pH 3.75 in Rio Negro water, they experienced a smaller inhibition of J_{in}^{Na} relative to fish in deionized water, and they recovered more quickly on return to high pH (Fig. 5*A*). At the same time, J_{out}^{Na} was significantly stimulated on low pH exposure in deionized water but not in Rio Negro water (Fig. 5*B*). Interestingly, *Pimelodes* exhibited a very different response to low pH exposure in different water types (Fig. 6). During low pH exposure, water type made very little difference. However, on return to pH 6.5, fish in Rio Negro water appeared to avoid a significant stimulation of J_{out}^{Na}. These results may help explain some earlier findings. In a previous trip to the Rio Negro, net loss rates of Na^+ or Cl^- were un-

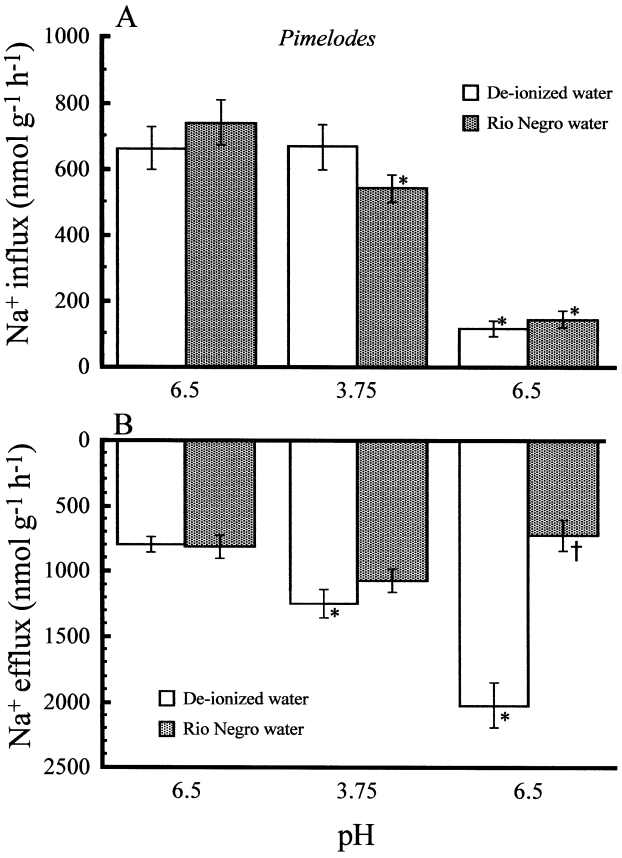

Figure 6. Sodium influx (*A*) and efflux (*B*) of *Pimelodes* in deionized water or Rio Negro water during exposure to low pH and during recovery at pH 6.5. Values are means ± SE ($N = 7$). Asterisks indicate significant differences from initial pH 6.5 group (control), and crosses indicate significant differences between water types.

affected by water Ca^{2+} concentration when species were exposed to low pH in Rio Negro water (Gonzalez et al. 1998).

It is not clear why ion regulation of *Pimelodes* was disturbed during recovery but was not disturbed during low pH exposure or why *Pimelodes* should respond differently from *Geophagus*. Nonetheless, it is clear that Rio Negro water offered some sort of protection at low pH. So what is it about Rio Negro water that offers protection, and what exactly is it doing? The most obvious difference between the water types is the presence in Rio Negro water of large quantities of dissolved organic molecules from partially broken down plant material, which give the river its dark tea color and name. It has been previously demonstrated that dissolved organic compounds can interact with and alter biological membranes (Karns 1983; Freda et al. 1989). The challenge in determining the exact nature of the interactions is the extreme complexity of the organic compounds that are leached out of a variety of plants and are in various stages of breakdown. It seems likely that no two liters of water from the Rio Negro are exactly the same in this regard. Nonetheless, our results suggest that alteration in paracellular tight junctions, which govern rates of efflux, and apical transporters responsible for ion transport are two good places to start.

While most species examined here seem able to ion regulate in extremely dilute waters of low pH, not all species demonstrated this ability. Two species with very low rates of uptake in dilute water were unable to match rates of diffusive loss until the water Na^+ concentration was well above ambient levels (Table 2). Similarly, inhibition of ion transport at low pH would cause an imbalance. Still, these fish are found in the Rio Negro. Therefore, it is likely that they are somehow able to maintain ion balance. We suggest that, for these species, dietary salt intake plays a key role. Several studies have shown that dietary salt significantly affects ion balance and can play a crucial role in maintenance on internal levels during exposure to low pH (Smith et al. 1989; D'Cruz and Wood 1998). We suggest that some species with particularly poor ion transport capabilities in dilute, acidic waters regularly depend on dietary salt to maintain overall ion balance. Of course, it is equally plausible that species that rely on dietary salt intake have less need for strong transport capabilities. It would be of interest to know the salt content of the food of these species, but we are not aware of any study with such information. Certainly, carnivorous species should be able to get enough salt from diet, but this is not necessarily so for herbivorous species.

There is one final issue. In several species examined here, J_{out}^{Na} rose in hyperbolic fashion parallel to influx with rising water Na^+ concentration (Fig. 2*A*). In a variety of previous studies, this sort of result has been cited as evidence for carrier-mediated self-exchange of ions (i.e., Na^+/Na^+ exchange; exchange diffusion) across the apical membrane of the gill epithelium (Maetz 1972; Wood and Randall 1973; DeRenzis 1975; Goss and Wood 1990). This sort of interpretation was common when the mechanism of Na^+ uptake across the apical membrane was thought to be a Na^+/H^+ (NH_4^+) antiporter. However, more recently a H^+ pump/Na^+ channel arrangement has been hypothesized (Lin and Randall 1991, 1993), and it is difficult to envision how exchange diffusion would occur with this mechanism. Further confusing the issue is the fact that some species tested here, as well as others (Patrick et al. 1997), show a constant J_{out}^{Na} with rising water Na^+ concentration (Fig. 2*B*). Either some of these species are using a Na^+/H^+ antiporter while others are using a H^+ pump/Na^+ channel system, or an alternate explanation is required.

One possible explanation is that rising J_{out}^{Na} with water Na^+ levels indicates a modulation of branchial permeability. Loss of Na^+ is usually considered to occur primarily by diffusion through paracellular channels (McDonald et al. 1989). The main determinants of transepithelial diffusion are the concentration gradient, transepithelial potential (TEP), branchial surface area, and epithelial Na^+ permeability. The gradient did not change appreciably, and it does not seem likely that surface area or TEP would change significantly (McWilliams and Potts 1978). That leaves branchial permeability. We propose that some species are increasing branchial permeability with climbing J_{in}^{Na}. Consequently, J_{out}^{Na} rises, possibly to avoid a large net gain of Na^+. However, some species appear to lack the ability to modulate permeability and, therefore, maintain a constant J_{out}^{Na} despite the rising J_{in}^{Na}. There is some evidence to indicate that some species can rapidly adjust branchial permeability (Gonzalez and McDonald 1992), and it seems that these adjustments can occur very quickly.

In sum, these data begin to flesh out the picture of ion regulation in fish native to the Rio Negro. We see that while all species are able to maintain ion balance in extremely dilute waters, the way in which they accomplish it varies among the species. These results confirm the presence of two basic patterns of ion regulation, but others are possible. Further, while the species are generally tolerant of low pH, they still differ significantly in the degree of tolerance of their uptake and efflux mechanisms. Perhaps most interestingly, it appears that Rio Negro water offers some protection at low pH for the fish. This raises the interesting possibility that the blackwater nature of the river has played a key role in allowing the high degree of diversity that is observed in the River (Val and de Almeida-Val 1995).

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