Scientia Amazonia, v. 3, n.1, 15-24, 2014
Revista on-line http://www.scientia.ufam.edu.br
Jan-Abr ISSN:2238.1910

EFFECT OF CLEARWATER ON OSMOREGULATION OF CURURU RAY, *POTAMOTRYGON* SP. (CHONDRICHTHYES; POTAMOTRYGONIDAE), AN ENDEMIC SPECIE FROM BLACKWATER RIVER[1]

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Submetido 02/05/2013 – Aceito 17/10/2013 – Publicado on-line 03/04/2014

Resumo

As diferenças nas características químicas dos rios da região amazônica podem representar barreiras geográficas para a distribuição das arraias de água doce. Para testar essa hipótese, o presente estudo teve por objetivo avaliar a influência da água clara/esverdeada (Rio Branco) sobre os mecanismos osmorregulatórios da arraia "cururu", *Potamotrygon* sp., uma espécie endêmica da água preta do Rio Negro. Os íons Na^+, K^+, Ca^{+2}, teores de uréia e osmolalidade foram analisados no plasma e fluido perivisceral. Além disso, a atividade da Na^+/K^+-ATPase (NKA) foi estimada nas brânquias e rins. A concentração de Na^+, Cl^- no plasma e no fluido perivisceral foi reduzida. Os níveis da NKA renal também foram significativamente reduzidos nos exemplares expostos às águas do Rio Branco. Por outro lado, a exposição aguda às águas do Rio Branco não provocaram alterações significativas nas concentrações dos íons K^+ e Ca^{+2} e uréia, e nem da atividade da NKA branquial. Uma análise de regressão revelou uma relação inversa entre a atividade da NKA branquial e renal quando estes peixes foram expostos às águas do Rio Branco. Esta integração funcional foi interpretada como sendo uma tentativa no mecanismo de ajuste para manter a homeostase iônica. A elevada abundância de *Potamotrygon* sp. no Rio Negro e a sua baixa abundância no Rio Branco sugere a existência de uma barreira hidrológica físico-química que limita a distribuição dessa espécie. Possivelmente essa barreira está relacionada com a inabilidade da homeostase iônica em uma água que é mais rica em íons e cujo pH é neutro.

Palavras-chave: Arraias de água doce, Barreira hidrológica, Na^+/K^+ ATPase, Osmorregulação.

Abstract

The differences in the chemical characteristics of the Amazonian Region Rivers may represent geographical barriers for freshwater stingray distributions. To test this hypothesis, the present study aimed to evaluate the influence of the clear/green-colored water river (Rio Branco) on the osmoregulatory mechanisms of "cururu" stingray, *Potamotrygon* sp., an endemic species of the Rio Negro black water. The Na^+, K^+, Ca^{+2}, urea and osmolality were analyzed in plasma and perivisceral fluid. Furthermore, the Na^+/K^+-ATPase (NKA) activity was estimated on gills and kidneys. The Na^+, and Cl^- concentration in plasma and perivisceral fluid were reduced. The renal NKA levels also were significantly reduced in the specimens exposed to the waters of the Rio Branco. On the other hand, the acute exposure to the waters of the Rio Branco River did not cause significant changes in K^+, Ca^{2+}, and urea concentrations, nor the activity of NKA in gills. A regression analysis revealed an inverse relationship between the NKA activity in gills and kidney when these fishes were exposed to the waters of the Rio Branco. This functional integration has been interpreted as an attempt in setting mechanism for maintaining ion homeostasis. The high abundance of *Potamotrygon* sp. in the Rio Negro and its low abundance in Rio Branco suggest the existence of a physicochemical hydrological barrier limiting for the species distribution. Possibly this barrier is related to the inability of ionic homeostasis in water that is richer in ions and whose pH is neutral.

Key words: Freshwater stingray, Hydrographical barrier, Na^+/K^+ ATPase, Osmoregulation.

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1. INTRODUCTION

The Amazon basin presents a large diversity of aquatic habitats, geographically separated by physical and/or hydrographical barriers, which may limit the flow of genes between individuals and populations. This condition favors allopatric speciation and/or divergent adaptation. As examples, *Potamotrygon* sp. ("cururu" ray, an undescribed species) and *P. schroederi* Fernandéz-Yépez 1958 are stingray species endemic to the black waters of the Rio Negro, while *Plesiotrygon iwamae*, Rosa, Castello & Thorson, 1987 is restricted to the white waters of the Rio Amazonas (MARTIN 2005; DUNCAN & FERNANDES 2010). The speciation of these stingrays occurred between 7.7 and 6.8 million years ago. The absence of a geographic barrier to explain the *P. iwamae* isolation from the stingrays of the Rio Negro and vice-versa leads us to consider the physical-chemical water characteristics of the Rio Negro and Rio Amazonas as the possible selective pressures for such divergent speciation (TOFFOLI et al. 2008).

For example, in salmonid fishes the migration depend on the physical and chemical characteristics of water, such as temperature, salinity and sea level changes, which act as natural barriers (GREGORY & QUINN 1990). In the Amazon basin, no study has been done to analyze the physical and chemical characteristics of aquatic systems as hydrographic barriers. Several migratory and non-migratory fish species have large geographic distributions in the Amazonian aquatic system and are well adapted to different habitats with different physical and chemical characteristics (RAMIREZ-GIL et al. 1998). In general, such species exhibit high plasticity in order to explore different environments (DUNCAN et al. 2009). On the other hand, some species are restricted to a given type of Amazonian water, indicating the absence of adaptive mechanisms to permit their migration, for example *Potamotrygon henlei* which is endemic to the Rio Tocantins drainage, another specie with that characteristic is *P. leopoldi*, originally from Rio Xingu drainage, we can also mention *P. magdalenae* which is restricted to Magdalena River drainage (ROSA et al. 2010).

The water of the Rio Negro and most of its tributaries is transparent and black in color, has very low pH (3.5-4.5) and conductivity, and has high concentrations of dissolved organic carbon (DOC) and low concentrations of solids and dissolved ions (KONHAUSER et al. 1994; AUCOUR et al. 2003). However, Rio Branco, a tributary river on the left side of Rio Negro, with pH 6 to 7, low DOC and higher dissolved ion concentrations than the Rio Negro. Although Rio Branco is considered a clearwater river, it has green-colored water (this term will be used throughout the text). The stingray *Potamotrygon* sp. is restricted to the Rio Negro water and its acidic tributaries. Studies on the ion regulation of cichlid teleost species from the Rio Negro (GONZALEZ & WILSON 2001; GONZALEZ et al. 2002) and the stingray *Potamotrygon* sp. evidenced some similarities in their ion regulation (WOOD et al. 2002). These authors suggested that cururu ray has an ion transport system with low affinity (high $_{Km}$ and low $_{Jmax}$), but it also has a system of low diffusive ion loss. Although the mechanism is not totally understood, Gonzalez & Preest (1999) point out that such system may be associated with reduced gill permeability via paracellular junctions.

Ion regulation depends on gill and kidney function. The gill epithelium in freshwater fish is the main site for diffusive ion loss, and the mitochondria-rich cells localized mainly in the filament epithelium of gills are the site of active ion uptake from water (WILSON et al. 2002). The kidneys have, in general, a secondary function in Na^+ and Cl^- regulation in fish. In both organs, the Na^+/K^+-ATPase (NKA) activity transports Na^+ against an electron osmotic gradient, generating an electrogenic force driving ion uptake in the gills and reabsorption in the convoluted renal tubules (LIN et al. 2003; LIN et al. 2004). In the euryhaline stingray *Dasyatis sabina* Lesueur 1824, NKA enzyme units in the basolateral membrane of MRCs, are directly involved in Na^+ uptake and H^+ excretion (PIERMARINI & EVANS 2000; EVANS et al. 2004; EVANS et al. 2005), and in the teleost *Tetraodon nigroviridis* Marion de Procé 1822, the increase of NKA activity in the convoluted renal tubules is essential for ion reabsorption, reducing their loss (LIN et al. 2004). These data evidence the importance of the integrated functions by gills and kidneys to maintain homeostasis and perform the necessary adjustments to overcome environmental challenges. Nevertheless, some species are highly specialized and show little capacity for restoring ionic and osmotic disturbances. Wood et al.

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(2002) demonstrated that the rate of Na^+, Cl^- and Ca^{2+} loss in the stingray *Potamotrygon* sp. was strongly influenced by water pH.

Considering the fact that *Potamotrygon* sp. are restricted to the acidic waters of the Rio Negro and its tributaries and are rarely found in the green-colored/clear waters of Rio Branco, the main goal of this study was to determine the changes in NKA in the gills and kidneys of stingrays exposed to Rio Branco water. We evaluated the effect of the possible restriction of ionic and osmoregulatory processes on the distribution of this species in other Amazonian habitats.

2. Material and methods

2.1 Site

Specimens of *Potamotrygon* sp. were collected by beach seine on the beach of Rio Negro (Figure 1), Igarapé do Zalala (a small-stream), (S00°42'11,2"; W62°58'47,8"), 120 km upstream of the Rio Branco mouth (S01º23'42,7"; W61º51'04,9"), during the dry season. The specimens were kept in their native water (S00º40'628" W63º03'506") to allow recovering from stress of capture. The physical-chemical characteristics of water were similar to the Igarapé do Zalala (pH 4.4, oxygen 5.8 mg/L, Total Dissolved Solid (TDS) 8.7 mg/L, electrical conductivity 16.3 µS/cm, temperature 31.0 °C). The experiment was done at Igarapé do Budarizinho (small stream), and the plasma, chemical analyses were done at Laboratório de Morfologia Funcional da Universidade Federal do Amazonas (LMF/UFAM).

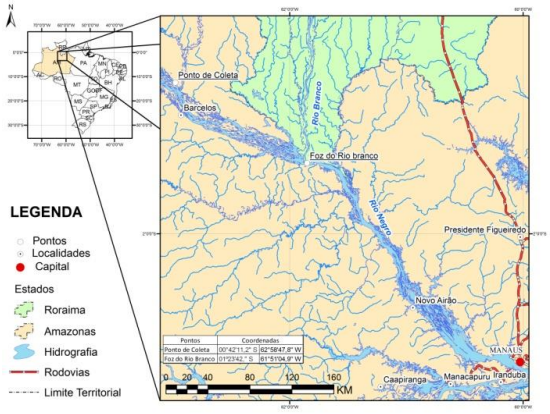

Figure 1: Confluence of Rio Branco and Rio Negro at the Mariuá Archipelago, Barcelos region (Middle of Rio Negro).

2.2 Experimental Protocol

The animals were randomly divided into two groups. One was the control group (n = 6, body mass = 416.0 ± 9.3 g; disk length = 20.6 ± 1.7 cm), and these stingrays were transferred to the experimental units (2 animals per experimental unit) and kept in their native water, Rio Negro. The other one was the experimental group (n = 6, body mass = 354.1 ± 11.8 g, disk length = 17.8 ± 2.3 cm), and the specimens in this group were transferred to experimental units containing Rio Branco water. The experimental units consisted of plastic boxes (20 liters) with continuous running water to avoid the accumulation of undesirable wastes. The physical and chemical characteristics of the Rio Negro and the Rio Branco waters are given in Table 1. All water analyses were done using a Consort C535 multiparameter analyzer (Consort, Turnhout, Belgium), except for the Na+ and K+ concentrations, which were measured using a Digimed DM-62 flame photometer (Digimed, SP, Brazil).

Table 1: Physical and chemical variables of the water from the Rio Negro (S00º40'628", W63º03'506") and Rio Branco waters (Rio Branco mouth, S01º23'42,7"; W61º51'04,9"), used in the experiments. (*) Indicate significant differences among waters (Student t test, $P < 0.05$).

Variables	Rio Negro (Blackwater)	Rio Branco (Green-colored water)
pH	4.1±0.3	6.8±0.4*
Temperature (ºC)	31.4±0.2	30.5±0.3
Dissolved O_2 (mg/L)	5.2±0.4	5.3±0.4
Conductivity (µS/cm)	9.3±1.4	27.5±1.1*
Total Dissolved Solid (mg/L)	5.2±0.7	14.7±0.2*
[Na^+] (mM)	11.8±2.1	72.3±9.5*
[K^+] (mM)	14.8±2.4	23.8±7.9*

After a 12h exposure to the Rio Negro (control) and Rio Branco (experimental group) waters, the stingrays from each group were slightly anaesthetized with 0.05 g/l of buffered 3-amino-benzoic acid ethyl ester (Sigma Chemical Co, St. Louis, MI, U.S.A), and blood samples were taken via cardiac puncture. Then, the animals were euthanized, and the perivisceral fluid, gill and kidney tissues were sampled through a body ventral incision.

2.3 Plasma and perivisceral measurements

The blood was immediately centrifuged and the plasma removed. Plasma and the perisvisceral fluid were frozen for ion, urea and osmolality analyses. Na^+ and K^+ concentrations were measured using a flame photometer, and Cl^- concentration was determined using colorimetric titration. Calcium concentration was measured using the orthocresolphthalein complexone at 570 nm, and urea concentration was assayed using a modification of Berthelot's reaction measured at 578 nm (urease method). All assays were done in duplicate using In Vitro Diagnostica kits (In Vitro Diagnostica, Barbacena, SP, Brazil) and a Spectrum SP-2000 UV spectrophotometer (Spectrum, Shanghai, P.R. China). The osmolality was determined with a µ-Osmette Precision Systems microsmometer (Precision Systems, Natick, MA, U.S.A.).

2.4 Specific activity of Na^+/K^+-ATPase (NKA)

The sampled gill and kidney tissues were stored in SEI buffer (0.3 M Sacarose, 20 mM EDTA, 10 mM 2-mercaptoethanol and 0.1 M imidazole, pH 7.4) in liquid nitrogen until analysis. Unfrozen gill and kidney samples were homogenated using a Dispersor Extratur (Quimis, Diadema, SP, Brazil) at 4 ^oC and centrifuged (Sigma 30-K, Osterode am Harz, Germany) at confirmar 17.500g at the same temperature for 30 min. Subsamples of supernatant were used for protein measurements and determination of enzyme activity.

The NKA activity assay was done using 1 µg protein: 1µl buffer containing NaCl (100 mM), MgCl2 (5 mM), KCl (13 mM), ATP (3 mM), and imidazole (30 mM) pH 7.4, at 25^oC. Ouabain (2 mM) was added in duplicate to determine ouabain-sensitive ATPase activity (NKA activity). The reaction was stopped by the addition of ice-cold 25% trichloroacetic acid, and the inorganic phosphate (Pi) produced by ATP hydrolyse was determined according to Fiske-Subbarow's method at 620 nm in a Spectrum SP-2000 UV spectrophotometer. The enzyme activity was defined as the difference between the inorganic phosphates released in the presence and absence of ouabain in the reaction mixture and expressed as specific activity in µmol Pi/mg protein/h.

2.4 Immunohistochemistry against NKA

Gill samples fixed in Bouin solution were dehydrated in ethanol crescent series until pure ethanol and embedded in paraffin. Filament sections (6 µm in thickness) were glued to glass slides with poly-L-lysine (Sigma Chemical Co.), deparaffinized and immunohistochemically stained using a monoclonal antibody against the α-subunit of chicken NKA (Development Studies Hybridoma Bank, Iowa City, Iowa, U.S.A.). Briefly, after paraffin removal and tissue rehydration, the sections were washed in phosphate buffer saline-Triton (PBS-T, pH 7.4) and incubated with 20% normal goat serum and 0.1% Triton X-100 to inhibit endogenous peroxidase activity. The primary antibody α5 diluted to 1:100 in PBS-T was placed on the sections overnight at room temperature in a dark, humid chamber. The following day, the sections were washed in PBS-T and incubated with the second antibody, anti-mouse IgG anti peroxidase (GAMPO, Sigma Chemical Co.) diluted to 1:150 in PBS for 1h. After washing with PBS, the antibody complex was visualized by staining sections with filtered DAB-Ni (3,3'-diaminobenzidine tetrahydrochloride, Sigma) in PB with the addition of 0.0125% H_2O_2 immediately prior to use at room temperature. Negative controls were obtained by omission of either the first or the second antibody and were incubated and stained as described above.

The number of Na^+/K^+-ATPase-rich cells were counted on 50 randomly sorted lamellae and filament. The results were expressed as the number of immunopositive-NKA cells per millimeter of gill lamellae or filament.

2.5 Statistical analysis

Data were presented as means ± standard error of mean. The water physical and chemical parameters as well as plasma and perivisceral data between the two groups, control (Rio Negro) and experimental (Rio Branco), were compared using the Student's t test. When appropriate, the Mann-Whitney test (non-parametric) was used. Regression analyses to evaluate the relationship between gill and renal NKA activity were calculated by the least-squares method, and the correlation coefficient (r) was estimated to determine the goodness of fit. The acceptable significance level for all tests was $P < 0.05$.

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3. Results

The exposure of *Potamotrygon* sp. to green-colored water from the Rio Branco significantly decreased ($p<0.05$) the Na^+ and Cl^- plasma concentrations as well as the Cl^- perivisceral concentration. The ratios of Na^+/Cl^- in plasma were 0.98 and 0.92 and in perivisceral fluid were 0.50 and 0.52 for the stingrays kept in the Rio Negro and Rio Branco waters, respectively, offering evidence of Na^+ loss in fish kept in the Rio Branco water and an imbalance between the Na^+ and Cl^- concentrations in plasma. No changes were found in the concentrations of K^+, Ca^{2+} and urea in the plasma and perivisceral fluid (Table 2). The reduction of Na^+ and Cl^- levels in the plasma of stingrays exposed to the Rio Branco water resulted in a significant reduction of plasma osmolality.

Table 2: Means (± SEM) of ion concentration (Na^+, Cl^-, K^+ and Ca^{+2}), urea and osmolality in plasma and perivisceral fluid of the freshwater stingray *Potamotrygon* sp. maintained in the Rio Negro water and exposed to Rio Branco water. N=6 in each group. (*) indicates significant differences (Student t test, $P < 0.05$).

	Plasma		Perivisceral Fluid	
	Rio Negro	Rio Branco	Rio Negro	Rio Branco
$[Na^+]$ (mM)	152,7± 13,3	124,8±1 1,9*	83,5±1 0,1	77,2±11, 7
$[Cl^-]$ (mM)	156,4± 14,4	135,3±1 1,3*	168,4± 8,9	147,6±1 3,4*
$[K^+]$ (mM)	4,7±0,5	4,9±0,6	6,1±0,9	6,9±0,8
$[Ca^{+2}]$ (mM)	2,8±0,2	2,7±0,3	2,0±0,2	1,8±0,3
Uréia (mmol/L)	2,0±0,3	2,5±0,4	17,3±1, 6	16,9±1,3
Osmolality(mOsmol/kg)	298,2± 17,1	254,7±2 2,9*	329,5± 15,1	307,2±2 1,4

The positive NKA-immuno reactivity in the MRCs was intense in the basolateral cell periphery. Most MRCs were found in the filament epithelium, forming clusters (follicular-like) of 8-10 or 12 MRCs and sharing the same apical pit (Figure 2). Usually, these clusters form a short channel that contacts the epithelial surface. Some cells are distributed in the lamellar epithelium, most of them localized at the base of a lamella. This pattern of MRCs distribution did not change when the fish were exposed to Rio Branco water, although a tendency to increase the MRCs in the lamellar epithelium was observed in these fish. Nevertheless, there was no significant change in the number of NKA-immuno-positive MRCs in the lamellae and filaments in the stingrays exposed to the Rio Branco water (Figure 3), and neither was there a significant change in the gill NKA activity. The NKA activity of the gills was 1.8 ± 0.1 and 1.7 ± 0.1 µmol Pi/mg pt/h in stingrays kept in the Rio Negro and Rio Branco waters, respectively (Figure 4A). The renal NKA activity was higher in the gills (12.7 ± 0.7 µmol Pi/mg pt/h) in stingrays kept in the Rio Negro water than in the gills of those kept in the Rio Branco water (5.7 ± 0.9 µmol Pi/mg pt/h) (Figure 4B).

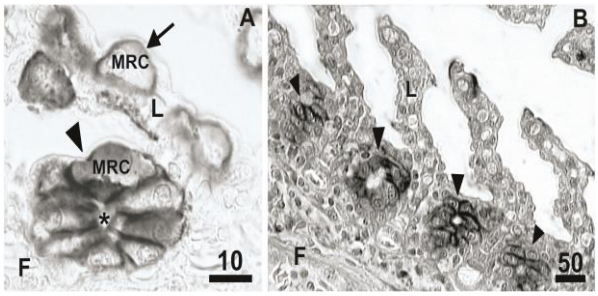

Figure 2: A. Mitochondria-rich cells (MRC) in the filament (arrowhead) and lamella (arrow) of the stingray *Potamotrygon* sp. (cururu ray) showing strong immunoreactivity against Na^+/K^+-ATPase in the periphery of cell. Note the MRC cluster in the filament epithelium showing short channel (*) in the center of cluster. B. Filament epithelium showing numerous clusters (arrowheads). Scale bars in µm. F, filament; L, lamellae

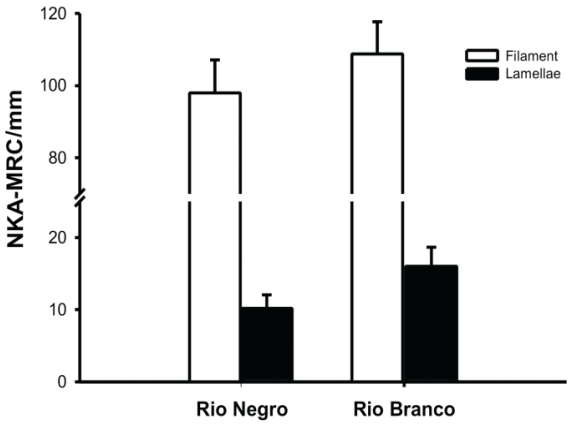

Figure 3: Number per millimeter of filament (mean ± SEM) of mitochondria-rich cells immunopositive against Na^+/K^+-ATPase (NKA-MRC) in the filament and lamella of the stingray *Potamotrygon* sp. kept in the Rio Negro (black water) and exposed to the Rio Branco water (green-colored water).

The NKA activity of stingrays kept in their native water (Rio Negro) did not show any relationship between gill and kidney NKA activity (Kidney NKA activity = 13.60 – (0.51 Gill NKA

activity), r^2 = 0.00; P > 0.05); however, those exposed to the Rio Branco water exhibited a negative relationship between gill and kidney NKA activity; which can be expressed as kidney NKA activity = 15.76 – (5.98 Gill NKA activity), r^2 = 0.73, P < 0.05 (Figure 5).

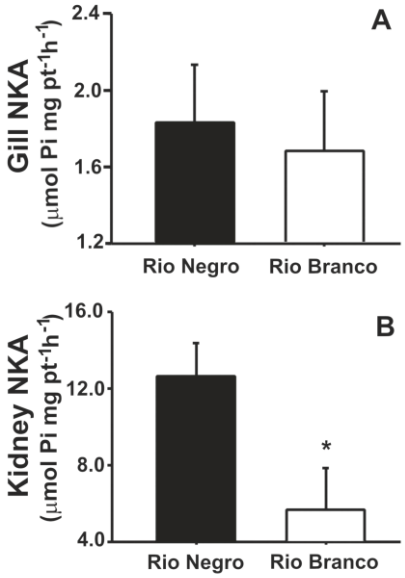

Figure 4. Na^+/K^+-ATPase activity (mean ± SEM) in the gills (A) and kidney (B) of the stingray *Potamotrygon* sp. kept in the Rio Negro (native water) and exposed to the Rio Branco water. (*) indicates significant differences (Student t test, P < 0.05).

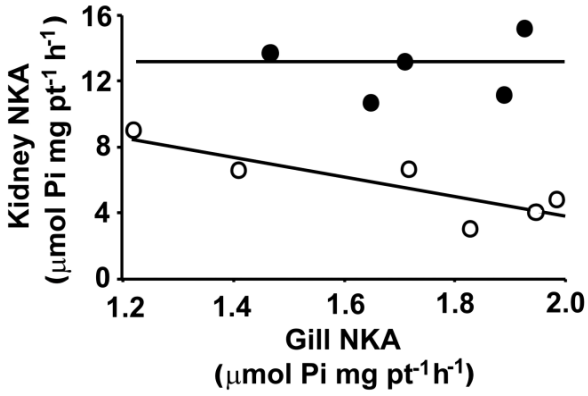

Figure 5: Relationships between the kidney and gill Na^+/K^+-ATPase activity (mean ± SEM) in the stingray *Potamotrygon* sp. kept in the Rio Negro (native water) and exposed to the Rio Branco water. The relationship was Kidney NKA activity = 13.60 – (0.51 Gill NKA activity), r^2 = 0.00; P > 0.05).

4. Discussion

The inability of *Potamotrygon* sp. to maintain ion regulation when kept in the Rio Branco water supports the early reports of Wood et al. (2002) on the ion transport mechanism in this species acclimated to their native ion-poor and soft water (Rio Negro) when exposed to high (5.9-6.1) and low (4.0) pH and to ion-rich hard water at pH 6.5. These data for *Potamotrygon* sp. and those of Gonzalez et al. (2002), Gonzalez & Wilson (2001) for the Rio Negro teleosts evidence a physicochemical (not geological) hydrographic barrier for the dispersion of some species in the Amazon basin.

The lower plasma and perivisceral Na^+ and Cl^- concentrations in *Potamotrygon* sp. kept in the Rio Branco water, in the present study, evidence a disturbance of plasma ions marked by ion loss and, consequently, osmolality reduction. The reduction of the Na^+/Cl^+ ratio in the plasma and the increase in the perivisceral fluid indicate a slight Na^+ shift to this extracellular compartment but an overall Na^+ and Cl^- loss to environmental water in stingrays kept into Rio Branco water.

The reduction of Na^+ and Cl^- in the plasma of *Potamotrygon* sp. kept in Rio Branco water disagrees with the plasma changes in stenohaline and euryhaline elasmobranches, in which these ions increased or decreased accompanying the changes in ion concentration in the environment. The stenohaline freshwater stingray, *Paratrygon aiereba* Müller & Henle 1841, living in both the Rio Negro (black water, acidic and ion-poor) and the Rio Solimões (white water, circumneutral pH and ion-richer), shows higher plasma ion (Na^+ and Cl^-) concentrations in the white water populations than in those from the Rio Negro (DUNCAN et al. 2009). In the euryhaline elasmobranchs, the bull shark, *Carcharhinus leucas* Müller & Henle 1839 (PILLANS & FRANKLIN 2004), yellow stingray, *Urolophus jamaicensis* Curvier 1816, (SULIKOWSKI & MAGINNIS 2001) and winter shark, *Leucoraja ocellata* Mitchill 1815, (SULIKOWSKI et al. 2003), the plasma Na^+ and Cl^- concentrations were reduced as the salinity decreased.

As the ion concentration of the Rio Branco water is significantly higher than that of the Rio Negro, an increase in the ions in the plasma of *Potamotrygon* sp. exposed to Rio Branco water was expected. However, the lower Na^+ and Cl^- plasma concentrations in the animals exposed to Rio Branco water may be explained by the overall ionic flux in *Potamotrygon* sp. reported by Wood et al. (2002). According to these authors, there are no changes in the Na+ and Cl^- influx in the stingrays acclimated in the Rio Negro water at pH 5.9-6.1 and those acclimated to

hard water at pH 6.5, although the efflux rates were lower in the Rio Negro water. Furthermore, the acute exposure to Rio Negro water at pH 4.0 caused sharp reductions in ion influx (approximately 80%), and did not increase the ion net rate as the efflux was also reduced. The dissolved organic carbon (DOC) in the Rio Negro (DOC = 11.4 mgL-1) is higher than that of the Rio Branco water (DOC = 2.7 mgL-1) (KÜCHLER et al. 2000), and it has been considered an important factor that acts in the gills, protecting them against ion loss in the acidic water of the Rio Negro (WOOD et al. 2003). This hypothesis was also accepted to explain the low ion loss of the teleost cardinal tetras, *Paracheirodon axelrodi* Schultz 1956, from the Rio Negro water (MATSUO et al. 2007).

In the present study, we demonstrated that the differences in the physical and chemical characteristics between the Rio Negro and Rio Branco waters did not affect the specific NKA activity in the gills of *Potamotrygon* sp. This fact, at least in part, explains the absence of changes in the kinetics of $_{Jin}Na^+$ and $_{Jin}Cl^-$ in this species when exposed to low and rich ion concentrations in water, as reported by Wood et al. (2002). However, the negative relationship between gill and kidney NKA activities in *Potamotrygon* sp. exposed to the Rio Branco water suggest an increase of ion loss through the kidneys in ion-rich and circumneutral pH water, resulting in an electrolyte (Na^+ and Cl^-) reduction in plasma and perivisceral fluid, whereas it did not occur in the stingrays kept in the Rio Negro water.

In general, fish living in or exposed to ion poor environments have high gill NKA expression, MRC proliferation, large MRC fractional surface area and increased kidney NKA activity to maintain hydrolytic homeostasis (FERNANDES et al. 1998; MORON et al. 2003; HAZON et al. 2003; LIN et al. 2004). In the case of the stingray *Potamotrygon* sp., endemic to the acidic and ion-poor water of the Rio Negro and exposed to the neutral pH and ion-rich water of the Rio Branco, the absence of changes in the number of MRC, probably related to an unusual organization of their MRC forming a follicular-like structure (DUNCAN et al. 2010; DUNCAN et al. 2011) or even due to the short-time exposure. On the other hand, the NKA activity in the gills was accompanied by a sharp reduction in the kidney NKA activity, which reduces ion reabsorption, seem to be an important factor in the restricted distribution of these species in the Rio Negro, a black water river.

The abundance of *Potamotrygon* sp. in the Rio Negro and its low biomass density in the mouth of Rio Branco (S01°23'42,7", W61°51'04,9") indicates a physicochemical hydrographic barrier for the distribution of this species, probably related to their inability to maintain osmotic and ionic homeostasis in circumneutral pH and ion-rich water. Excessive ion loss has been associated with fish mortality in different environmental conditions and pollution (CLAIBORNE et al. 1994; GROSELL et al. 2002; SCOTT et al. 2004). The physicochemical hydrographic barrier hypothesis is supported by the zoogeographical distribution pattern of Gymnotiformes species, since 17 species of the family Apteronotidae occur in the Rio Negro water near the Rio Branco mouth, and only 3 of them were collected in the Rio Branco (THOMÉ-SOUZA & CHAO 2004).

5. Conclusions

The short-time exposure (12h) of *Potamotrygon* sp. to Rio Branco water suggests an inability of this species to maintain osmotic and ionic homeostasis in circumneutral pH and ion-rich water.

It is important to emphasize that the limnological characteristics of a given body of water, together with ecological niche, predation competition and habitat structure, are important factors limiting fish distribution without a physical barrier. At present, further studies using long term experiments with *Potamotrygon* sp. exposed to Rio Branco water are necessary to confirm its inability to maintain ion balance in ion-rich water at neutral pH to confirm our hypothesis.

Acknowledgments

This study was supported by grants from CNPq (proc. #484374/2011-7; and FAPEAM (Edital UNIVERSAL AMAZONAS and Edital PROGRAMA DE INFRAESTRUTURA PARA JOVENS PESQUISADORES PROGRAMA PRIMEIROS PROJETOS – PPP) to WPD. The authors acknowledge Dra. Maria Lúcia Góes de Araújo for helpful discussions and assistance. The stingrays were collected with a wild animal license from the IBAMA/ICMBio/SISBIO

(#127/2004; #10085-1/2007; #22055-2/2010; #22055-3/2012).

Divulgação

Este artigo é inédito e não está sendo considerado para qualquer outra publicação. Os autores e revisores não relataram qualquer conflito de interesse durante a sua avaliação. Logo, a revista *Scientia Amazonia* detém os direitos autorais, tem a aprovação e a permissão dos autores para divulgação, deste artigo, por meio eletrônico.

References

AUCOUR, A.M.; TAO, F.X.; MOREIRA-TURCQ, P.; SEYLER, P.; SHEPPARD, S.; BENEDETTI, M.F. The Amazon river: behaviour of metals (Fe, Al, Mn) and dissolved organic matter in the initial mixing at the Rio Negro/Solimões confluence. **Chemical Geology**, 2003, v. 197, p. 271-285.

CLAIBORNE, J.B.; WALTON, J. S.; COMPTON-MCCULLOUGH, D. Acid-base regulation, branchial transfers and renal output in a marine teleost fish (the long-horned sculpin *Myoxocephalus octodecimspinosus)* during exposure to low salinity. **Journal of Experimental Biology**, 1994, v. 193, p. 79-95.

DUNCAN W.P.; COSTA, O.T.F.; ARAÚJO, M.L.G.; FERNANDES, M.N. Ionic regulation and Na+-K+ -ATPase activity in gills and kidney of the freshwater stingray *Paratrygon aiereba* living in white and blackwaters in the Amazon basin. **Journal of Fish Biology**, 2099, v. 74, n. 2, p. 956-960.

DUNCAN, W.P.; FERNANDES, M. N. Physicochemical characterization of the white, black, and clearwater rivers of the Amazon Basin and its implications on the distribution of freshwater stingrays (Chondrichthyes, Potamotrygonidae)**. Pan-American Journal of Aquatic Sciences**, 2010, v. 53, n. 5, p. 454-464.

DUNCAN, W.P.; COSTA, O.T.F.; SAKURAGUI, M.M.; FERNANDES, M.N. Functional morphology of the gill in Amazonian freshwater stingrays (Chondrichthyes: Potamotrygonidae): implication for adaptation to freshwater. **Physiological and Biochemical Zoology**, 2010, v. 83, n. 1, p.19-32.

DUNCAN, W.P., SILVA, N.F.; FERNANDES, M.N. Mitochondrion-rich cells distribution, Na+/K+-ATPase activity and gill morphometry of the Amazonian freshwater stingrays (Chondrichthyes: Potamotrygonidae). **Fish Physiology and Biochemistry**. 2011, v. 37, p. 523-531.

EVANS, D.H.; PIERMARINI, P.M. & CHOE, K.P. Homeostasis: osmoregulation, pH regulation and nitrogen excretion**.** In: Carrier, J.C, Musick, J.A. & Heithaus, M.R. (Eds.) Biology of sharks and their relatives. CRC Press. Boca Raton. 2004, p. 247-268.

EVANS, D.H.; PIERMARINI, P.M.; CHOE, K.P. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous wastes**. Physiological Reviews**, 2005, v. 85, p.: 97-177.

FERNANDES, M.N.; PERNA, S.A. & MORON, S.E. Chloride cell apical surface changes in gill epithelia of the armoured catfish *Hypostomus plecostomus* during exposure to distilled water. **Journal of Fish Biology**, 1998, v. 52, p. 844-849.

GONZALEZ, R.J.; PREEST, M. Mechanisms for exceptional tolerance of ion-poor, acidic waters in the neon tetra (*Paracheirodon innesi*). **Physiological and Biochemical Zoology**, 1999, v. 72, p. 156-163.

GONZALEZ, R.J.; WILSON, R.W. Patterns of ion regulation in acidophilic fish native to the ion-poor, acid Rio Negro. **Journal of Fish Biology**, 2001, v. 58, p. 1680-1690.

GONZALEZ, R.J.; WILSON, R.W.; WOOD, C.M.; PATRICK, M.L.; VAL, A.L. Diverse strategies for ion regulation in fish collected from the ion-poor, acidic Rio Negro. **Physiological and Biochemical Zoology**, 2002, v. 75, p. 37-47.

GREGORY, T.R.; QUINN, P.T. Horizontal and vertical movements of adult steelhead trout, *Oncorhynchus mykiss*, in the Dean and Fisher Channels, Britain Columbia. **Canadian Journal of Fisheries and Aquatic Sciences**, 1990, v. 149, p. 1963-1968.

GROSELL, M.; NIELSEN, C.; BIANCHINI, A. Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. **Comparative Biochemistry and Physiology,** 2002, v. C 133, p. 287-303.

HAZON, N.; WELLS, A.; PILLANS, R.D.; GOOD, J.P.; ANDERSON, W.G.; FRANKLIN, C.E. Urea based osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity. **Comparative Biochemistry and Physiology B**, 2003, v. 136, p. 685-700.

KONHAUSER, K.O.; FYFE, W.S.; KRONBERG, B.I. Multi-element chemistry of some Amazonian

water and soils. **Chemical Geology,** 1994, v. 111, p. 155-175.

KÜCHLER, I.L.; MIEKELY, N.; FORSBERG, B.R. A Contribution to the Chemical Characterization of Rivers in the Rio Negro Basin, Brazil. **Journal of Brazilian Chemical Society**, 2000, v. 11, p. 286-292.

LIN, Y.M.; CHEN, C.N.; LEE, T.H. The expression of gill Na, K-ATPase in milkfish, *Chanos chanos*, acclimated to seawater, brackish water and freshwater. **Comparative Biochemistry and Physiology A,** 2003, v. 135, p. 489-497.

LIN, C.H.; TSAI, R.S.; LEE, T.H. Expression and distribution of Na^+, K^+-ATPase in gill and kidney of the spotted green pufferfish, *Tetraodon nigroviridis*, in response to salinity challenge. **Comparative Biochemistry and Physiology A**, 2004, v. 138, p. 287-295.

MARTIN, R.A. Conservation of freshwater and euryhaline elasmobranchs: a review. **Journal of Marine Biology**, 2005, v. 85, p.1049-1073.

MATSUO, A.; VAL, A.L. Acclimation to humic substances prevents whole body sodium loss and stimulates branchial calcium uptake capacity in cardinal tetras *Paracheirodon axelrodi* (Schultz) subjected to extremely low pH. **Journal of Fish Biology**, 2007, v. 70, p. 989-1000.

MORON, S.E; OBA, E.T.; ANDRADE, C.A.; FERNANDES, M.N. Chloride cell responses to ion challenge in two tropical freshwater fish, the erythrinids *Hoplias malabaricus* and *Hoplerythrinus unitaeniatus*. **Journal of Experimental Zoology A**, 2003, v. 298, p. 93-104.

PIERMARINI, P.M.; EVANS, D.H. Effects of environmental salinity on Na^+/K^+ - ATPase in the gills and rectal gland of a euryhaline elasmobranch (*Dasyatis sabina*). **Journal of Experimental Biology**, 2000, v. 203, p.: 2957-2966.

PILLANS, R.D.; FRANKLIN, C.E. Plasma osmolyte concentration and rectal gland mass of bull sharks *Carcharhinus leucas*, captured along a salinity gradient. **Comparative Biochemistry and Physiology A**, 2004, v. 138, p. 363-371.

RAMIREZ-GIL, H.; FELDBERG, E.; ALMEIDA-VAL, V.M.F.; Val, A.L. Karyological, biochemical, and physiological aspects of *Callophysus macropterus* (Siluriformes, Pimelodidae) from the Solimões and Negro Rivers (Central Amazon). **Brazilian Journal of Medical and Biological Research**, 1998, v. 31, n.1449-1458,

SCOTOT, G.R.; ROGERS, J.T.; RICHARDS, J.G.; WOOD, C.M.; SCHULTE, P.M. Intraspecific divergence of ionoregulatory physiology in the euryhaline teleost *Fundulus heteroclitus*: possible mechanisms of freshwater adaptation. **Journal of Experimental Biology**, 2004, v. 207, p. 3399-3410.

SULIKOWSKI, J.A.; MAGINNISS, L.A. Effects of environmental dilution on body fluid regulation in the yellow stingray, *Urolophus jamaicensis*. **Comparative Biochemistry and Physiology A**, 2001, v. 128, p. 223-232.

SULIKOWSKI, J.A.; TREBERG, J.R.; HOWELL, W.H. Fluid regulation and physiological adjustments in the winter skates, *Leucoraja ocellata*, following exposure to reduce environmental salinities. **Environmental Biology of Fishes**, 2003, v. 66, p. 339-348.

THOMÉ-SOUZA, M.J.F.; CHAO, N.L. Spatial and temporal variation of benthic fish assemblages during the extreme drought of 1997-98 (El Niño) in the middle rio Negro, Amazonia, Brazil. **Neotropical Ichthyology**, 2004, v. 2, p. 127-136.

TOFFOLI, D.; HRBEK, T.; ARAÚJO, M.L.G.; ALMEIDA, M.P.; CHARVET-ALMEIDA, P.; FARIAS, I.P. **A test of the utility of DNA barcoding** in the radiation of the freshwater stingray genus *Potamotrygon* (Potamotrygonidae, Myliobatiformes). **Genetics and Molecular Biology**, 2008, v. 31, p. 324-336.

WILSON, J.M.; MORGAN, J.D; VOGL, A.W. & RANDALL, D.J. Branchial mitochondria-rich cells in the dogfish *Squalus acanthias*. **Comparative Biochemistry and Physiology A**, 2002, v. 132, p. 365–374.

WOOD, C.M.; MATSUO, A.Y.O.; GONZALEZ, R.J.; WILSON, R.W.; PATRICK, M.L.; VAL, A.L. Mechanisms of ion transport in *Potamotrygon*, a stenohaline freshwater elasmobranch native to the ion-poor blackwater of the Rio Negro. **Journal of Experimental Biology**, 2002, v. 205, p. 3039-3054.

WOOD, C.M.; MATSUO, A.Y.O; WILSON, R.W.; GONZALEZ, R.J.; PATRICK, M.L.; PLAYLE, R.C.; VAL, A.L. Protection by natural blackwater against disturbances in ions fluxes caused by low pH exposure in freshwater stingrays endemic to the Rio Negro. **Physiological and Biochemical Zoology,** 2003, v. 76, p. 12–27.