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BRIEF COMMUNICATIONS

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

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During low-water period, freshwater stingray *Paratrygon aiereba* collected in the whitewater (WW) of the River Amazon showed higher urea content, osmolality, Na^+ and Cl^- concentrations in plasma and perivisceral fluid than those caught in blackwater (BW) of the River Negro. Gills and kidney Na^+–K^+-ATPase activities were significantly lower in WW than in BW fish. The high level of kidney Na^+–K^+-ATPase activity in *P. aiereba* may minimize ion loss and generate diluted solute-free urine in ion-poor BW environment.

Key words: freshwater elasmobranch; osmoregulation; River Amazon; River Negro.

Neotropical freshwater stingrays (Potamotrygonidae) are the only group of Elasmobranchii completely adapted to living and reproducing exclusively in freshwater environments (Lovejoy, 1996). This group of stingrays developed several physiological characteristics during its adaptation to freshwater environments, such as no urea retention as an osmoregulatory solute (Treberg *et al.*, 2006), the presence of albumin in plasma (Griffith *et al.*, 1973) and a vestigial rectal gland, setting it apart from its marine counterparts (Thorson *et al.*, 1978). Several studies have demonstrated that osmoregulation in potamotrygonids is similar to that of freshwater teleosts and is characterized by low electrolyte concentrations (Griffith *et al.*, 1973; Wood *et al.*, 2002).

Paratrygon aiereba (Müller & Henle) is a monotypical species widely distributed in the river systems of the Amazon Basin. This species constitutes local populations with low genetic variability, a non-migratory pattern and low

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(or absent) genetic fluxes between the populations (Frederico, 2006). Therefore, this species constitutes an excellent model for studies of osmoregulatory strategies in different Amazonian aquatic environments. The whitewater (WW) rivers of the central Amazon region are rich in dissolved solutes and extremely turbid owing to their high concentrations of suspended sediment. In contrast, blackwater (BW) rivers are characterized by low suspended sediment, very low ion content and extremely acidic water due to high content of humic, fulvic and other organic acids (Konhauser *et al.*, 1994). Hence, the aim of this study was to characterize the osmolytes composition of body fluids and the gill and the kidney Na^+–K^+-ATPase activity in the potamotrygonid species *P. aiereba* collected from their native BW (the River Negro) and WW (the River Amazon) rivers.

Eight juveniles of *P. aiereba* (mean ± s.e. body mass = 177 ± 15 g) were collected using a beach seine in the River Negro (near Barcelos, Amazonas, Brazil, 0°51′ S; 62°45′ W), and six specimens (211 ± 19 g) were caught in the River Amazon (Lake Janauacá, 3°21′ S; 60°15′ W). The fish sampling was performed during the low-water period (dry season). Concurrent with the capture of the fish, the pH, oxygen concentration, total dissolved solids (TDS), conductivity and temperature of the local water were measured using a Consort C535 multiparameter analyser (Consort nv; www.consort.be).

The *P. aiereba* were held in their native river water without food for 24–72 h prior to tissue sampling. The fish were slightly anaesthetized in buffered 3-aminobenzoic acid ethyl ester (0·05 g^{-1} MS-222; Sigma-Aldrich; www.sigmaaldrich.com) and blood was sampled by cardiac puncture. Then, the animals were killed by spinal contusion and the perivisceral fluid, and gill and kidney tissues were sampled through a ventral body incision. Perivisceral fluid and plasma were frozen in liquid nitrogen until analysis.

The total protein, albumin, glucose, urea, chloride and calcium concentrations were determined by colorimetric methods and were assayed using *in vitro* diagnostic kits (In Vitro Diagnostica SA; www.invitro.com.br) with two replicates using a Spectrum SP-2000 UV spectrophotometer (Shanghai Spectrum Instruments Co. Ltd; www.spectrum-cn.com). Sodium and potassium ion concentrations were measured using a Digimed model DM-61 flame photometer (Digimed, São Paulo, Brazil) and the osmolality (mOsm kg^{-1}) was determined by freezing point depression using a μ-Osmette Precision System microsmometer (Precision System Inc; www.precisionsysteminc.com).

Gills and kidney were individually homogenized on ice in sucrose ethylene diaminetetraacetic acid (EDTA) and imidazole (SEI) buffer (0·3 mol l^{-1} sucrose, 20 mmol l^{-1} EDTA, 10 mmol l^{-1} and 0·1 mol l^{-1} imidazole and pH 7·4) and centrifuged at 17 500 *g* at 4° C for 30 min. Homogenates were used for protein measurement and determination of Na^+/K^+-ATPase activity according by the method of Ewing *et al.* (2001). The enzyme activity assay was performed using 100 mmol l^{-1} NaCl, 5 mmol l^{-1} $MgCl_2$, 13 mmol l^{-1} KCl, 3 mmol l^{-1} ATP and 30 mmol l^{-1} imidazole and pH 7·4 at 25° C for 30 min. Ouabain (2 mmol l^{-1}) was added in duplicate to determine ouabain-sensitive ATPase activity. The inorganic phosphate in the supernatant was measured at 620 nm, and the Na^+/K^+-ATPase activity was expressed as μmol Pi mg $protein^{-1}$ h^{-1}.

The Kolmogorov–Smirnov test was used prior to analysing the data normality. Non-parametric Mann–Whitney *U*-tests were performed to compare data, and significance was set at 0·05.

No significant differences were found in the concentrations of electrolytes (Na^+, Cl^-, K^+ and Ca^{2+}), urea, osmolality, total protein, albumin and glucose values in the perivisceral fluid of BW and WW stingrays (Table I). Similarly there were no significant differences in the plasma total-protein concentration and the plasma glucose levels in both populations of *P. aiereba* (Table I). The total-protein and glucose concentrations fell within the same range as those reported for *Potamotrygon* sp. (Wood *et al.*, 2002) and other potamotrygonids (Thorson *et al.*, 1967). Albumin, which is extremely low or absent in marine Elasmobranchii (Griffith *et al.*, 1973; Zammit & Newsholme, 1979), was present in the plasma of both populations of *P. aiereba* varying from 20 to 40% of total plasma protein (Table I) as found in other potamotrygonids (Griffith *et al.*, 1973).

Although BW and WW *P. aiereba* showed significant difference in the plasma urea concentration (Table I), the very low plasma urea in this species suggests that the overall osmoregulation involves mainly the blood electrolytes (Na^+ and Cl^-), as reported for other Amazonian potamotrygonidae (Thorson *et al.*, 1967; Wood *et al.*, 2002). The significantly high urea content in the perivisceral fluid of *P. aiereba* (Table I), however, suggests that urea may play an important role as an osmolyte in this fluid, unlike *Potamotrygon* spp., whose perivisceral fluid shows a urea content *c*. 135-fold lower (Thorson *et al.*, 1967).

Plasma [Na^+], [Cl^-], urea concentrations and osmolality were significantly higher in WW than in BW *P. aiereba* (Table I). These differences can be explained as phenotypic plasticity usually expressed in aquatic animals living in environments with different aquatic compositions. There is a clear contrast in the physico-chemical variables between BW of the River Negro [mean ± s.e. pH 4·5 ± 0·9, [O_2] 5·1 ± 0·2 mg l^{-1}, conductivity 9·4 ± 0·7 μS cm^{-1}, total

TABLE I. Chemical composition and osmolality of the plasma and perivisceral fluid of *Paratrygon aiereba* captured in the River Negro (BW, blackwater; $n = 8$) and the River Amazon (WW, whitewater; $n = 6$). Values are means ± s.e.

	Plasma		Perivisceral fluid	
	BW	WW	BW	WW
[Na^+] (mM)	138·9 ± 2·2	156·6 ± 0·7*	133·7 ± 2·6	144·5 ± 1·8
[Cl^-] (mM)	146·2 ± 2·8	177·5 ± 2·9*	132·9 ± 1·8	127·7 ± 2·6
[K^+] (mM)	4·8 ± 0·3	5·7 ± 0·4	5·1 ± 0·3	5·8 ± 0·1
[Ca^{+2}] (mM)	2·6 ± 0·3	2·9 ± 0·4	1·9 ± 0·7	1·5 ± 0·5
[Urea] (mM)	1·3 ± 0·2	4·2 ± 0·3*	39·5 ± 2·9	43·8 ± 2·1
Osmolality (mOsm kg^1)	263·1 ± 5·2	304·7 ± 15·5*	252·5 ± 6·2	278 ± 14·5
Total protein (g 100 ml^{-1})	1·6 ± 0·2	1·2 ± 0·2	0·2 ± 0·0	0·3 ± 0·0
Albumin (g 100 ml^{-1})	0·3 ± 0·1	0·5 ± 0·1	n.d.	n.d.
Glucose (mM)	1·7 ± 0·2	1·5 ± 0·3	0·4 ± 0·0	0·5 ± 0·0

n.d., not detected by the analytical method.
*$P < 0·05$, difference between BW and WW means.

TABLE II. Na^+–K^+-ATPase in gills and kidney homogenates of *Paratrygon aiereba* captured in the River Negro (BW, blackwater; $n = 8$) and the River Amazon (WW, whitewater; $n = 6$). Values are means ± S.E.

	Specific activity (μmol Pi mg protein^{-1} h^{-1})	
	BW	WW
Gill	1·2 ± 0·1	0·5 ± 0·1*
Kidney	12·8 ± 0·5	8·2 ± 0·4*

*$P < 0{\cdot}05$, difference between BW and WW means.

dissolved solids (TDS) 5·2 ± 0·1 and temperature: 29·6 ± 0·7° C] and WW of the River Amazon (pH 6·4 ± 0·7, [O_2] 3·2 ± 0·3 mg l^{-1}, conductivity 18·9 ± 0·5 μS cm^{-1}, TDS: 17·3 ± 0·6 and temperature: 28·3 ± 0·9° C). In addition, the River Amazon has 6·2 times more Cl^-, 7·3-fold more Na^+, 3·3 times more K^+ and 39·4-fold more Ca^{+2} than the River Negro (Aucour *et al.*, 2003) being ion richer compared with the River Negro.

In the specimens from BW, the gill Na^+/K^+-ATPase activity was 2·4-fold higher, and the kidney Na^+/K^+-ATPase activity was *c.* 1·6-fold higher than those from WW (Table II). In the kidney, the activity of Na^+/K^+-ATPase was higher than in the gills. It suggests high tubular NaCl reabsorption to minimize ion loss generating diluted solute-free urine. In the case of *P. aiereba* living in BW, it may be an important adaptation to retain ions in acidic and ion-poor waters, as suggested by Wood *et al.* (2002) for *Potamotrygon* sp. It is important to emphasize that *P. aiereba* is strongly ichthyophagous (Lasso *et al.*, 1996) and may obtain significant supplementary ions from the diet in both BW and WW environments. Finally, *P. aiereba* exhibited a marked ability to adjust to the ionic characteristics of fresh water, osmoregulating to a wide range of ion challenges in their natural environment in the Amazon Basin.

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