

Plasma ion levels of freshwater and marine/estuarine teleosts from Southern Brazil

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The purpose of this study was to investigate Na⁺, Cl⁻, K⁺, Ca²⁺, and Mg²⁺ levels in the plasma of freshwater and marine/estuarine teleosts collected at different salinities (0 to 34) from the estuarine and freshwater portions of the São Gonçalo channel in Southern Brazil. Any relationship between plasma ion levels and salinity and the capacity of ionic regulation of teleosts found at three or more different salinities (*Genidens barbatus* and *Micropogonias furnieri*) was also investigated. Results showed no relationship between plasma ion levels and salinity when considering all species together, but the two species collected from three or more different salinities showed a significant positive relationship between plasma ion levels and salinity, indicating that *G. barbatus* and *M. furnieri* have a high capacity to regulate plasma ion levels at both low and high salinities.

A proposta deste estudo foi investigar os níveis de Na⁺, Cl⁻, K⁺, Ca²⁺, and Mg²⁺ no plasma de teleósteos de água doce e marinhos/estuarinos coletados em diferentes salinidades (0 a 34) nas porções de água doce e estuarinas do Canal São Gonçalo, sul do Brasil. Uma possível relação entre os níveis iônicos plasmáticos e a salinidade também foi investigada bem como a capacidade de regulação iônica dos teleósteos coletados em três ou mais salinidades (*Genidens barbatus* e *Micropogonias furnieri*). Os resultados mostraram que não houve relação entre os níveis iônicos no plasma com a salinidade quando considerando todas as espécies juntas, mas as duas espécies coletadas em três ou mais salinidades mostraram uma relação significativamente positiva entre níveis iônicos no plasma e salinidade, mostrando que *G. barbatus* e *M. furnieri* apresentaram uma grande capacidade para regular os íons do plasma em baixas e altas salinidades.

Keywords: Ariidae, Estuaries, Ionoregulation, Osmoregulation, Salinity, Sciaenidae.

Introduction

The capacity to regulate plasma ion levels in conditions of changing external salinity is necessary for fish that either live in estuaries or that migrate between freshwater and seawater as part of their normal life cycle. The response to changes in salinity may need to be rapid (such as during tidal cycles or rapid movements through estuaries) or slow (such as for the seasonal or ontogenetic acquisition of salinity tolerance in anadromous fish) (McCormick, 2001). Thus, estuarine teleosts are euryhaline species and efficient osmoregulators by necessity (Prodócimo & Freire, 2001).

In Southern Brazil, the most important estuary is the Patos Lagoon (10,227 km²), which is connected to the Mirim Lagoon (3,750 km²) by the São Gonçalo Channel. This natural channel is 75 km long, 200-500 m wide, 6 m deep, and is obstructed by the São Gonçalo dam to prevent salt water intrusion into the

Mirim Lagoon during the dry season (Burns *et al.*, 2006; Da Rocha *et al.*, 2009).

Analysis of plasma ion levels in neotropical teleosts has generally been performed in the laboratory on specimens acclimated to either freshwater (Baldisserotto & Mimura, 1997; Borges *et al.*, 2004; Becker *et al.*, 2009) or seawater (Altinok *et al.*, 1998; Prodócimo & Freire, 2001; Sampaio & Bianchini, 2002). The ionic regulation of two species of pufferfish (*Sphoeroides testudineus* and *S. greeleyi*) (Prodócimo & Freire, 2001) and a flounder (*Paralichthys orbignyanus*) (Sampaio & Bianchini, 2002) was also studied when fish were subjected to salinity changes in the laboratory. Only one study has analyzed the plasma ion content of fish immediately collected from their natural freshwater environments (Becker *et al.*, 2006).

The purpose of this study was to investigate Na⁺, Cl⁻, K⁺, Ca²⁺, and Mg²⁺ levels in the plasma of freshwater and marine/estuarine teleosts collected from the freshwater and estuarine

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regions of the São Gonçalo channel in Southern Brazil from water at different salinities (0 to 34). Any relationship between plasma ion levels and salinity and the capacity of ionic regulation of teleosts found at three or more different salinities was also investigated. Therefore, this study provides a better understanding of plasma ion regulation in freshwater and marine/estuarine teleosts in the wild.

Material and Methods

During three sampling periods (February 2005, April 2006, and December 2006), fish were collected from water at different salinities from the estuarine (downstream) and freshwater (upstream) regions of the São Gonçalo channel (Table 1). Freshwater and marine/estuarine teleosts were collected using a shrimp trawl (10.5 m head rope, 0.5 cm bar mesh in the center, 1.3 cm bar mesh along the wings) deployed for 5 min by a wood boat with a 60 hp engine. Samples were also taken from shallow water on both sides of the dam using a beach seine (9 m).

Fish were classified into four ecological guilds (see Table 1) as previously described by Garcia *et al.* (2003a): 1) estuarine residents - species typically residing occurring and breeding within the estuary; 2) estuarine dependents - marine- or freshwater-spawning species found in large numbers within the estuary during certain periods of their life cycle; 3) marine vagrants - typically inhabiting marine habitats and rarely occurring within the estuary; 4) freshwater vagrants - typically inhabiting freshwater habitats and rarely occurring within the estuary. The last guild was further split into first-order freshwater fish, (ecologically restricted to freshwater) and second-order freshwater fish (generally restricted to freshwater but able to survive, and occasionally occur, in saltwater) (Vieira *et al.*, 1998).

After collection, fish were stunned with a blow to the head. Blood was collected from the caudal vein with heparinized 1-mL syringes and refrigerated in Eppendorf tubes. Specimens were then euthanized by severing the spinal cord, weighed and measured (Table 1). All blood samples were stored on ice and transported to the Fish Physiology Laboratory/UFSM. Samples were then centrifuged at 2,000 rpm for 5 min to separate the plasma and were stored at -20°C for subsequent analysis.

Field water salinity was measured with a handheld salinity meter, and plasma chloride concentration was measured according to Zall *et al.* (1956). Sodium and potassium ion concentrations were measured with a B262 flame spectrophotometer (Micronal, São Paulo, Brazil); calcium and magnesium ion concentrations were measured using a GBC 932AA atomic absorption spectrophotometer (GBC Scientific Equipment, Victoria, Australia). Standard solutions were made with analytical grade reagents (Vetec or Merck) dissolved in deionized water, and standard curves of each ion were calculated using five different concentrations. This study was approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria (process number 23081.008434/2007-85). The identification of the fishes was performed by comparisons with existing vouchers in the Laboratório de Ictiologia (FURG).

All data are expressed as the means \pm standard error of the mean (SEM). Relationships between plasma ion levels, salinity and ion regulation curves were made using the Sigma Plot 11.0 software set at a minimum significance level of $P < 0.05$.

Results

Fish were categorized into the following groups: estuarine residents (one species), estuarine dependents (six species), marine vagrants (one species) and freshwater vagrants (five species) (Table 1).

Although there was no relationship between plasma ion levels and water salinity when considering all species together (Table 2), a significant relationship was observed for two species collected from water at three or more different salinities. *Micropogonias furnieri* and *Genidens barbatus* exhibited a positive relationship between plasma Na^+ , Cl^- , Ca^{2+} , and Mg^{2+} levels and water salinity and a negative relationship between plasma K^+ levels and salinity (Fig. 1).

The ionic regulatory capacity was also verified in the two species collected from water at three or more different salinities (*G. barbatus* and *M. furnieri*) (Fig. 1). *Genidens barbatus* and *M. furnieri* hyperregulated Na^+ and Cl^- levels to keep plasma values above the isoionic line at a salinity of 5 or below, but

Table 1. Freshwater and marine/estuarine teleosts collected from water at different salinities from the São Gonçalo channel. Specimens were classified according to ecological guild: ESR: estuarine residents; ESD: estuarine dependents (diadromous); MARV: marine vagrants and FRV: freshwater vagrants (Vieira *et al.*, 1998; Garcia *et al.*, 2003a). Values are presented as the means \pm SEM.

Species	Voucher numbers	Ecological guild	Salinity	Weight (g)	Length (cm)	N
<i>Hypostomus commersoni</i>	FURG 88034	FRV	0	515.20 \pm 1.86	37.35 \pm 1.20	12
<i>Micropogonias furnieri</i>	FURG 2499	ESD	0; 5; 24; 26; 29; 34	56.15 \pm 2.41	12.10 \pm 1.14	12
<i>Parapimelodus nigribarbis</i>	FURG 2545	FRV	0	305.15 \pm 1.26	24.86 \pm 1.33	12
<i>Pimelodus pintado</i>	FURG 1216	FRV	0; 5	816.13 \pm 3.49	30.14 \pm 1.41	12
<i>Rhamdia quelen</i>	FURG 2241	FRV	0	620.10 \pm 4.51	35.24 \pm 1.56	12
<i>Cyphocharax voga</i>	FURG 2539	FRV	0; 5	155.15 \pm 2.12	28.23 \pm 1.23	12
<i>Genidens barbatus</i>	FURG 94018	ESD	5; 24; 29	570.21 \pm 2.85	28.35 \pm 1.18	12
<i>Atherinella brasiliensis</i>	FURG 1191	ESR	8	10.45 \pm 1.11	11.71 \pm 2.13	12
<i>Brevoortia pectinata</i>	FURG 2274	ESD	8	11.67 \pm 1.03	10.17 \pm 2.59	12
<i>Menticirrhus americanus</i>	FURG 2048	MARV	24	70.18 \pm 1.28	20.52 \pm 2.12	12
<i>Paralichthys orbignyanus</i>	FURG 94020	ESD	24	520.13 \pm 2.89	33.27 \pm 1.86	12
<i>Trichiurus lepturus</i>	FURG 5013	MARV	29	223.89 \pm 2.10	50.48 \pm 3.49	12
<i>Mugil curema</i>	FURG 95147	ESD	34	980.10 \pm 3.51	44.51 \pm 0.91	12

hyporegulated these ion levels at all other salinities (Figs. 1a and 1b); the isoionic line was crossed at approximately a salinity of 17. Plasma Ca^{2+} levels of *G. barbuis* and *M. furnieri* were hyporegulated at salinities above 24 (Fig. 1c), but hyperregulated at lower salinities (0 and 5) with values near the isoionic line. *Genidens barbuis* and *M. furnieri* also strongly hyporegulated Mg^{2+} levels, continuously keeping plasma levels below the isoionic line (Fig. 1d). Plasma K^+ levels were hyperregulated in *M. furnieri* collected from water at salinities of 0, 5, 24, and 26, but they were hyporegulated at higher salt concentrations and crossed the isoionic line at approximately a salinity of 27 (Fig. 1e). In *G. barbuis* plasma K^+ levels were hyperregulated at salinity 5 and hyporegulated at salinities 24 and 29.

Discussion

Classification by ecological guilds has previously been used to simplify information and allow for a better comparison of estuarine use between different systems (freshwater, estuarine and marine). In addition, this approach confirms the physiological and ionoregulatory mechanisms reported for fish found in estuarine regions (Elliott & Dewailly, 1995; Prodóximo & Freire, 2001; Garcia *et al.*, 2003a, 2003b, 2004; Burns *et al.*, 2006; Freire & Prodóximo, 2007; Martinho *et al.*, 2007; Da Rocha *et al.*, 2009). Our findings also confirm results

from earlier studies regarding the environments used by these fish species for feeding, reproduction and nursery grounds (Chao *et al.*, 1985; Vieira & Castello, 1996; Garcia *et al.*, 2003a, 2003b, 2004; Burns *et al.*, 2006).

The analysis of spatiotemporal distribution patterns of freshwater fish in the Patos Lagoon by Garcia *et al.* (2003b; 2004) and Vieira *et al.* (2010) showed that primary freshwater fish inhabiting the freshwater site (upper region of the Patos Lagoon) can be passively flushed into the estuarine zone during periods of high freshwater discharge and briefly remain in this zone. However, Garcia *et al.* (2003a) reported that when the estuarine zone returns to normal hydrological conditions primary freshwater fish that remain in the estuary may suffer from high physiological stress due to the intrusion of saline water into the estuary. In addition, only secondary freshwater fish, such as Cyprinodontiformes and Cichlidae, are able to remain in the estuary, and some euryhaline estuarine fish can move into freshwater sites of the Patos Lagoon during part of their life cycle (Chao *et al.*, 1985; Vieira & Castello, 1996; Barletta *et al.*, 2010; Vieira *et al.*, 2010).

The plasma ion levels of all fish species collected in our study (see Table 2) were similar to the values reported for other freshwater, estuarine and seawater teleosts (Chew & Ip, 1990; Baldisserotto *et al.*, 1990; Jensen *et al.*, 1998; Prodóximo & Freire, 2001; Borges *et al.*, 2004; Becker *et al.*, 2006; McDonald & Grosell, 2006).

Table 2. Plasma ion levels of teleosts collected from water at different salinities from the São Gonçalo channel. Values in parentheses represent water ion levels. Values are presented as the means \pm SEM ($n = 12$); r^2 is related to the salinity x plasma ion level relationship.

Species	(mmol L ⁻¹)				
	Na ⁺	K ⁺	Cl ⁻	Ca ²⁺	Mg ²⁺
Salinity 0	(0.75 \pm 0.15)	(0.08 \pm 0.01)	(0.91 \pm 0.12)	(1.16 \pm 0.04)	(1.27 \pm 0.12)
<i>Cyphocharax voga</i>	223.38 \pm 3.90	0.54 \pm 0.04	129.36 \pm 0.84	2.75 \pm 0.20	1.06 \pm 0.11
<i>Hypostomus commersoni</i>	159.27 \pm 1.04	6.07 \pm 0.14	120.73 \pm 1.31	3.03 \pm 0.15	1.27 \pm 0.16
<i>Micropogonias furnieri</i>	217.22 \pm 2.70	14.39 \pm 0.73	126.43 \pm 3.31	1.43 \pm 0.21	0.70 \pm 0.06
<i>Parapimelodus nigribarbuis</i>	198.73 \pm 2.33	3.87 \pm 0.60	142.61 \pm 3.67	2.19 \pm 0.11	1.33 \pm 0.21
<i>Pimelodus pintado</i>	180.33 \pm 2.24	2.28 \pm 0.22	168.45 \pm 2.99	2.82 \pm 0.15	1.54 \pm 0.22
<i>Rhamdia quelen</i>	165.28 \pm 2.05	1.36 \pm 0.11	112.63 \pm 2.51	3.26 \pm 0.19	2.17 \pm 0.17
Salinity 5	(64.64 \pm 2.56)	(2.04 \pm 0.15)	(78.61 \pm 2.26)	(2.37 \pm 0.22)	(5.08 \pm 0.51)
<i>Cyphocharax voga</i>	197.64 \pm 2.03	0.95 \pm 0.14	133.63 \pm 4.42	2.84 \pm 0.21	1.67 \pm 0.28
<i>Genidens barbuis</i>	228.02 \pm 1.65	13.46 \pm 1.36	161.92 \pm 1.62	2.66 \pm 0.21	0.89 \pm 0.20
<i>Micropogonias furnieri</i>	214.86 \pm 2.57	12.72 \pm 1.62	144.35 \pm 2.83	2.44 \pm 0.14	0.79 \pm 0.20
<i>Pimelodus pintado</i>	201.06 \pm 2.97	0.54 \pm 0.04	127.53 \pm 2.75	2.97 \pm 0.21	2.31 \pm 0.20
Salinity 8	(105.33 \pm 3.12)	(3.20 \pm 0.26)	(125.77 \pm 3.05)	(3.79 \pm 0.32)	(8.13 \pm 0.35)
<i>Brevoortia pectinata</i>	200.58 \pm 1.49	6.92 \pm 0.05	161.79 \pm 4.00	2.62 \pm 0.13	2.27 \pm 0.09
Salinity 24	(310.29 \pm 4.21)	(9.81 \pm 0.35)	(377.32 \pm 4.54)	(11.36 \pm 0.84)	(24.38 \pm 0.25)
<i>Genidens barbuis</i>	233.08 \pm 4.07	9.74 \pm 0.52	196.45 \pm 2.73	3.03 \pm 0.13	1.34 \pm 0.09
<i>Menticirrhus americanus</i>	204.92 \pm 0.90	2.77 \pm 0.04	204.60 \pm 0.86	3.44 \pm 0.20	1.03 \pm 0.20
<i>Micropogonias furnieri</i>	225.47 \pm 2.97	12.00 \pm 0.66	166.50 \pm 0.92	2.48 \pm 0.16	0.91 \pm 0.23
<i>Paralichthys orbignyanus</i>	203.59 \pm 2.13	3.69 \pm 0.32	203.12 \pm 3.81	2.71 \pm 0.16	1.10 \pm 0.19
Salinity 26	(336.15 \pm 4.31)	(10.63 \pm 0.37)	(408.76 \pm 6.31)	(12.31 \pm 0.95)	(26.42 \pm 0.28)
<i>Micropogonias furnieri</i>	234.73 \pm 2.63	11.73 \pm 0.88	174.64 \pm 0.73	2.60 \pm 0.29	0.95 \pm 0.27
Salinity 29	(374.94 \pm 4.26)	(11.86 \pm 0.26)	(455.93 \pm 5.34)	(13.73 \pm 0.89)	(29.46 \pm 0.31)
<i>Genidens barbuis</i>	247.23 \pm 4.14	9.57 \pm 0.72	214.68 \pm 2.74	3.75 \pm 0.14	1.92 \pm 0.19
<i>Micropogonias furnieri</i>	236.65 \pm 1.91	9.94 \pm 0.56	189.28 \pm 2.94	2.67 \pm 0.13	1.28 \pm 0.11
<i>Trichiurus lepturus</i>	236.65 \pm 2.24	27.39 \pm 1.42	136.21 \pm 3.28	2.45 \pm 0.30	2.11 \pm 0.28
Salinity 34	(439.58 \pm 5.12)	(13.90 \pm 0.29)	(534.54 \pm 6.25)	(16.10 \pm 1.06)	(34.54 \pm 0.35)
<i>Micropogonias furnieri</i>	235.47 \pm 0.61	6.75 \pm 0.40	185.30 \pm 3.33	2.85 \pm 0.11	1.56 \pm 0.23
<i>Mugil curema</i>	225.47 \pm 0.65	0.54 \pm 0.04	150.57 \pm 0.78	2.35 \pm 0.20	0.98 \pm 0.20
r^2	0.47	0.08	0.47	0.04	0.003

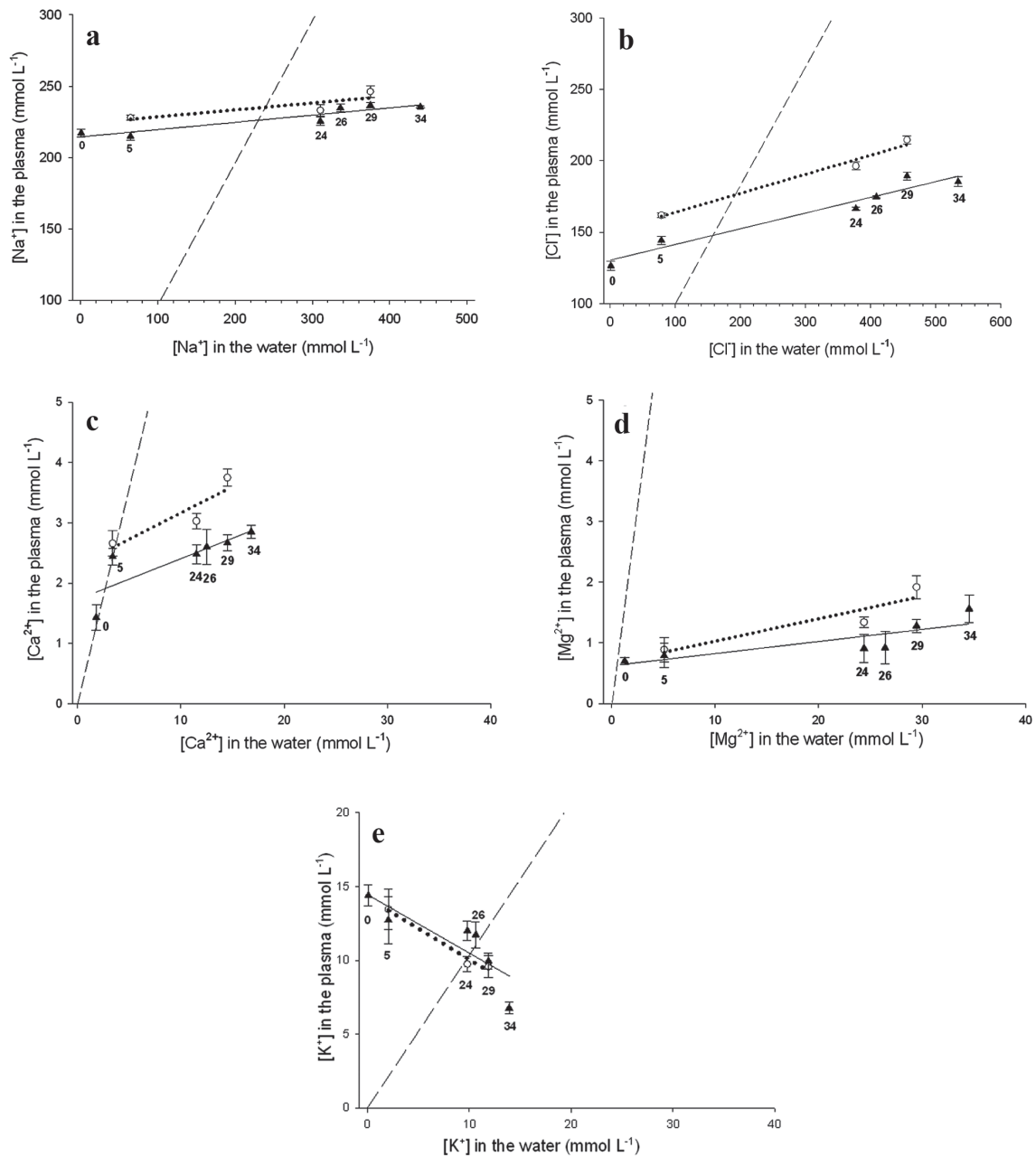


Fig. 1. Ion regulation curves for Na^+ (a), Cl^- (b), Ca^{2+} (c), Mg^{2+} (d), and K^+ (e) in the plasma of *M. furnieri* (\blacktriangle) and *G. barbus* (\circ) collected from water at different salinities from the São Gonçalo channel. Data are expressed as the means \pm SEM ($n = 12$) ($P < 0.05$); the traced line represents the isoionic line. The following equations were fitted to the data: *Micropogonias furnieri* (Na^+ : $y = 214.52 + 0.051x$, $r^2 = 0.87$; Cl^- : $y = 130.45 + 0.110x$, $r^2 = 0.94$; K^+ : $y = 14.44 - 0.396x$, $r^2 = 0.71$; Ca^{2+} : $y = 1.73 + 0.067x$, $r^2 = 0.70$; Mg^{2+} : $y = -0.62 + 0.020x$, $r^2 = 0.70$); *Genidens barbus* (Na^+ : $y = 223.74 + 0.048x$, $r^2 = 0.70$; Cl^- : $y = 150.56 + 0.133x$, $r^2 = 0.98$; K^+ : $y = 14.23 - 0.419x$, $r^2 = 0.97$; Ca^{2+} : $y = 2.29 + 0.087x$, $r^2 = 0.82$; Mg^{2+} : $y = 0.66 + 0.037x$, $r^2 = 0.85$), where x = salinity and y = plasma ion concentration (mmol L^{-1}).

The capacity to adapt to different environmental salinities, *i.e.*, to move between freshwater and seawater, is determined by the ability of fish to regulate ion uptake and excretion by the gills, gut and kidney and to maintain hydromineral balance (McCormick, 2001; Rodrigues *et al.*, 2002; Martínez-Álvarez *et al.*, 2005; McDonald & Grosell, 2006).

In the present study, no relationship was observed between plasma ion levels and salinity when considering all collected species together. However, an individual analysis of each of the two species collected from water at three or more different salinities (*M. furnieri* and *G. barbus*) revealed a significant relationship between plasma ion levels and salinity. Previous

studies reported a slight, but significant, positive linear relationship (over the salinity range tested) between plasma osmolality and ionic composition of the euryhaline flounder, *Paralichthys orbignyanus*, acclimated to different salinities (0-40) for 15 days (Sampaio & Bianchini, 2002) and *Pagrus auratus* transferred from ambient seawater (salinity of 30) to either concentrated hyperosmotic (salinity 45) or diluted hyperosmotic (salinity of 15) environments for 168 h (Fielder *et al.*, 2007).

All measured ions in the plasma of *M. furnieri* (at salinities of 29 and 34; except Mg^{2+} at a salinity of 29) and *G. barbuis* (at a salinity of 29) were higher than those reported for both an estuarine fish, *Sphoeroides testudineus*, maintained in the laboratory at a salinity of 30 for 6 h (Prodóximo & Freire, 2001) and a marine teleost, *Opsanus beta*, maintained in the laboratory at a salinity of 33 for 7 days (McDonald & Grosell, 2006). These differences may be due to the fact that in our study blood was collected immediately after the fish were caught with a shrimp trawl deployed for 5 min. Therefore, fish were subjected to a very stressful situation, and in stressed individuals there is an increase of blood flow to the gill and paracellular permeability (Cech *et al.*, 1996; McDonald *et al.*, 1991), which lead to dehydration and ion influx by diffusion because seawater is more concentrated than plasma (Gallaughier *et al.*, 2001).

Both *M. furnieri* and *G. barbuis* demonstrated a high capacity to regulate their plasma ion levels at low and high salinities. In marine/estuarine teleosts of different origin (*Myoxocephalus octodecemspinosus*, *Acipenser naccarii*, *P. orbignyanus*, *S. testudineus*, and *S. greeleyi*), plasma ion concentration is kept within narrow limits in diluted seawater or even in freshwater, indicating euryhalinity and a well-developed capacity of osmoionic regulation (Claiborne *et al.*, 1994; Cataldi *et al.*, 1995; Prodóximo & Freire, 2001; Sampaio & Bianchini, 2002), as found in our study.

Plasma ion levels for *M. furnieri* and *G. barbuis* were similar to those reported by Prodóximo & Freire (2001) for pufferfish (*S. testudineus* and *S. greeleyi*), where Na^+ and Cl^- were hyperregulated at low salinities but hyporegulated at high salinities, and Mg^{2+} was continuously and tightly hyporegulated. This hyporegulation of Mg^{2+} is likely accomplished through renal tubular Mg^{2+} secretion, as observed by Beyenbach *et al.* (1993, 1997), Hentschel & Zierold (1994) and Beyenbach (1995).

The inability of most freshwater fish species to enter hyperosmotic salinities may be caused by absence of hormonal, renal or intestinal control mechanisms (*i.e.*, no neuroendocrine axis to stimulate oral ingestion) (Evans *et al.*, 2005). Recent studies report that polyvalent cation receptors (CaR) in the gill epithelium stimulate osmoregulatory changes by responding to changes in internal or external Ca^{2+} concentrations. Therefore, the osmoregulatory machinery controlled by these ion receptors could explain the capacity of both *G. barbuis* and *M. furnieri* to hyporegulate plasma Ca^{2+} levels at higher salinities.

In summary, our data show that plasma ion levels were not directly related to water salinity when considering several species together (*i.e.*, a general pattern could not be established). However, a relationship between plasma ion

levels and salinity was observed when individually analyzing each species collected from a range of salinities, indicating that this pattern is species-specific. Moreover, similarities in the plasma ion regulatory capacity of both *G. barbuis* and *M. furnieri* were observed at all salinity levels considered, allowing those fish to live in estuarine environments. Furthermore, these findings are completely consistent with the environmental distribution records of both species.

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