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Muscle water control in crustaceans and fishes as a function of habitat, osmoregulatory capacity, and degree of euryhalinity

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Abstract

This study aimed at detecting possible patterns in the relationship between Anisosmotic Extracellular Regulation (AER) and Isosmotic Intracellular Regulation (IIR) in crustaceans and teleost fish from different habitats and evolutionary histories in fresh water (FW), thus different osmoregulatory capabilities, and degrees of euryhalinity. Crustaceans used were the hololimnetic FW *Aegla schmitti*, and *Macrobrachium potiuna,* the diadromous FW *Macrobrachium acanthurus*, the estuarine *Palaemon pandaliformis* and the marine *Hepatus pudibundus*; fishes used were the FW *Corydoras ehrhardti*, *Mimagoniates microlepis*, and *Geophagus brasiliensis*, and the marine-estuarine *Diapterus auratus*. The capacity for IIR was assessed *in vitro* following wet weight changes of isolated muscle slices incubated in anisosmotic saline (~50% change). *M. potiuna* was the crustacean with the highest capacity for IIR; the euryhaline perciforms *G. brasiliensis* and *D. auratus* displayed total capacity for IIR. It is proposed that a high capacity for IIR is required for invading a new habitat, but that it is later lost after a long time of evolution in a stable habitat, such as in the FW anomuran crab *A. schmitti*, and the Ostariophysian fishes *C. ehrhardti* and *M. microlepis*. More recent FW invaders such as the palaemonid shrimps (*M. potiuna* and *M. acanthurus*) and the cichlid *G. brasiliensis* are euryhaline and still display a high capacity for IIR.

Keywords: Anisosmotic extracellular regulation; Crustacean; Fish; Isosmotic intracellular regulation; Muscle hydration; Osmoregulation

1. Introduction

The function of osmoregulation relates to the osmotic (and ionic) homeostasis of the extracellular medium. In the case of aquatic animals, it involves a series of epithelial mechanisms, which end up being effective in the maintenance of a significant osmotic gradient between the extracellular medium and the external medium. Animals that invest energy into those mechanisms (mostly located in the gills) are osmoregulators, and perform Anisosmotic Extracellular Regulation (AER, term coined by Florkin, 1962; Evans, 1993; Péqueux, 1995). All freshwater animals are by necessity osmoregulators, but marine species may osmoregulate (all teleostean fishes) or osmoconform (marine invertebrates in general, many crustaceans in particular). Osmoconformers do not perform AER, they do not invest energy into transport mechanisms in their interface epithelia and are thus unable to keep significant osmotic gradients between their internal medium and the surrounding water, mainly upon salinity fluctuations.

If the osmolality of the extracellular medium changes along time, all cells will be osmotically challenged and will be forced to regulate their volume. A significant volume disturbance will

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compromise the physiological performance of the cells. Several membrane transporters are employed to promote solute (inorganic and organic) flux which will result in corrective water movements. The ensemble of mechanisms is called Isosmotic Intracellular Regulation (IRR, term coined by Florkin, 1962), and may involve solute and water efflux (Regulatory Volume Decrease, RVD) upon hyposmotic conditions, and solute and water influx (Regulatory Volume Increase, RVI) upon hyperosmotic conditions (e.g., Chamberlin and Strange, 1989; Hoffmann and Dunham, 1995; Péqueux, 1995; Russell, 2000; Wehner et al., 2003).

Crustaceans are the most diverse and successful fully aquatic invertebrates that invaded freshwater bodies from the sea (Ruppert and Barnes, 1994). Thus, they display all types of osmoregulatory strategies, ranging from marine stenohaline osmoconformers to stenohaline freshwater regulators. Frequently, a species may switch from osmoregulation to osmoconformation, depending on the salinity challenge presented, and the time of exposure (Péqueux, 1995; Freire et al., 2003; Freire et al., in press). The order Decapoda is the largest order of crustaceans, and has the largest sized specimens, with several lineages that invaded the freshwater biotope (Ruppert and Barnes, 1994). Freshwater decapods demonstrate a great capacity for salt absorption in the gills, coupled to very low permeabilities, attaining over 400 mOsm/kgH_2O of hemolymph osmolality (Subramanian, 1975; Kirschner, 1991; Rasmussen and Andersen, 1996; Freire et al., 2003).

Teleostean fishes are osmoregulators, both in fresh water and in sea water (Evans, 1993; Jobling, 1995). Marine species are strongly hyposmotic to sea water, with plasma osmolalities in temperate, subtropical, and tropical species ranging between 370 and 480 mOsm/kgH_2O, while freshwater species are hyper-osmotic, with plasma osmolalities between 230 and 330 mOsm/kgH_2O (Evans, 1993; Jobling, 1995; Freire and Prodocimo, 2007). Teleosts (Division Teleostei) represent the vertebrate group with the largest number of species (Nelson, 2006). The superorder Ostariophysi is the second largest teleost superorder (Saitoh et al., 2003), comprising 68% of all fishes that live in fresh water (Nelson, 2006; Froese and Pauly, 2007 FishBase version 10/2007).

This study aimed at detecting possible patterns in the relationship between AER and IIR in invertebrates (decapod crustaceans) and vertebrates (teleost fish) from different habitats and evolutionary histories in fresh water, different osmoregulatory capabilities, and degrees of euryhalinity. The research questions raised were: 1) Is there a difference in the relationship between AER and IIR between crustaceans and fishes? 2) How do AER and IIR capacity relate to migrations between habitats and the presumed evolutionary history of a species in a certain habitat?

2. Materials and methods

2.1. Animals and field characteristics

The source locality of the species used, with geographical coordinates, species habit, habitat salinity, as well as the osmoregulatory behaviour of the species, and size of the specimens used are summarized in Table 1.

2.2. Crustaceans

The family Aeglidae represents the only group of freshwater anomuran crustaceans, and occupy clear and well oxygenated waters in subtropical and temperate latitudes within a restricted geographical range in South America (Melo, 2003; Castro-Souza and Bond-Buckup, 2004; Ferreira et al., 2005; Gonçalves et al., 2006). The freshwater crab *Aegla schmitti* Hobbs III, 1979 (Decapoda, Anomura, Aeglidae) is hololimnetic and displays few large lecithotrophic eggs (Table 1).

Differently, one of the most diverse and successful groups of freshwater decapods in South America is that of the caridean shrimps of the family Palaemonidae, a lineage still undergoing freshwater invasion (Moreira et al., 1983; Freire et al., 2003; Melo, 2003; Augusto et al., 2007a,b). The genus *Palaemon* is restricted to more coastal Atlantic waters, and the species *Palaemon pandaliformis* is an estuarine resident, a strong hypo-hyper osmoregulator, displays numerous oligolecithic eggs, and is necessarily very euryhaline (Teixeira and Sá, 1998; Freire et al., 2003). The salinity in the estuary where *Palaemon pandaliformis*

Table 1
Habitat location and characteristics, life habit and osmoregulatory behaviour of the species used in this study

Species (length, mm)	Source locality	Coordinates	Habitat type and habit	Habitat salinity	Osmoregulatory behaviour
Crustaceans					
A. schmitti (27.3±1.2, 24)	Piraquara	25°29′S 49°03′W	FW hololimnetic	<0.5‰	FW regul., euryhaline
M. potiuna (43.2±2.3, 17)	Piraquara	25°29′S 49°03′W	FW hololimnetic	<0.5‰	FW regul., euryhaline
M. acanthurus (58.7±2.9, 13)	Pontal do Sul	25°34′S 48°21′W	FW diadromous	<0.5‰	FW regul., euryhaline
P. pandaliformis (35.6±0.6, 31)	Pontal do Sul	25°34′S 48°21′W	Estuarine resident	~8–29‰	Estuarine regul., euryhaline
H. pudibundus (57.7±3.6, 6)	Ipanema	25°37′S 48°25′W	Marine	32–34‰	Marine confor., stenohaline
Fishes					
C. ehrhardti (47.3±1.5, 26)	Piraquara	25°29′S 49°03′W	FW hololimnetic	<0.5‰	FW regul., stenohaline
M. microlepis (45.3±1.1, 44)	Piraquara	25°29′S 49°03′W	FW hololimnetic	<0.5‰	FW regul., stenohaline
G. brasiliensis (66.8±5.3, 16)	Piraquara	25°29′S 49°03′W	FW hololimnetic	<0.5‰	FW regul., euryhaline
D. auratus (90.2±3.0, 15)	Pontal do Sul	25°34′S 48°21′W	Marine/estuarine	11–32‰	Estuarine regul., euryhaline

The mean (±SEM) length, and the total number of individuals used are provided below the species name. FW = fresh water; regul. = osmoregulator; confor. = osmoconformer.

has been obtained varies in the range between 8 and 29‰ (Freire et al., 2003). The freshwater *Macrobrachium acanthurus* inhabits coastal freshwater rivers, close to estuarine areas, displays numerous oligolecithic eggs, and its larvae depend on brackish water for development (Moreira et al., 1983; Teixeira and Sá, 1998), thus being referred as diadromous. It is very euryhaline, and can survive more than 10 days in 20‰ sea water (Cavassin, F. and Freire, C.A., unpublished results). The hololimnetic *Macrobrachium potiuna* develops entirely in fresh water, occurring in fresh waters more distant from the estuaries, displaying few large lecithotrophic eggs and abbreviated development. It is very euryhaline, and a very strong osmoregulator, maintaining its hemolymph homeostasis when facing salinity increase even more efficiently than the marine coastal *Palaemon northropi*, the estuarine *Palaemon pandaliformis*, the continental freshwater stenohaline *Macrobrachium brasiliense*, and the diadromous *Macrobrachium olfersi* (Freire et al., 2003) (Table 1).

Within the brachyuran crabs, most species are strictly marine, although some have ventured into diluted waters (Ruppert and Barnes, 1994). The super-family Calappoidea gathers species of strictly marine crabs ranging from shallow coastal areas down to hundreds of meters (Melo, 1996; Hebling and Rieger, 2003). The marine crab *Hepatus pudibundus* Herbst, 1785 (Decapoda, Brachyura, Hepatidae) is distributed along the West Atlantic coast from Georgia (USA) down to Rio Grande do Sul (Brazil). It inhabits muddy or sandy substrates in depths extending down to 160 m (Melo, 1996). *H. pudibundus* is the main Calappoidid (super-family Calappoidea) crab in the by-catch fauna of trawl net fishing in Southern Brazil (Fracasso and Branco, 2005). After 6 h in diluted sea water of salinity 25‰, its hemolymph osmolality decreased from 962 mOsm/kg H_2O (control in 33‰ sea water) to 684 mOsm/kg H_2O (Foster, C., Amado, E.M., Souza, M.M, Freire, C.A., unpublished results), thus behaving as osmoconformer and relatively stenohaline (Table 1).

2.3. Fishes

Within the essentially freshwater super-order Ostariophysi, the group Otophysi (including the orders Cypriniformes, Characiformes, Siluriformes, and Gymnotiformes) has originated in fresh waters during the Jurassic Period (Nelson, 2006; Froese and Pauly, 2007 FishBase version 10/2007). The Siluriformes (catfishes) is of primary freshwater origin, assembling 33 families, 27 of which are exclusively freshwater; only Ariidae has most of its species inhabiting marine or estuarine areas (Bruton, 1996; Froese and Pauly, 2007 FishBase version 10/2007). The freshwater catfish *Corydoras ehrhardti* Steindachner, 1910 (Ostariophysi, Siluriformes, Callichthyidae) is a common ornamental fish (Nelson, 2006; Froese and Pauly, 2007 FishBase version 10/2007) (Table 1).

All species of the order Characiformes inhabit fresh water. The family Characidae is widely spread in the Neotropical region (Vazzoler and Menezes, 1992; Lowe-McConnell, 1999; Nelson, 2006). The blue tetra *Mimagoniates microlepis* Steindachner, 1877 (Ostariophysi, Characiformes, Characidae) is a small freshwater fish, occurring in shallow coastal streams of clear water in Eastern Brazil, ranging in distribution from Southern Bahia to Northern Rio Grande do Sul (Weitzman, 2003; Froese and Pauly, 2007 FishBase version 10/2007) (Table 1).

Within the Superorder Acanthopterygii, only 23% of the species are freshwater (Nelson, 2006). The order Perciformes, the largest fish order in number of species (~7,000 species, mostly marine), originated in the late Cretaceous period, with a primary marine origin. The family Cichlidae is typically freshwater, but its species are frequently euryhaline and dwell in brackish waters in the Neotropical region, Africa and India (Nelson, 2006; Lowe-McConnell, 1999; Froese and Pauly, 2007 FishBase version 10/2007). *Geophagus brasiliensis* Quoy and Gaimard, 1824 (Acanthopterygii, Perciformes, Cichlidae) is a widespread freshwater cichlid in Southeastern and Southern Brazil, also found in coastal streams down to estuaries (Lowe-McConnell, 1999; Mazzoni and Iglesias-Rios, 2002; Froese and Pauly, 2007 FishBase version 10/2007) (Table 1).

Fishes from the family Gerreidae (mojarras) are marine and coastal, also occurring in water bodies of reduced salinity (Deckert and Greenfield, 1987; Castillo-Rivera et al., 2005). *Diapterus auratus* Ranzani, 1842 (Acanthopterygii, Perciformes, Gerreidae) is a marine coastal species that is very frequent in estuaries and even in lower reaches of rivers, occurring along the Western Atlantic coast, from Florida down to Southern Brazil (Deckert and Greenfield, 1987; Gilmore and Greenfield, 2002; Castillo-Rivera et al., 2005; Froese and Pauly, 2007 FishBase version 10/2007). From its distribution its euryhalinity can already be ascertained. The salinity in the estuary when it was caught was 11‰, but as it is a marine/estuarine species, its habitat salinity has been proposed as 11–32‰ (Table 1).

2.4. Laboratory maintenance

The freshwater crab *A. schmitti* and the palaemonid shrimps were caught from the marginal vegetation of the shallow freshwater streams or estuary through manual sieving. The marine crab *Hepatus pudibundus* was purchased from fishermen, as it is by-catch of the shrimp fishing activity. The freshwater fishes *Corydoras ehrhardti*, *Mimagoniates microlepis* and *Geophagus brasiliensis* were obtained using sieves, from the marginal vegetation, and the marine/estuarine *Diapterus auratus* was obtained from the estuary using cast nets.

Animals were brought to the laboratory (within 30 min–3 h) where they were maintained in stock aquaria with filtered and aerated fresh water (all freshwater species), or diluted sea water of salinity 12‰ (*Palaemon pandaliformis*). The marine crab *Hepatus pudibundus* was kept in sea water of salinity 30‰. The marine/estuarine fish *Diapterus auratus* was obtained from the estuary and brought to the laboratory in salinity 11‰. In the laboratory, the fish were kept for 2 h in sea water of salinity 20‰, and were then transferred to the stock tank with sea water of salinity 30‰. All individuals were allowed to acclimate to laboratory conditions (water temperature of 21 ± 1 °C, natural photoperiod) for 3–8 days before being used in the experiments. During this acclimation period, they were fed on alternate days with shrimps, fish, and commercially available flocculated fish food.

2.5. In vivo determinations of osmoregulatory behaviour

With those freshwater species for which no previous information on their salt tolerance was available, quick *in vivo* osmoregulatory experiments were performed. No thorough investigation of salinity acclimation capabilities was intended, but only a brief comparative investigation upon 7 h of steep salinity increase, from fresh water to diluted sea water of salinity 15‰. Thus, individuals of the freshwater crab *A. schmitti*, and the freshwater fish *Geophagus brasiliensis* were submitted for 7 h to 15‰ sea water (obtained by diluting full-strength sea water with filtered tap water). The freshwater fishes *Corydoras ehrhardti* and *Mimagoniates microlepis* began to show locomotory impairment already after ~2 h of exposure, and have thus been sacrificed and sampled after 4:20 h. Two or 3 individuals were exposed to water of increased salinity in a 1-liter aquarium, with experiments performed in duplicate or triplicate. Control individuals for the *in vivo* experiments were sampled directly from their stock aquaria (fresh water).

2.6. Extracellular fluid (ECF) and muscle sampling

Both after the *in vivo* experiments for the assessment of osmoregulatory capabilities described above, and before the *in vitro* experiments described below, specimens were anaesthetized in ice; duration ranging from 2 min for the small *Palaemon pandaliformis* to 10 min for the large *Hepatus pudibundus*. A sample of hemolymph was obtained from the shrimps using a pipette through cardiac puncture, or from the crabs using an insulin syringe through puncture of the arthrodial membrane of a pereopod. The hemolymph sample was intensely vortexed to prevent clotting. The fish blood sample was obtained from the caudal vein, and was centrifuged to yield plasma. Extracellular fluids were stored in −20 °C until assayed for osmolality. Osmolality was determined in undiluted hemolymph and plasma samples, using the vapor-pressure micro-osmometer VAPRO 5520 (Wescor, USA).

A muscle sample was removed, either for the determination of total water content (*in vivo* experiments, from control and experimental animals), or for the wet weight change evaluation (*in vitro* experiments, from control animals). For all shrimps, the whole abdomen had the cuticle removed and was used: 0.3221±0.0011 g (n=16) for *M. potiuna*, 0.6430±0.0262 g (n=15) for *M. acanthurus*, and 0.1229±0.0071 g (n=19) for *P. pandaliformis*. For the freshwater crab *A. schmitti*, the abdominal flexor muscle was dissected: 0.0347±0.0045 g (n=12) for the *in vivo* experiments and 0.0407±0.0052 g (n=16) for the *in vitro* experiments. For the marine crab *H. pudibundus*, the penniform muscle of the chelipeds was used: 0.0759±0.0180 g (n=13). From the fishes, a piece of lateral trunk muscle was removed, respectively for the *in vivo* and the *in vitro* experiments: 0.0561±0.0046 g (n=19) and 0.0801±0.0049 g (n=14) for *C. ehrhardti*; 0.0539±0.0057 g (n=18) and 0.0495±0.0050 g (n=15) for *M. microlepis;* 0.0835±0.0080 g (n=14) and 0.1083±0.0065 g (n=14) for *G. brasiliensis*; 0.5181±0.0463 g (n=16) for *D. auratus*. The muscle slice obtained was either immediately weighed and dried (60 °C for 24 h) for the determination of percentage of water content (*in vivo* experiments, Table 3), or transferred to the respective control saline (*in vitro* experiments), according to the species (Table 2).

2.7. In vitro muscle wet weight experiments

The composition of the control salines was determined according to previous reports from the literature (Robertson, 1960; Péqueux and Gilles, 1981; Tan and Choong, 1981; Barbe and Sevilla, 1987; Freire et al., 1995, 2003; Patrick et al., 1997; Prodocimo and Freire, 2001, 2006; Borges et al., 2004; Evans et al., 2005; Amado et al., 2006; Bolner and Baldisserotto, 2007; Prodocimo et al., 2007), or unpublished results from our laboratory (Foster, C., Amado, E.M., Souza, M.M., Freire, C.A., unpublished results). Glycine was added to prevent the impairment of volume regulation eventually dependent on free amino acid uptake from the extracellular fluid (e.g., Tan and Choong, 1981; Lang, 1987; Freire et al., 1995; Amado et al., 2006). Tissues from all freshwater species (and from the estuarine shrimp) were thus submitted to osmolality increases (i.e., hyper-osmotic, challenged with RVI), while tissues of the marine crab and the

Table 2
Composition of control (Cont) and experimental (Exp) salines (60% change in NaCl with respect to the respective control saline) used for the *in vitro* experiments

Species	NaCl (mM)		KCl (mM)		$MgCl_2$ (mM)		$CaCl_2$ (mM)		Measured Osmolality (mOsm/kg H_2O)	
	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp (% change)
Crustaceans										
FW and estuarine species: As, Mp, Ma, Pp	190	304	5	5	3	3	10	10	408	613 (50%)
Marine species: Hp	475	190	11	11	60	60	18	18	1119	585 (48%)
Fishes										
FW species: Ce, Mm, Gb	130	208	3	3	1	1	2	2	265	406 (53%)
Marine/estuarine species: Da	155	62	3	3	1	1	3	3	324	149 (54%)

Species used: the freshwater (FW) crustaceans *Aegla schmitti* (As), *Macrobrachium potiuna* (Mp), *Macrobrachium acanthurus* (Ma), the estuarine crustacean *Palaemon pandaliformis* (Pp), and the freshwater fishes *Corydoras ehrhardti* (Ce), *Mimagoniates microlepis* (Mm), *Geophagus brasiliensis* (Gb), and the marine/estuarine *Diapterus auratus* (Da).
Additional components, of constant concentration in all salines: glucose (5 mM), $NaHCO_3$ (2 mM), HEPES acid (5 mM), glycine (5 mM), pH 7.6.

marine/estuarine fish were submitted to osmolality decreases (i.e., hyposmotic, challenged with RVD) (Table 2, Figs. 1 and 2).

The wet weight change of the muscle slices under the anisosmotic conditions of the experimental saline (always ~50% osmolality change) was assayed in freshly dissected muscle slices from control specimens. The muscle slices were incubated for at least 1 h in the control saline. The muscle pieces were then carefully blotted on filter paper, weighed, and then individually immersed (small vials of 10 mL, under room temperature, 21–22 °C) either in control saline or in experimental saline (Table 2). The time course of the wet weight of the muscle pieces was followed for 2 h, with measurements every 15 min (Balance Bioprecisa FA2104 N, Brazil, precision 0.1 mg). The initial weight of the muscle piece (time zero) was used as reference (100%) for all subsequent values obtained for the same fragment. For the crab *H. pudibundus* and the fish *G. brasiliensis*, 2 muscle samples have been obtained from each single animal, one for the control saline, and another for the anisosmotic experimental saline (paired protocol). For all other species, each muscle slice used was obtained from a different individual.

2.8. Statistics

Data are presented in Table 3 and in Figs. 1 and 2 as mean± standard error of the mean. Unpaired Student's *t*-tests (or the Mann–Whitney *U*-tests, when the assumptions of data normality and/or equal variance were not met) were employed to

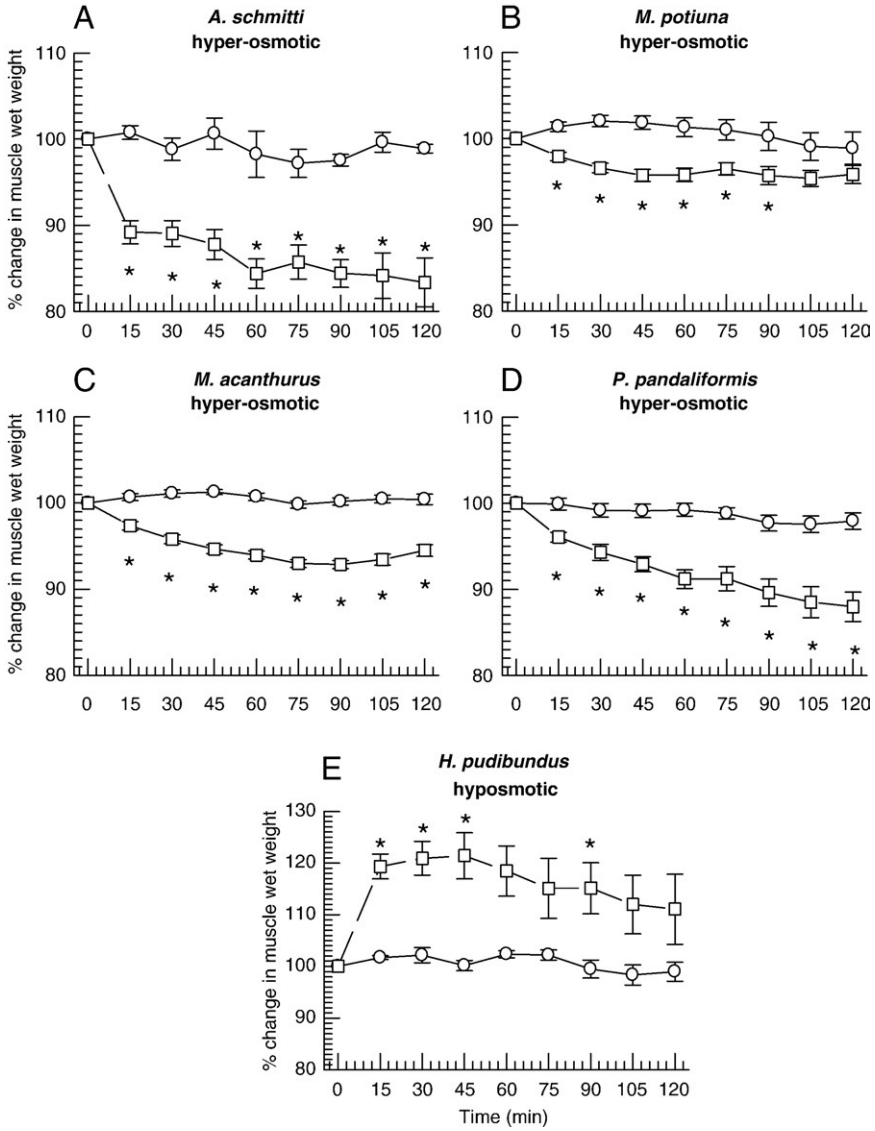

Fig. 1. Time course of muscle wet weight change (as % of initial weight) in freshwater (A, B, C), estuarine (D), and marine (E) crustaceans. Muscle slices were incubated *in vitro* in either control (-○-) or experimental (-□-) saline; * indicates significant differences ($P<0.05$) between values in experimental and control saline, for the same time of exposure (Student's *t*-test). The experimental saline was hyper-osmotic for the freshwater and estuarine species, and hyposmotic for the marine species. Differences detected along the experimental series through the ANOVA and the *post hoc* test of Tukey are described in the results text, and were not included in the graphs to avoid crowding the traces.

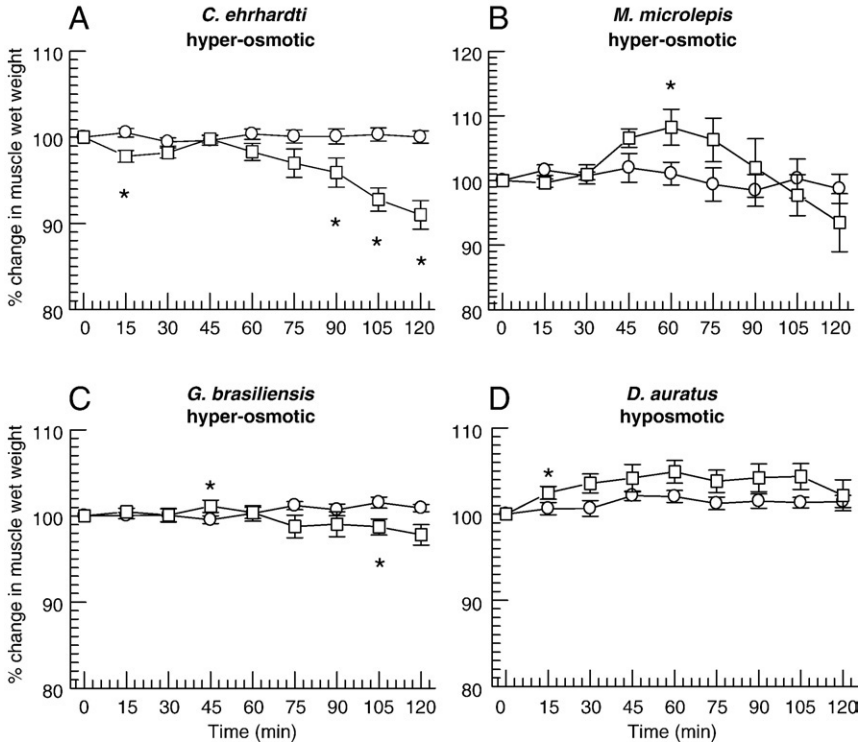

Fig. 2. Time course of muscle wet weight change (as % of initial weight) in freshwater (A, B, C), and marine/estuarine (D) fishes. Muscle slices were incubated *in vitro* in either control (-○-) or experimental (-□-) saline; * indicates significant differences ($P<0.05$) between values in experimental and control saline, for the same time of exposure (Student's *t*-test). The experimental saline was hyper-osmotic for the freshwater species, and hyposmotic for the marine/estuarine species. Differences detected along the experimental series through the ANOVA and the *post hoc* test of Tukey are described in the results text, and were not included in the graphs to avoid crowding the traces.

compare control and experimental data, both for the *in vivo* and for the *in vitro* experiments. For *H. pudibundus* and *G. brasiliensis in vitro* results, paired Student's *t*-tests were employed. With data from Figs. 1 and 2 (*in vitro* experiments), two-way repeated measures ANOVAs were performed to evaluate the effects of treatment (saline: control or experimental) and time (sampling times every 15 min, until 120 min) on muscle wet weight. The *post hoc* test of Tukey was performed to locate differences within the experimental time series, to allow a better characterization of the time course of water regulation by the muscle tissue when challenged with anisosmotic (experimental) medium. Level of significance was always set at 0.05.

3. Results

3.1. Crustaceans

3.1.1. Aegla schmitti

After 7 h in sea water of salinity 15‰ (*in vivo* experiment), specimens of the freshwater anomuran crab *A. schmitti* showed

Table 3
Results of the *in vivo* experiments

Species	ECF osmolality (mOsm/kg H_2O) (FW)	ECF osmolality (mOsm/kg H_2O) (15‰)	Percentage of water in muscle (FW)	Percentage of water in muscle (15‰)
Crustaceans				
Aegla schmitti (7 h)	453±6.3 (19)	512±20.7 * (5)	79.9±0.9 (6)	80.2±1.9 (6)
Fishes				
Corydoras ehrhardti (4:20 h)	259±1.5 (14)	465[§] (2)	81.8±0.8 (10)	74.8±0.4 * (9)
Mimagoniates microlepis (4:20 h)	258±13.5 (9)	439[§] (2)	78.9±0.3 (10)	73.7±0.2 * (8)
Geophagus brasiliensis (7 h)	313±7.3 (8)	348±7.8 * (5)	79.4±1.1 (6)	79.1±0.6 (8)

Osmolality of extracellular fluid and percentage of water in muscle of *Aegla schmitti*, *Corydoras ehrhardi*, *Mimagoniates microlepis*, and *Geophagus brasiliensis*, when in fresh water (FW, control, natural habitat), and after exposure (time indicated below the species name) to sea water of salinity 15‰. The number of measurements (*n*) appears below the mean±SEM, in parenthesis.

[§]Due to the very small size of the fishes, samples of blood or plasma had to be pooled, and the number of osmolality measurements was sometimes still very low ($n=2$), preventing the use of statistics.

* Mean in 15‰ is different from the mean in fresh water ($P<0.05$), Student's *t*-test.

no visible signs of distress, their hemolymph displayed a little increased osmolality (13% increase), and the percentage of water in the muscle remained unchanged (Table 3). Its muscle slices submitted to the hyper-osmotic saline (*in vitro* experiment) displayed progressive weight reduction already after 15 min of exposure, when compared to respective controls kept in control saline (Student's *t*-test, Fig. 1A). Exposure to the hyper-osmotic saline, the time of exposure, and their interaction were all significant (Two-way ANOVA). Differences were located only along the experimental time series: the value after 120 min was lower than values after 15 and 30 min (Fig. 1A).

3.1.2. Macrobrachium potiuna

The muscle slices of the hololimnetic freshwater palaemonid shrimp *M. potiuna* (control hemolymph osmolality 466± 16.6 mOsm/kgH_2O, $n=14$) submitted to the hyper-osmotic saline (*in vitro* experiment) displayed reduced weight from 15 to 90 min of exposure, when compared to the response of the muscle in control saline (Fig. 1B). Exposure to the hyper-osmotic saline, the time of exposure, and their interaction were all significant, but no individual differences were located by the post-hoc test.

3.1.3. Macrobrachium acanthurus

The muscle slices of the diadromous freshwater palaemonid shrimp *M. acanthurus* (control hemolymph osmolality 453± 4.6 mOsm/kgH_2O, $n=11$) submitted to the hyper-osmotic saline (*in vitro* experiment) displayed reduced weight from 15 min until the end of the experiment, when compared to the respective response of the muscle in control saline (Fig. 1C). Exposure to the hyper-osmotic saline, the time of exposure, and their interaction were all significant. Along the experimental series, there was a progressive weight loss that reached a maximum at 75–90 min, with a later increase noted after 120 min (Fig. 1C).

3.1.4. Palaemon pandaliformis

The muscle slices of the estuarine shrimp *P. pandaliformis* (control hemolymph osmolality 512±8.4 mOsm/kgH_2O, $n=8$) submitted to the hyper-osmotic saline (*in vitro* experiment) lost weight from 15 min until the end of the experiment, when compared to the respective response of the muscle in control saline (Fig. 1D). Exposure to the hyper-osmotic saline, the time of exposure, and their interaction were all significant. Along the experimental series, the *post hoc* test confirmed the progressive and basically linear weight loss of the muscle slices in the hyper-osmotic saline (Fig. 1D).

3.1.5. Hepatus pudibundus

The muscle slices of the marine calappoid flecked box crab *H. pudibundus* (control hemolymph osmolality 908± 1.9 mOsm/kgH_2O, $n=6$) submitted to the hyposmotic saline (*in vitro* experiment) displayed increased weight from 15 min until 45 min, and then again after 90 min, when compared to the respective response of the muscle in control saline (Fig. 1E). Exposure to the hyposmotic saline, the time of exposure, and their interaction were all significant. Along the experimental series, values after 30 and 45 min were higher than values after 105 and 120 min (Fig. 1E).

3.2. Fishes

3.2.1. Corydoras ehrhardti

After 4 h and 20 min in sea water of salinity 15‰ (*in vivo* experiment), the blood/plasma of the freshwater siluriform *C. ehrhardti* displayed increased osmolality (80% increase, no statistics possible), concomitant with decreased water content of the muscle (Table 3). Its muscle slices submitted to the hyper-osmotic saline (*in vitro* experiment) displayed weight reduction after 15 min of exposure, and again after 90 min to 120 min, when compared to respective controls kept in control saline (Student's *t*-test, Fig. 2A). Exposure to the hyper-osmotic saline, the time of exposure, and their interaction were all significant (Two-way ANOVA). Along the experimental time series, the last values, after 105 and 120 min, were lower than all the previous values; the value after 90 was lower than the value after 45 min (Fig. 2A).

3.2.2. Mimagoniates microlepis

After 4 h and 20 min in sea water of salinity 15‰ (*in vivo* experiment), the blood/plasma of the freshwater characiform *M. microlepis* displayed increased osmolality (70% increase, no statistics possible), and decreased water content of the muscle (Table 3). Its muscle slices submitted to the hyper-osmotic saline (*in vitro* experiment) surprisingly displayed weight increase after 60 min of exposure, when compared to respective controls kept in control saline (Fig. 2B). The time of exposure and its interaction with the treatment were significant. Along the experimental time series, the *post hoc* test confirmed that there was an increase in weight from 45–75 min, with a peak at 60 min, after which a progressive reduction ensued, until the end of the experiment (Fig. 2B).

3.2.3. Geophagus brasiliensis

After 7 h in sea water of salinity 15‰ (*in vivo* experiment), the perciform cichlid *G. brasiliensis* showed no visible signs of distress, its plasma displayed slightly increased osmolality (11% increase), and the percentage of water in the muscle remained unchanged (Table 3). Its muscle slices submitted to the hyper-osmotic saline (*in vitro* experiment) displayed increased weight after 45 min, and reduced weight after 105 min, when compared to respective controls kept in control saline (Fig. 2C). From the ANOVA result, only the interaction between treatment and time of exposure was significant.

3.2.4. Diapterus auratus

The muscle slices of the perciform gerreid *D. auratus* (control hemolymph osmolality 349±6.6 mOsm/kgH_2O, $n=9$) submitted to the hyposmotic saline (*in vitro* experiment) displayed increased weight only after 15 min, when compared to respective controls kept in control saline (Fig. 2D). From the ANOVA result, only the time of exposure was significant.

4. Discussion

4.1. In vitro method

The method for assaying the tissue water regulatory capacity as a tool to compare several species of crustaceans and fishes was a rather simple, inexpensive method, although reliable, and reproducible. As no isolated cells were analyzed, the time course response was "buffered" and macroscopic. Thus, a classic RVD or RVI response after a transient volume disturbance was not observed. Still, the averaged weight change of the muscle slice can be considered to represent major water movements to/from cells of the muscle tissue (Amado et al., 2006), as also previously inferred in whole animal studies (see Kirschner, 1991; Dunbar and Coates, 2004). Furthermore, exactly the same procedure (and performed by the same individual, using the same balance) was employed with the 9 species used here, conferring reliability and reproducibility to the results. It should be pointed out that the osmolality of the control saline of freshwater crustaceans (measured: 408 mOsm/kgH_2O) was not equal to the measured control osmolality of the freshwater (and estuarine) crustaceans used here, which ranged between 453 (*A. schmitti* and *M. acanthurus)* and 512 mOsm/kgH_2O (*P. pandaliformis*), and which could be responsible for some slight fluctuation noted in some control data (e.g., *M. potiuna* and *M. acanthurus*). The same happened for the marine crab *H. pudibundus*: 908 mOsm/kgH_2O measured in the hemolymph of the used animals, and 1119 mOsm/kgH_2O measured in the control saline employed. For the fishes the difference was negligible (see Tables 2 and 3, and the results text). Moreover, gases (pO_2 and pCO_2) were not measured or monitored along the time course of the experiment, and the build up of metabolic end products could interfere with IIR. Still, the follow up and direct comparison of the weight response of the muscle slices bathed by the control salines allows confidence in the response observed in the muscle slices exposed to the experimental salines. All muscle slices were pre-incubated in this same control saline during dissection until the start of the experiment. The focus of this study was entirely comparative, submitting the tissues of 9 species to basically the same anisosmotic stress; no absolute quantification of IIR capacity for these species was intended.

4.2. Crustaceans

The freshwater anomuran crab *A. schmitti* has shown itself to be relatively euryhaline, in that it survived and sustained hemolymph osmolality (increased only 13%) after 7 h of exposure to half-strength sea water (15‰). Upon this small increase in hemolymph osmolality, muscle tissue did not lose water *in vivo*. Consistently, *in vitro*, muscle tissue of this crab lost water upon a much steeper hyper-osmotic challenge, of ~50%, and did not recover its volume until the end of the experiment. *A. schmitti* belongs to a very ancient freshwater group, but one which did not reach high diversity and did not vastly spread in continental freshwater (Melo, 2003). Similar to what has been described and proposed for the trichodactylid brachyuran red crab *Dilocarcinus pagei* (Augusto et al., 2007b), these decapods with a long evolutionary history in fresh water are still relatively euryhaline (at least in the case of *D. pagei* and *A. schmitti*), but they have lost their capacity for IIR (Augusto et al., 2007b).

The shrimps examined here belong to the family Palaemonidae, which is still undergoing fresh water invasion (Freire et al., 2003; Augusto et al., 2007a,b). This family is extremely successful in estuarine and continental fresh waters in Brazil and neighbouring South American countries, showing high diversity and abundance (Moreira et al., 1983; Freire et al., 2003; Melo, 2003). Being thus much more recent in fresh water, although displaying hololimnetic habit, producing few large eggs, *Macrobrachium potiuna* possesses a high capacity for IIR, the strongest here among the 3 palaemonids examined: *M. potiuna* displayed some muscle weight reduction, but recovered in the end of the experiment; *M. acanthurus* has shown a similar reduction, which stabilized in the end of the experiment, and the estuarine *P. pandaliformis* displayed a linear progression of muscle weight loss.

It seems than reasonable to propose that a high capacity for cell volume regulation, or IIR, is a pre-requisite for the invasion of the freshwater biotope from the sea. *M. potiuna* probably still displays this capacity, given its relatively recent ancestry in fresh water. Having been described as the palaemonid shrimp with the highest capacity for IIR (within this study), this same species has also been described as the palaemonid with the highest capacity for AER, and also as being extremely euryhaline, in a previous multi-species study on palaemonids (Freire et al., 2003). The other *Macrobrachium* species, the larger *M. acanthurus*, is not as much freshwater-adapted as *M. potiuna*, given its diadromous habit, lengthy development of saline-water dependent larval stages, large number of small eggs, and adult habitat in coastal freshwater bodies (Moreira et al., 1983; Brailovsky and Galera, 1997). It would also be expected not to be as powerful a regulator (AER) as *M. potiuna*, similar to the other abundant coastal diadromous palaemonid, *M. olfersi* (McNamara, 1987; Freire et al., 2003).

The estuarine *P. pandaliformis* is necessarily very euryhaline, is a powerful osmoregulator (AER, see Freire et al., 2003), but surprisingly displays low capacity of IIR. This small shrimp, being an estuarine resident, deals very well with intermediate salinities (3–30‰), but not as well with extreme salinities (0 and 35‰) (Freire et al., 2003). Being so competent in AER, and displaying the structural machinery for salt transport in its branchial epithelia (McNamara, J.C. and Freire, C.A., unpublished results), it does not have the "need" for a high capacity of IIR, representing a lineage of palaemonids that shows no trend of upstream migration/colonization, thus diverting from the trend observed in the genus *Macrobrachium*. In fact, the genus *Palaemon* does not occur in fully freshwater biotopes, but is restricted to more saline brackish waters of estuaries and intertidal coastal habitats (Parry, 1957; Bond-Buckup and Buckup, 1989; Campbell and Jones, 1989; Dalla Via, 1989; Rasmussen and Andersen, 1996; Freire et al., 2003). Therefore, the pattern observed in the 3 species of palaemonids examined fits the hypothesis that this family of caridean shrimps is in the

process of fresh water invasion, thus being a more recent group in the freshwater biotope, when compared to the aeglids and trichodactylid crabs (Freire et al., 2003; Augusto et al., 2007a,b).

Data on muscle free amino acids reported for palaemonids (Augusto et al., 2007a), and the trichodactylid *D. pagei* (Augusto et al., 2007b) and several marine crustaceans (Burton and Feldman, 1982; Goolish and Burton, 1988; data compiled in Augusto et al., 2007b) are strikingly consistent with the results presented here. Being an extant descendent of a very old freshwater lineage, *D. pagei* has lost its capacity for strong IIR, a fact that reflects on very low levels of muscle free amino acids that can be mobilized for IIR (Augusto et al., 2007b). The more recent fresh water invaders, the palaemonids, display intermediate levels, between those low levels of *D. pagei*, and the high levels detected in marine crustaceans such as the lobsters, shrimps, and copepods (Augusto et al., 2007a, b). The anomuran crab *A. schmitti* presumably also displays low levels of organic effectors of IIR (free amino acids) in its muscle. It is important to add that the family Palaemonidae has many other species that occupy more continental freshwater bodies. One such species is *M. brasiliense*, shown to be rather stenohaline (Freire et al., 2003), which also probably lost the capacity for IIR. More thorough investigations could shed more light on why some old freshwater species of decapods remain so euryhaline and tolerant of salinity increase *in vivo*, but rather its cells and tissues lose the capacity for IIR. Could *M. brasiliense* be older in the freshwater biotope? Showing loss of IIR, concomitant with loss of euryhalinity? Would it be at the last stage of fresh water adaptation, further away than *D. pagei* and *A. schmitti*? In any case, different lineages may have different genetic background for natural selection to act upon, thus making different solutions possible (Kirschner, 1991; Freire et al., in press), and generalizations should be made cautiously.

The marine crab *Hepatus pudibundus* is a typical osmoconformer, unable to perform AER (Foster, C., Amado, E.M., Souza, M.M., Freire, C.A., unpublished results). The crab displayed limited capacity for IIR under the stress imposed here, muscle weight initially increased ~20%. Osmoconformers in fact depend more on a good capacity of IIR (Kirschner, 1991), if they are to be euryhaline; stenohaline osmoconformers end up dying when faced with a salinity challenge that overcomes their capacity of IIR. We should never forget that these terms are mostly relevant on a relative basis, it all depends on the degree and duration of the salinity stress imposed. Considering that approximately the same anisosmotic challenge was presented to the muscle slices of all species (~50%), the *H. pudibundus* muscle could recover from significant (20%) water entry, while that of the estuarine *P. pandaliformis* or that of the hololimnetic *A. schmitti*, while showing lower values of weight change, could not recover from water loss. However, the maximum weight change in *A. schmitti* and *P. pandaliformis* never reached 20%. The muscle of *H. pudibundus* was thus challenged for a RVD response, while the muscles of both *P. pandaliformis* and *A. schmitti* were challenged for a RVI response. It is interesting to point out here that RVD seems mechanistically universally easier than RVI (e.g., Kévers et al., 1979; Hoffmann and Dunham, 1995; Deaton, 1997; Freire and Prodocimo, 2007). However, in the present set of data, muscles from *M. potiuna* and *M. acanthurus* did very well upon a steep RVI challenge.

4.3. Fishes

Among the species of fishes examined, the siluriform *C. ehrhardti* could maintain its muscle weight stable until 75 min of exposure to the hyper-osmotic saline, then losing the capacity to hold water. The other Ostariophysi examined, the characiform *M. microlepis*, has shown impaired capacity to hold muscle water after 45 min of exposure to the same hyper-osmotic saline, with a surprising increase in muscle weight until 60 min, followed by a steady decrease. There must have been some solute uptake that led to water influx, possibly glycine. Despite this different *in vitro* response between the muscles from the two Ostariophysi species examined, they behaved exactly the same way in the *in vivo* experiment. They have shown signs of swimming impairment after the same time of exposure (~2 h) to increased salinity (15‰), were sampled at the same time (4:20 h), and have shown similar increases in plasma/blood osmolality (80% for *C. ehrhardti* and 70% for *M. microlepis*). This extracellular osmolality increase was actually higher than the *in vitro* hyper-osmotic challenge imposed (~50%) to the isolated muscle slices, and consistently led to reduced water content in their muscle tissue (*in vivo*), leading them to morbidity and death. Thus, the *in vivo* results were in agreement with the *in vitro* results (at least for *C. ehrhardti*), in that both species have shown limited capacity for muscle water homeostasis upon a steep hyper-osmotic challenge. These responses are entirely compatible with the long evolution of Ostariophysi fish in fresh water. Differently, Perciforms originated in sea water (Nelson, 2006), and even if *G. brasiliensis* is found and lives in coastal fresh waters, it displays marked euryhalinity, and the water content of its muscle was fully controlled both *in vivo,* with only 11% increase in plasma osmolality, and *in vitro* with the much steeper hyper-osmotic stress, of ~50%. Interestingly, cichlids are perciforms that returned to fresh water at a later stage (Chakrabarty, 2004; Sparks and Smith, 2004). They thus remained euryhaline and capable of IIR upon hyper-osmotic challenge; like the palaemonid shrimps, they can be considered as recent freshwater invaders. Other cichlids, the tilapias, are among the most studied freshwater teleosts, famous for their euryhalinity, including tolerance of salinities even higher than full-strength sea water (Sardella et al., 2004; Freire and Prodocimo, 2007). The isolated muscle of the other perciform studied, the euryhaline marine/estuarine gerreid *D. auratus*, also consistently displayed excellent capacity to maintain the water homeostasis upon hyposmotic challenge.

Although partially speculative, it is in general agreed that there is consistent evidence for a period of evolution (~250 million years) of the bony fish lineage in fresh water or diluted sea water before their radiation into groups that either remained in fresh water for a long time until now, such as the Ostariophysi, or other groups that later returned to the sea in different times (and eventually even returned secondarily to fresh water) (Griffith, 1985; Fyhn et al., 1999; Vize, 2004).

Low blood and intracellular osmolality (NaCl) (Kirschner, 1991; Fyhn et al., 1999), the presence of the filtering glomeruli efficient in the elimination of water (Griffith, 1985; Vize, 2004), the lower osmotic permeability shown by marine teleosts when compared to freshwater teleosts (Evans, 1969; Kirschner, 1991), the need for significant hydration of pelagic eggs in marine teleost fishes (>90% water) through the accumulation of free amino acids from vitellogenin degradation (Fyhn et al., 1999; Finn et al., 2000; Finn and Kristoffersen, 2007), and the fact that all primitive lineages of Actinopterygii spawn in freshwater, can all be listed as available evidence for this general freshwater ancestry of teleosts. An additional evidence could be the fact that the Ostariophysi, besides this very long time of evolution in fresh water, leading putatively to the loss of IIR capacity, as argued above, also had no fresh water to sea water migration in their evolutionary past, a fact which may genetically determine their lack of capacity to deal with increased salinity. In agreement, most migratory movements seen across estuaries nowadays are of marine teleosts heading upriver, rather than freshwater fish going down to the sea (Nordlie, 2003; Freire and Prodocimo, 2007).

4.4. IIR capacity needed for invasions, but later lost

Thus, it appears that IIR capacity is a pre-requisite for invading an osmotically different and thereby challenging environment, but is later lost, after a long time of evolution in that (stable) environment. In what concerns the invasion of fresh water by lineages of marine crustaceans, or marine teleosts, IIR capacity is observed in recent invaders such as freshwater palaemonids and cichlid fishes, but not in those species that are "old" in those invaded biotopes, such as the freshwater anomuran crab *A. schmitti*, or Ostariophysian fishes. These extant teleost fish species, speculatively descendent from the original lineages that evolved only in freshwater since the origins of bony fish (Kirschner, 1991; Fyhn et al., 1999; Finn et al., 2000; Vize, 2004; Finn and Kristoffersen, 2007; Freire and Prodocimo, 2007), they consistently lost the capacity for IIR, at least this was the response of the species studied here.

A high capacity for IIR was needed in lineages of marine crustaceans that invaded fresh water, being found in the recent invaders *Macrobrachium potiuna* and *M. acanthurus*, and also in teleost lineages that returned to the sea after presumably evolving and osmoregulating in fresh water, such as the Perciformes, here represented by the euryhaline teleosts, the cichlid *Geophagus brasiliensis* and the gerreid *Diapterus auratus*. Actually, the cichlids display a more recent secondary migration, return to fresh water from sea water, thus consistently showing a high degree of euryhalinity and capacity for IIR. Obviously, a clearer picture could emerge from the study of additional perciforms, and an interesting way to widen the conclusions drawn here would be to investigate marine stenohaline fish, which could presumably exhibit low IIR capacity. In accordance with this proposal, extant groups of marine crustaceans, unrelated to lineages that invaded fresh water, show less IIR capacity, being osmoconformers and stenohaline, after a long time of evolution in stable sea water, as typical for marine invertebrates, and as here found for the marine crab *Hepatus pudibundus*. However, *H. pudibundus* has shown a trend for recovery, and additional osmoconformer crabs should be studied for a better evaluation. Actually, the degree of steno- and euryhalinity in marine osmoconformer invertebrates is quite variable (Kirschner, 1991) and relative upon the stress imposed; those that are more euryhaline, display higher capacity for IIR.

Teleost fish always show high capacity for AER, and have been here shown to display higher capacity for IIR as well, in agreement with the higher complexity of vertebrates and thus need for tighter homeostatic mechanisms. This high AER capacity is taken as part of the evidence for the long teleost ancestrality in diluted sea water or fresh water, as discussed above. A similar pattern could be detected between crustaceans and fishes, so that extant groups of strictly freshwater fishes such as lineages within the Ostariophysi, which likely evolved essentially in fresh water, show poor IIR capacity, being stenohaline freshwater species. In summary, the results gathered here seem to indicate that the degree of euryhalinity of a species is not only proportional to the osmotic stability of its current habitat, but is mainly a function of its time of evolution in that habitat. IIR capacity is apparently a significant part of the reason. It thus appears that the capacity for IIR limits (or extends) euryhalinity (salinity tolerance range) away from the limits set by the homeostatic AER mechanisms.

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References

Amado, E.M., Freire, C.A., Souza, M.M., 2006. Osmoregulation and tissue water regulation in the freshwater red crab *Dilocarcinus pagei* (Crustacea, Decapoda), and the effect of waterborne inorganic lead. Aquat. Toxicol. 79, 1–8.

Augusto, A., Greene, L.J., Laure, H.J., McNamara, J.C., 2007a. The ontogeny of isosmotic intracellular regulation in the diadromous, freshwater palaemonid shrimps, *Macrobrachium amazonicum* and *M. olfersi* (Decapoda). J. Crustac. Biol. 27, 626–634.

Augusto, A., Greene, L.J., Laure, H.J., McNamara, J.C., 2007b. Adaptive shifts in osmoregulatory strategy and the invasion of freshwater by Brachyuran crabs: evidence from *Dilocarcinus pagei* (Trichodactylidae). J. Exp. Zool. 307A, 688–698.

Barbe, L., Sevilla, C., 1987. Evolution des acides amines libres seriques. Biochem. Syst. Ecol. 15, 133–137.

Bolner, K.C.S., Baldisserotto, B., 2007. Water pH and urinary excretion in silver catfish *Rhamdia quelen*. J. Fish Biol. 70, 50–64.

Bond-Buckup, G., Buckup, L., 1989. Os palaemonidae de águas continentais do Brasil meridional (Crustacea, Decapoda). Rev. Bras. Biol. 49, 883–896.

Borges, A., Scotti, L.V., Siqueira, D.R., Jurinitz, D.F., Wassermann, G.F., 2004. Hematologic and serum biochemical values for jundiá (*Rhamdia quelen*). Fish Physiol. Biochem. 30, 21–25.

Brailovsky, G.S.P., Galera, E.S., 1997. Comportamento osmorregulador de *Macrobrachium tenellum* y *Macrobrachium acanthurus* (Decapoda: Palaemonidae) em diferentes salinidades. Rev. Biol. Trop. 45, 1085–1091.

Bruton, M.N., 1996. Alternative life-history strategies of catfishes. Aquat. Living Resour. 9, 35–41.

Burton, R.S., Feldman, M.W., 1982. Changes in free amino acid concentrations during osmotic response in the intertidal copepod *Tigriopus californicus*. Comp. Biochem. Physiol., A 73, 441–445.

Campbell, P.J., Jones, M.B., 1989. Osmoregulation of the estuarine prawn *Palaemon longirostris* (Caridea: Palaemonidae). 69, 261−272.

Castillo-Rivera, M., Montiel, M., Sanvicente Añorve, L., Zárate, R., 2005. Spatial, seasonal and diel distribution patterns of two species of mojarras (Pisces: Gerreidae) in a Mexican tropical coastal lagoon. J. Appl. Ichthyol. 21, 498–503.

Castro-Souza, T., Bond-Buckup, G., 2004. O nicho trófico de duas espécies simpátricas de *Aegla* Leach (Crustacea, Aeglidae) no tributário da bacia hidrográfica do Rio Pelotas, Rio Grande do Sul, Brasil. Rev. Bras. Zool. 21, 805–813.

Chakrabarty, P., 2004. Cichlid biogeography: comment and review. Fish Fish. 5, 97–119.

Chamberlin, M.E., Strange, K., 1989. Anisosmotic cell volume regulation: a comparative review. Am. J. Physiol. 257, C159–C173.

Dalla Via, G., 1989. The effect of salinity on free amino acids in the prawn *Palaemon elegans* (Rathke). Arch. Hydrobiol. 115, 125–135.

Deaton, L.E., 1997. Comparative aspects of cellular volume regulation in cardiomyocytes. Physiol. Zool. 70, 379–390.

Deckert, G.D., Greenfield, D.W., 1987. A review of the Western Atlantic species of the genera *Diapterus* and *Eugerres* (Pisces: Gerreidae). Copeia 1, 182–194.

Dunbar, S.G., Coates, M., 2004. Differential tolerance of body fluid dilution in two species of tropical hermit crabs: not due to osmotic/ionic regulation. Comp. Biochem. Physiol. A 137, 321–337.

Evans, D.H., 1969. Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. J. Exp. Biol. 50, 689–703.

Evans, D.H., 1993. The Physiology of Fishes. CRC Press, Boca Raton, USA.

Evans, D., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.

Ferreira, B.D.P., Hack, C., Oliveira, G.T., Bond-Buckup, G., 2005. Perfil metabólico de *Aegla platensis* Schmitt (Crustacea, Aeglidae, Anomura) submetidas a dietas ricas em carboidratos ou proteínas. Rev. Bras. Zool. 22, 161–168.

Finn, R.N., Kristoffersen, B.A., 2007. Vertebrate vitellogenin gene duplication in relation to the ''3R Hypothesis'': correlation to the pelagic egg and the oceanic radiation of teleosts. PLoS ONE 2 (1), e169. doi:10.1371/journal.pone.0000169.

Finn, R.N., Fyhn, H.J., Norberg, B., Munholland, J., Reith, M., 2000. Oocyte hydration as a key feature in the adaptive evolution of teleost fishes to seawater. In: Norberg, B., Kjesbu, O.S., Taranger, G.L., Anderson, E., Stefansson, S.O. (Eds.), Proc. 6th Int. Symp. Reprod. Physiol. Fish Inst Mar Res & Univ Bergen, pp. 289–291.

Florkin, M., 1962. La régulation isosmotique intracellulaire chez lês invertébratés marins euryhalins. Bull. Acad. Roy. Belg. Cl. Sci. 48, 687–694.

Fracasso, H.A.A., Branco, J.O., 2005. Estrutura populacional de *Hepatus pudibundus* (Herbst) (Crustacea, Decapoda) na Armação do Itapocoroy, Penha, Santa Catarina, Brasil. Rev. Bras. Zool. 22, 342–348.

Freire, C.A., Prodocimo, V., 2007. Special challenges to teleost fish osmoregulation in environmentally extreme or unstable habitats. In: Baldisserotto, B., Mancera, J.M., Kapoor, B.G. (Eds.), Fish Osmoregulation, vol. 1. Science Publishers, Enfield, pp. 249–276.

Freire, C.A., McNamara, J.C., Rosa, J.C., Greene, L.J., 1995. Neuroendocrine control of osmotic regulation in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann) (Crustacea, Decapoda): free amino acid concentration in the hemolymph. Gen. Comp. Endocrinol. 100, 83–91.

Freire, C.A., Cavassin, F., Rodrigues, E.N., Torres, A.H., McNamara, J.C., 2003. Adaptive patterns of osmotic and ionic regulation, and the invasion of fresh water by the palaemonid shrimps. Comp. Biochem. Physiol. A 136, 771–778.

Freire, C.A., Onken, H., McNamara, J.C., in press. A structure-function analysis of ion transport in crustacean gills and excretory organs. Comp. Biochem. Physiol. A. doi:10.1016/j.cbpa.2007.05.008.

Froese, R., Pauly, D. 2007. FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2007)

Fyhn, H.J., Finn, R.N., Reith, M., Norberg, B., 1999. Yolk protein hydrolysis and oocyte free amino acids as key features in the adaptive evolution of teleost fishes to seawater. Sarsia 84, 451–456.

Gilmore Jr., R.G., Greenfield, D.W., 2002. Gerreidae. In: Carpenter, K.E. (Ed.), The Living Marine Resources of the Western Central Atlantic, vol. 3. Bony fishes part 2 (Opistognathidae to Molidae), sea turtles and marine mammals. FAO, Rome, pp. 1506–1513.

Gonçalves, R.S., Castiglioni, D.S., Bond-Buckup, G., 2006. Ecologia populacional de *Aegla franciscana* (Crustacea, Decapoda, Anomura) em São Francisco de Paula, RS, Brasil. Iheringia, Sér. Zool., Porto Alegre 96, 109–114.

Goolish, E.M., Burton, R.S., 1988. Exposure to fluctuating salinity enhances free amino acid accumulation in *Tigriopus californicus* (Copepoda). J. Comp. Physiol. 158, 99–105.

Griffith, R.W., 1985. Habitat, phylogeny and the evolution of osmoregulatory strategies in primitive fishes. In: Foreman, R.E., Gorbman, A., Dodd, J.M., Olsson, R. (Eds.), Evolutionary Biology of Primitive Fishes. Plenum Press, New York, pp. 69–80.

Hebling, N.J., Rieger, P.J., 2003. Desenvolvimento juvenil de *Hepatus pudibundus* (Herbst) (Crustacea, Decapoda, Calappidae) em laboratório. Rev. Bras. Zool. 20, 531–539.

Hoffmann, E.K., Dunham, P.B., 1995. Membrane mechanisms and intracellular signalling in cell volume regulation. In: Kwang, J.W. (Ed.), International Review of Cytology, vol. 161, pp. 172–262.

Jobling, M., 1995. Environmental Biology of Fishes. Chapman & Hall, London.

Kévers, C., Péqueux, A., Gilles, R., 1979. Effects of hypo- and hyperosmotic shocks on the volume and ion content of *Carcinus maenas* isolated axons. Comp. Biochem. Physiol. A 64, 427–431.

Kirschner, L.B., 1991. Water and ions. In: Prosser, C.L. (Ed.), Environmental and Metabolic Animal Physiology. Comparative Animal Physiology. Wiley-Liss, New York, pp. 13–107.

Lang, M.A., 1987. Correlation between osmoregulation and cell volume regulation. Am. J. Physiol. 252, R768–R773.

Lowe-McConnell, R.H., 1999. Estudos ecológicos de comunidades de peixes tropicais. Edusp, São Paulo.

Mazzoni, R., Iglesias-Rios, R., 2002. Environmentally related life history variations in *Geophagus brasiliensis*. J. Fish Biol. 61, 1606–1618.

McNamara, J.C., 1987. The time course of osmotic regulation in the freshwater shrimp *Macrobrachium olfersii* (Wiegmann) (Decapoda, Palaemonidae). J. Exp. Mar. Biol. Ecol. 107, 245–251.

Melo, G.A.S., 1996. Manual de identificação dos Brachyura (caranguejos e siris) do litoral brasileiro. Plêiade/FAPESP, São Paulo.

Melo, G.A.S., 2003. Manual de identificação dos Crustacea Decapoda de água doce do Brasil. Edições Loyola, São Paulo.

Moreira, G.S., McNamara, J.C., Shumway, S.E., Moreira, P.S., 1983. Osmoregulation and respiratory metabolism in Brazilian *Macrobrachium* (Decapoda, Palaemonidae). Comp. Biochem. Physiol. A 74, 57–62.

Nelson, J.S., 2006. Fishes of the World, 4th ed. Wiley, New York.

Nordlie, F.G., 2003. Fish communities of estuarine salt marshes of eastern North America, and comparisons with temperate estuaries of other continents. Rev. Fish Biol. Fish. 13, 281–325.

Parry, G., 1957. Osmoregulation in some freshwater prawns. J. Exp. Biol. 34, 417–423.

Patrick, M.L., Pärt, P., Marshall, W.S., Wood, C.M., 1997. Characterization of ion and acid–base transport in the fresh water adapted mummichog (*Fundulus heteroclitus*). J. Exp. Zool. 279, 208–219.

Péqueux, A., 1995. Osmotic regulation in crustaceans. J. Crustac. Biol. 5, 1–60.

Péqueux, A., Gilles, R., 1981. Na^+ fluxes across isolated perfused gills of the Chinese crab *Eriocheir sinensis*. J. Exp. Biol. 92, 173–186.

Prodocimo, V., Freire, C.A., 2001. Ionic regulation in aglomerular tropical estuarine pufferfish submitted to sea water dilution. J. Exp. Mar. Biol. Ecol. 262, 243–253.

Prodocimo, V., Freire, C.A., 2006. The Na^+,K^+,$2Cl^-$ cotransporter of estuarine pufferfishes (*Sphoeroides testudineus* and *S. greeleyi*) in hypo- and hyper-regulation of plasma osmolality. Comp. Biochem. Physiol. 142, 347–355.

Prodocimo, V., Galvez, F., Freire, C.A., Wood, C.M., 2007. Unidirectional Na^+ and Ca^{2+} fluxes in two euryhaline teleost fishes, *Fundulus heteroclitus* and

Oncorhynchus mykiss, acutely submitted to a progressive salinity increase. J. Comp. Physiol. 177, 519–528.

Rasmussen, A.D., Andersen, O., 1996. Apparent water permeability as a physiological parameter in crustaceans. J. Exp. Biol. 199, 2555–2564.

Robertson, J.D., 1960. Osmotic and ionic regulation. The Physiology of Crustacea. Metabolism and Growth, vol. 1. Academic Press, New York, pp. 319–339.

Ruppert, E.E., Barnes, R.D., 1994. Invertebrate Zoology, 6th ed. Saunders College Publishing, Orlando.

Russell, J.M., 2000. Sodium–potassium–chloride cotransport. Physiol. Rev. 80, 211–276.

Saitoh, K., Miya, M., Inoue, J.G., Ishiguro, N.B., Nishida, M.B., 2003. Mitochondrial genomics of Ostariophysan fishes: perspectives on phylogeny and biogeography. J. Mol. Evol. 56, 464–472.

Sardella, B.A., Cooper, J., Gonzalez, R.J., Brauner, C.J., 2004. The effect of temperature on juvenile Mozambique tilapia hybrids (*Oreochromis mossambicus* x *O. urolepis hornorum*) exposed to full-strength and hypersaline seawater. Comp. Biochem. Physiol. A 137, 621–629.

Sparks, J.S., Smith, W.L., 2004. Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). Cladistics 20, 501–517.

Subramanian, A., 1975. Sodium and water permeabilities in selected Crustacea. Physiol. Zool. 48, 398–403.

Tan, C.H., Choong, K.Y., 1981. Effect of hyperosmotic stress on hemolymph protein, muscle ninhydrin-positive substances and free aminoacids in (de man). Comp. Biochem. Physiol. 70, 485–489.

Teixeira, R.L., Sá, H.S., 1998. Abundância de macrocrustáceos decápodas nas áreas rasas do Complexo Lagunar Mundaú/Manguaba. Rev. Bras. Biol. 58, 393–404.

Vazzoler, A.E.A.M., Menezes, N.A., 1992. Síntese de conhecimento sobre o comportamento reprodutivo dos Characiformes da América do Sul (Teleostei: Characiformes). Rev. Bras. Biol. 52, 627–640.

Vize, P.D., 2004. A Homeric view of kidney evolution: A reprint of H.W. Smith´s classic essay with a new introduction. Anat. Rec. 277, 344–354.

Wehner, F., Olsen, H., Tinel, H., Kinne-Saffran, E., Kinne, R.K.H., 2003. Cell volume regulation: osmolytes, osmolyte transport, and signal transduction. Rev. Physiol., Biochem. Pharmacol. 148, 1–80.

Weitzman, S.H., 2003. Glandulocaudinae (Characins, tetras). In: Reis, R.E., Kullander, S.O., Ferraris Jr., C.J. (Eds.), Checklist of the Freshwater Fishes of South and Central America. EDIPUCRS, Porto Alegre, Brazil, pp. 222–230.