Relative mitochondrial area in branchial and renal epithelia of the tropical estuarine pufferfish *Sphoeroides testudineus* after acclimation to isosmotic seawater

Área mitocondrial relativa nos epitélios branquial e renal do baiacu estuarino tropical *Sphoeroides testudineus* após aclimatação à água do mar isosmótica

VIVIANE PRODOCIMO
& CAROLINA A. FREIRE

The gills of teleost fishes are responsible both for gas exchange in respiration and salt transport in osmoregulation (JOBLING, 1995; ZADUNAISKY, 1996; PERRY, 1997; VAN DER HEIJDEN *et al.*, 1997; EVANS *et al.*, 1999; EVANS *et al*., 2005). In marine teleosts the gill epithelium secretes salt mainly through chloride cells (JOBLING, 1995; ZADUNAISKY, 1996; PERRY, 1997; VAN DER HEIJDEN *et al.*, 1997; FERNANDES et *al.*, 1998; EVANS *et al.*, 2005). These cells are typically located between secondary lamellae at their insertion in the gill filament (inter-lamellar region) or in the gill filament itself (LAURENT & DUNEL, 1980; PERRY, 1997; FERNANDES *et al.*, 1998; EVANS *et al*., 2005). These cells are rounded, display abundant mitochondria, a tubular system of endomembranes, sub-apical vesicles, and extensive intercellular junctional complexes (LAURENT & DUNEL, 1980; JOBLING, 1995; ZADUNAISKY, 1996; PERRY, 1997; EVANS *et al.*, 2005).

Departamento de Fisiologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, Centro Politécnico, Curitiba, Paraná, CEP 81531-990, Brazil. CORRESPONDENCE: Dr. Carolina A. Freire, Departamento de Fisiologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, Centro Politécnico, Curitiba, Paraná, 81531-990, Brazil. Phone: +55-41-3361-1712, Fax: +55-41-3361-1714. E-mail: cafreire@ufpr.br

There are plenty of data on gill ultrastructural changes as related to the salinity of acclimation, but most studies compare teleosts in seawater (hypo-regulating) with fish in fresh water or very diluted seawater (hyper-regulating). The general pattern is of a higher density and size of chloride cells in gills of fishes acclimated to seawater (PISAM *et al.*, 1990; VAN DER HEIJDEN *et al*., 1997; KELLY & WOO, 1999; CARMONA, *et al.*, 2004). In marine species (*Scophthalmus maximus* and *Sparus sarba*) submitted to salinity reduction, an increase in the apical projections and a reduction in the system of endomembranes of chloride cells have also been detected (PISAM *et al.*, 1990; KELLY & WOO, 1999). Coherently, in conditions close to isosmoticity, the number and size of chloride cells in *Sparus auratus* were lower than when the fish were acclimated to very low or very high salinities (LAIZ-CARRIÓN *et al.*, 2005a). The paucity of analyses focusing on isosmotic conditions has prompted us to investigate the ultrastructure of the gills of the estuarine pufferfish *S. testudineus*. Furthermore, it is rather common that euryhaline fish display minimum rates of oxygen consumption and branchial Na,K-ATPase activities under isosmotic (brackish or intermediary salinities) conditions ("U-shaped" curves) (WOO & FUNG, 1981; KELLY *et al*., 1999; IMSLAND *et al*., 2003; LAIZ-CARRIÓN *et al*., 2005b). Thus, it would be interesting to examine whether this state of lower metabolism is reflected into a reduced area of mitochondria in chloride cells.

The kidney cooperates with the gills for osmoregulatory homeostasis. Most marine teleosts have simple renal tubule morphology, frequently with less numerous glomeruli as compared to freshwater species, to the extent that some species are entirely aglomerular (HICKMAN & TRUMP, 1969; HENTSCHEL & ELGER, 1989; EVANS, 1993; JOBLING, 1995; BEYENBACH, 2004), a characteristic previously reported for the Tetraodontidae family (HICKMAN AND TRUMP, 1969), but not confirmed for *S. testudineus*. The kidney of *S. testudineus* has a large cranial portion with glomeruli and tubules surrounded by haematopoietic tissue, and a very thin cauldal portion with a single large convoluted collecting duct (mesonephric duct) (PRODOCIMO & FREIRE, 2003).

Sphoeroides testudineus is a marine/estuarine pufferfish frequently found in low salinity areas in Paranaguá Bay estuarine

complex, where salinity can reach 0‰ at low tide, being a truly resident estuarine species (VENDEL *et al*., 2002). In the laboratory as well as in the field it was shown to be extremely euryhaline, regulating blood osmolality and ions as well as hematocrit and muscle water content, even after 5-15 days in diluted seawater (5‰), when compared to respective values in full-strength seawater (PRODOCIMO & FREIRE, 2001; 2004; 2006). *S. testudineus* is most probably approximately isosmotic at salinity 10‰, as it was demonstrated to be iso-ionic for Na^+ (133 mM) and Cl^- (115 mM) (PRODOCIMO & FREIRE, 2001).

This study aimed at investigating the effect of long-term (15 days) seawater dilution down to 10‰ on the structure of branchial and mesonephric duct epithelia of this species, focusing on the area occupied by the energy-supplying organelles, the mitochondria. Although this is not a physiological situation for this estuarine species which inhabits a dynamic environment subject to wide salinity fluctuations within hours, the long-term acclimation to seawater dilution can reveal morphological adaptations to reduced metabolism and decreased salt extrusion rates.

MATERIALS AND METHODS

Specimens of *Sphoeroides testudineus* (average 15 cm of body length and 40 g of body mass) were obtained from Baguaçu River tidal creek (25° 33' 6,33''S, 48° 23' 41,63''W), located in the south margin of Paranaguá Bay, state of Paraná, southern Brazil. The salinity at the collection site varies with the tidal cycle, ranging between 32‰ and 0‰ (VENDEL *et al*, 2002).

The caught fishes were transported to the laboratory in 30-liter plastic gallons, under constant aeration and temperature between 21-24 °C. In the laboratory they were maintained in 250-liter stock tank with seawater from the collection site and salinity adjusted to 30‰, temperature between 19-22 °C, and constant aeration. The salinity of acclimation was chosen as 30‰ because this is a marine/estuarine species, and the fish were caught during high tide. The fish were fed daily with shrimps and earthworms, and acclimation experiments were conducted under natural light regime.

After approximately 7 days of acclimation to laboratory conditions, 5 specimens of *S. testudineus* were transferred to 30-

liter aquaria containing seawater of salinity 30‰ (control), and 5 specimens were transferred to seawater of salinity 10‰ (approximately isosmotic dilute seawater), under constant aeration. After 15 days in seawater of salinity 30‰ (410 mM Na^+, 480 mM Cl^-), Na^+ and Cl^- measured in the plasma of the pufferfish were of respectively 150 and 120 mM, thus markedly hypo-ionic and necessarily hyposmotic to the water (PRODOCIMO & FREIRE, 2001). Seawater was diluted down to 10‰ with addition of filtered dechlorinated tap water overnight, to avoid osmotic shock to the animals. The 15-day period was counted after salinity had reached 10‰. After 15 days in either 30‰ or 10‰ seawater, fish were anesthesized using MS-222 0.04% dissolved in 1 liter of aquarium water. After complete anesthesia, they were dissected to have the fragments of gills and kidneys removed.

TRANSMISSION ELECTRON MICROSCOPY OF GILLS AND KIDNEYS OF *SPHOEROIDES TESTUDINEUS* — After fish were anesthesized, they were opened by ventral incision, had internal organs removed, and the thin kidney could be observed. The posterior, trunk kidney containing the mesonephric duct (collecting duct) (PRODOCIMO & FREIRE, 2003) was dissected out, as well as the second gill arch of the right side of the animal, from which a filament of the central portion of the arch was removed.

The medial portion of a gill filament and a fragment of the posterior trunk kidney were then fixed by immersion in primary fixative solution containing 2.5% glutaraldehyde and 200 mM paraformaldehyde in 100 mM sodium cacodylate buffer, containing additionally 100 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, and 3 mM $CaCl_2$, in proportions adequate for the species (Prodocimo & Freire, 2001), on ice for 2 hours. The tissue fragments were washed in cacodylate buffer, and post-fixed in 1% osmium tetroxide, on ice for 1.5 h. The fragments were then dehydrated in an ethanol series, transferred to propylene oxide, and embedded in Araldite 502 resin (Polysciences, Warrington). Thin (50 nm) sections were contrasted with 5% uranile acetate and lead citrate (REYNOLDS, 1963), and observed at the transmission electron microscope JEOL JEM 1200 EXII of the Electron Microscopy Center of the Federal University of Paraná.

MORPHOMETRIC ANALYSIS OF BRANCHIAL AND RENAL EPITHELIA OF *SPHOEROIDES TESTUDINEUS* — Electron micrographs were prepared of

the interlamelar region of the branchial filaments, where chloride cells are located, as well as of the collecting duct epithelium rich in mitochondria and membrane infoldings of fishes acclimated for 15 days to either 30‰ (control) or 10‰ (isosmotic). A single grid has been examined with the renal or branchial sections of each fish, in control or isosmotic seawater. One single, representative micrograph of a well preserved region of the chosen epithelia has been enlarged, for each grid. The morphometric parameters analysed in these tissues were: the area occupied by mitochondria and by the endomembrane tubular system in the branchial chloride cells (final enlargement ~27,000x); and area occupied by mitochondria in the mesonephric duct epithelial cells (final enlargement ~11,000x). All images were scanned, and analysed in Sigma Scan™ for Windows™, from Jandel Scientific Software.

STATISTICAL ANALYSIS — Statistical analysis of the data was performed using SigmaStat® for Windows™ version 2.03, always with significance limit set at 0.05. Student's t-tests were conducted to compare morphometric data between control (30‰) and experimental groups (10‰).

RESULTS

ULTRASTRUCTURE OF THE BRANCHIAL EPITHELIUM OF *SPHOEROIDES TESTUDINEUS* — After 15 days in 30‰ seawater (control condition), chloride cells were observed in the primary lamellae (gill filaments) in the interlamellar region (between secondary, respiratory lamellae). These cells displayed mitochondria spread all over their cytoplasm, surrounded by a well developed endomembrane tubular system (Figs 1A, 1B). Accessory cells could be observed (Fig. 1C). No obvious alterations upon acclimation to isosmotic seawater could be detected in the number, arrangement, or general characteristics of chloride cells. Additionally, no change was noted in the aspect of mitochondria, the tubular system, or the apical projections of chloride cells (Figs 1C, 1D), upon careful observation of all micrographs. The area occupied by mitochondria was unchanged in fishes acclimated to isosmotic seawater (Fig. 1D) (P=0.867), when compared to controls (Fig. 1B). The area occupied by mitochondria was (mean ± SEM) 10.6 ± 2.9 μm^2 (n=4) in 30‰, and 11.2 ± 1.5 μm^2 (n=4) in 10‰. Likewise, no difference was detected in the area occupied by the tubular system (P=0.855). The area occupied by

the tubular system was of 18.9 4.9 μm² (n=4) in 30‰, and 17.7 ± 4.1 μm² (n=4) in 10‰.

ULTRASTRUCTURE OF THE MESONEPHRIC DUCT EPITHELIUM OF *SPHOEROIDES TESTUDINEUS* — The mesonephric duct epithelium of *S.*

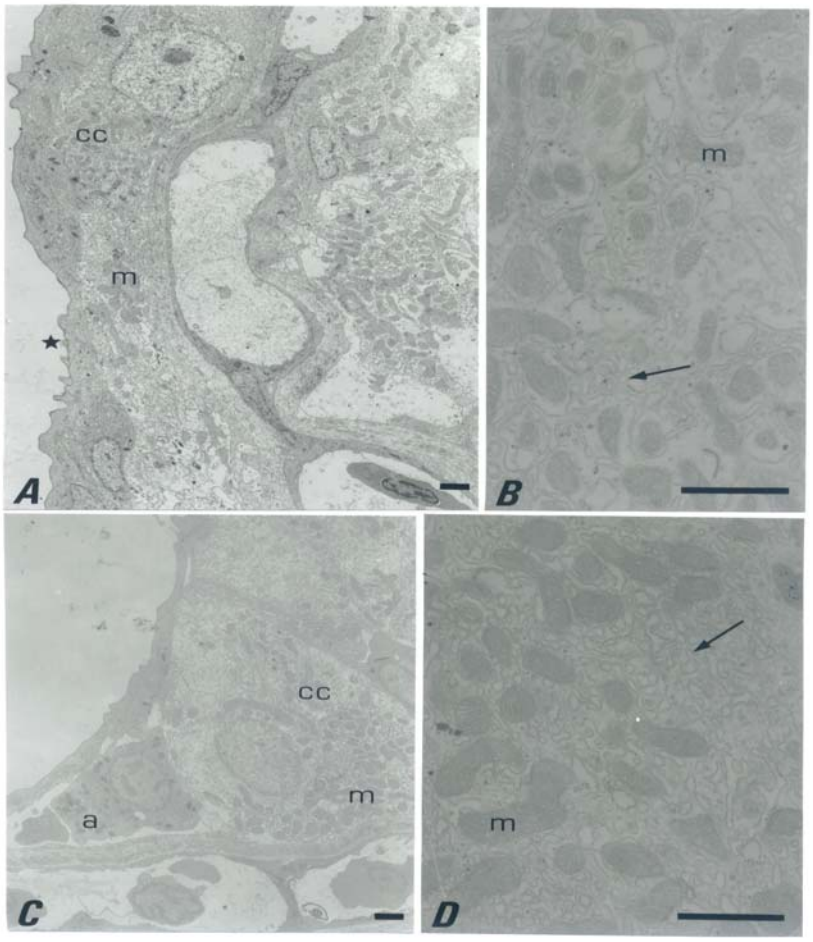

Fig. 1. Electron micrographs of the branchial epithelium of *S. testudineus*. 1A, primary lamella showing chloride cells (cc) with mitochondria (m) in 30‰ seawater. The star indicates the microridges of pavement cells scale bar: 1 μm; 1B, higher magnification of chloride cell cytoplasm with mitochondria (m) and tubular system of endomembranes (arrow) in 30‰ seawater, scale bar: 1 μm; 1C, primary lamella showing chloride cells (cc) with mitochondria (m), accessory cell with electron dense cytoplasm (a) in 10‰ seawater, scale bar: 1 μm; 1D, higher magnification of chloride cell cytoplasm with mitochondria (m) and tubular system of endomembranes (arrow) in 10‰ seawater, scale bar: 1 μm.

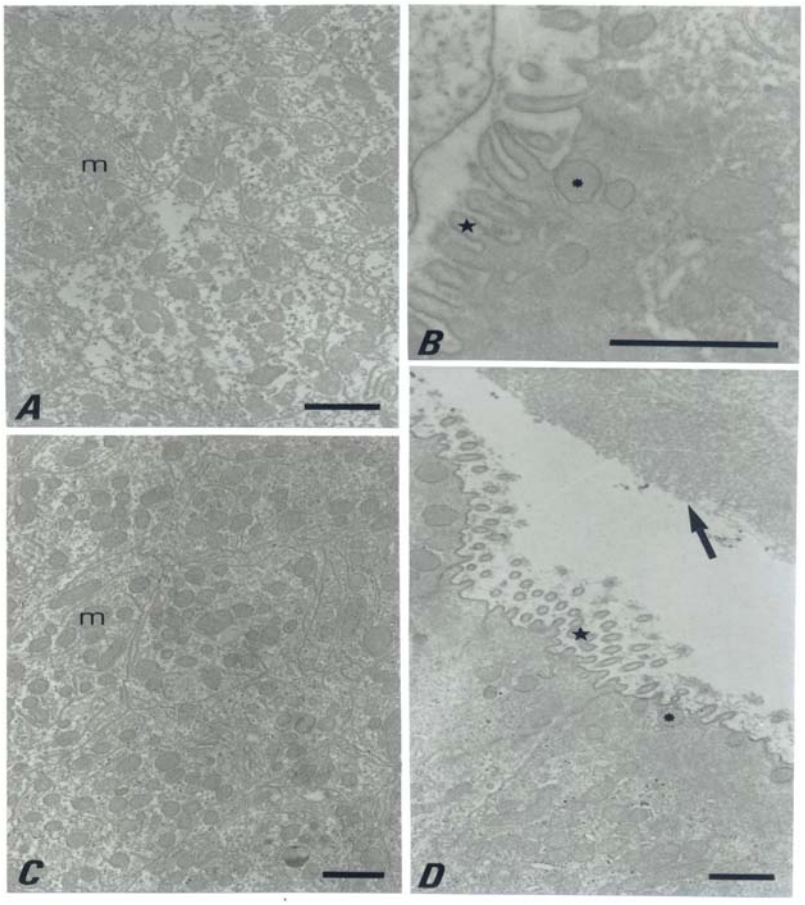

Fig. 2: Electron micrographs of the renal tubular epithelium *of S. testudineus*. 2A, basal cytoplasm with mitochondria (m) in 30‰ seawater, scale bar: 1 µm; 2B, detail of luminal region with apical microvillae (star), putative secretory vesicles in the sub-apical cytoplasm devoid of organelles (*) in 30‰ seawater, scale bar: 1 µm; 2C, basal cytoplasm with mitochondria (m) in 10‰ seawater, scale bar: 1µm; 2D: Same structures as in 2B plus amorphous material secreted into the lumen (arrow), but from fish in 10‰ seawater, scale bar: 1 µm.

testudineus observed under the transmission electron microscope revealed a tissue rich in mitochondria and membrane infoldings wrapping these mitochondria, both in control and experimental fishes (Figs 2A, 2C). The area occupied by mitochondria increased in fishe acclimated to isosmotic seawater, when compared to controls

(P=0.049). The area occupied by mitochondria was (mean ± SEM) of 21.5 ± 7.1 μm^2 (n=4) in 30‰ (Fig. 2A), and 57.6 ± 12.2 μm^2 (n=5) in 10‰ (Fig. 2C). Putative secretory subapical vesicles were observed in both controls (in 30 ‰ — Fig. 2B) and experimental animals (in 10‰ — Fig. 2D).

DISCUSSION

The morphology and epithelial structure of the gills of *S. testudineus* are typical of marine teleosts (LAURENT & DUNEL, 1980; PISAM *et al*., 1990; JOBLING, 1995; SHIRAISHI *et al*., 1997; EVANS *et al*., 2005). Chloride cells, as well as accessory cells of this species of pufferfish displayed the typical characteristics described for the cells of other teleosts (LAURENT & DUNEL, 1980; PISAM *et al*., 1990; EVANS, 1993; JOBLING, 1995; ZADUNAISKY, 1996; PERRY, 1997; SHIRAISHI *et al*., 1997; VAN DER HEIJDEN *et al*., 1997; FERNANDES *et al*., 1998; EVANS *et al*., 1999; EVANS *et al*., 2005), being located in the interlamellar region. The chloride cells observed in the gills of *S. testudineus* displayed apical projections that allowed them to be identified as chloride cells of the a type, the type normally present in marine species or freshwater species adapted to high salinity (JOBLING, 1995; PERRY, 1997; EVANS *et al*., 2005).

Acclimation to isosmotic seawater for 15 days induced no change either in the localization of chloride cells in the gill epithelium, or in the number or size of these cells, or else in the interdigitations with adjacent cells, when compared to gills of control pufferfish kept in full strength seawater. As a confirmation of this qualitative analysis, morphometric, quantitative analysis also did not reveal differences between the chloride cells of the gills of control and experimental animals, with respect to the area occupied by mitochondria and the system of tubular endomembranes. On the contrary, other marine species submitted to seawater dilution (hyposmotic), such as *Scophthalmus maximus* and *Sparus sarba*, have shown increase in the projections of the apical membrane, reduction in the tubular system of endomembranes, and reduction in the apical crypts (PISAM *et al*., 1990; KELLY & WOO, 1999), as morphological adaptations to reduction in the rate of salt secretion by the gills. It should be noted that *S. sarba* has been transferred from 33‰ to 6‰ for 120 hours,

and these described morphological differences were apparent already after 6 hours, returning to the control picture after 120 hours. Most studies compare the ultrastructure of salt-secretory gills to salt-absorptive gills. As an example of a study comparing the salt transporting gills to an epithelium separating isosmotic compartiments, data on *Sparus auratus* can be cited (LAIZ-CARRIÓN *et al*., 2005a). The number and size of chloride cells were higher at very low and very high salinities, and were lower at intermediate salinities (LAIZ-CARRIÓN *et al*., 2005a).

The kidney of fish has not been the target of so many ultrastructural studies, when compared to the gills, to the extent that no literature was found analysing structural changes in the renal epithelia associated with acclimation of a same species to different salinities. Acclimation to isosmotic salinity lead to a significant increase in the area occupied by mitochondria in the mesonephric (collecting) tubule cells. This result would be consistent with a putative increased metabolism/ATP supply for renal energy-consuming processes. Which renal process would require a larger ATP supply when the fish is isosmotic than when the fish is hyposmotic? One possible answer would be tubular solute reabsorption. With the possible relative increase in water load in reduced salinity, a putative increase in glomerular filtration would lead to increased ionic loss in the urine, unless the renal tubule is able to increase its rate of ion reabsorption. Indeed, the distal tubule of marine teleosts is known to actively reabsorb sodium (JOBLING, 1995; BEYENBACH, 2004). Further studies are necessary to clarify this issue.

In summary, long-term (15 days) transition between a plasma hyposmotic state (in 30‰) and a plasma isosmotic state (in 10‰), meaning at least in terms of salt regulation the shutdown of salt secretory mechanisms, apparently has no structural mitochondrial correlate in the gills, but has a correlate in the kidney. Adaptation to drastically reduced rates of salt secretion in the gills must involve mechanisms other than structurally-apparent ones, as for example, changes in the expression/regulation of transporter proteins, or changes in paracellular permeabilities (GORDON, 1959; 1963; POTTS & EVANS, 1967; EVANS, 1993; WOOD & MARSHALL, 1994; JOBLING, 1995; WOOD & LAURENT, 2003). These physiological/biochemical alterations may of course also be taking place in the kidney.

SUMMARY

The pufferfish *Sphoeroides testudineus* is an abundant euryhaline species in the estuaries of Paranaguá Bay (Paraná, Brazil), in waters where salinity ranges from 34‰ to 0‰. The present work aimed at morphometrically quantifying (transmission electron microscopy) the relative area occupied by mitochondria in gill and renal epithelia and the area of the tubular system of endomembranes of gill chloride cells. Fish in isosmotic salinity (10‰, 15 days) were compared to fish in 30‰, where they are hypo-osmotic. The two parameters quantified did not change in isosmotic conditions in the gills. In the basal region of the renal mesonephric duct there was an increase in the area occupied by mitochondria in fishes acclimated to 10‰. The low values of oxygen consumption and gill ATPase activity reported in the literature for fish acclimated to isosmotic media has not been mirrored in this study by a decrease in the relative area occupied by the energy supplying organelles, the mitochondria.

KEY WORDS**:** Gills, kidney, mitochondria, salinity, *Sphoeroides testudineus*, ultrastructure

RESUMO

O baiacu *Sphoeroides testudineus* é uma espécie eurihalina abundante em estuários da Baía de Paranaguá (Paraná, Brazil), em águas com salinidade variando entre 34‰ e 0‰. O presente trabalho teve como objetivo quantificar morfometricamente (microscopia eletrônica de transmissão) a área relativa ocupada pelas mitocôndrias nos epitélios branquial e renal e a área do sistema tubular de endomembranas das células de cloreto branquial. Peixes em salinidade isosmótica (10‰, 15 dias) foram comparados a peixes em 30‰, quando eles são hipo-osmóticos. Os dois parâmetros quantificados não sofreram alterações em condições isosmóticas na brânquia. Na região basal do duto mesonéfrico renal ocorreu aumento na área ocupada pelas mitocôndrias nos peixes aclimatados a 10‰. Os baixos valores de consumo de oxigênio e atividade ATPásica branquial relatados na literatura para peixes aclimatados a meio isosmótico não se refletiram em redução na área relativa ocupada pelas organelas responsáveis pelo suprimento de energia, as mitocôndrias.

PALAVRAS CHAVE: Brânquias, rim, mitocôndrias, salinidade, *Sphoeroides testudineus*, ultraestrutura

RÉSUMÉ

L'objectif du présent travail était de comparer, em utilisant la morphométrie, la surface occupée par lês mitochondries (microscopie électronique à transmission) dans la branchie et les épithéliums rénaux de compères corotuches après une acclimatation (15 jours) à une salinité iso-osmotique (10‰), par rapport à une acclimatation à 30‰, quand le milieu intérieur du poisson est hypo-osmotique. Dans les conditions iso-osmotiques, les deux paramètres évalués, la surface dês mitochondries et celle du système tubulaire des endo-membranes des cellules à chlorure branchiales – n'ont pas varié. En revanche, dans la región basale du canal mésonéphrique rénal, la superficie occupée par les mitochondries des poissons aclimatés à 10‰ a augmenté. La réduction de la concommation en oxygène et des activités des ATPases branchiales, normalement observée chez les poissons acclimatés en milieu iso-osmotique, ne se reflète pas par une diminution de la surface relative occupée dans des organites producteurs d'énergie comme les mitochondries.

Mots-Clé: branchies, rein, mitochondries, salinité, *Sphoeroides testudineus*, ultrastructure

ACKNOWLEDGMENTS — The authors wish to gratefully acknowledge the generous help of Dr Henry L. Spach of the Centro de Estudos do Mar of the Universidade Federal do Paraná (UFPR) in collecting the animals. This work represents part of a dissertation by VP in fulfillment of the requirements for the degree of Master of Science in Cell Biology at the Departamento de Biologia Celular, UFPR, and was financed by CAPES through a student fellowship to VP. The authors also wish to thank Dr Ciro A. Oliveira Ribeiro and Dr Heléne Roche for translating the resumé.

BIBLIOGRAPHY

BEYENBACH, K.W. 2004. Kidneys sans glomeruli. *American Journal of Physiology Renal Physiology 286*: F811-F827.

CARMONA, R.; M. GARCÍA-GALLEGO; A. SANZ; A. DOMEZAÍN & M.V. OSTOS-GARRIDO. 2004. Chloride cells and pavement cells in gill epithelia of *Acipenser naccarii*: ultrastructural modifications in seawater-acclimated specimens. *Journal of Fish Biology 64*: 553–566.

EVANS, D.H. 1993. *The Physiology of Fishes*. Boca Raton: CRC Press.

EVANS, D.H.; P.M. Piermarini & K.P. Choe. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews 85*: 97-177.

EVANS, D.H.; P.M. Piermarini & W.T.W. Potts. 1999. Ionic Transport in the Fish Gill Epithelium. *Journal of Experimental Zoology 283*: 641–652.

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

GORDON, M.S. 1959. Ionic regulation in the brown trout (*Salmo trutta* L.). *Journal of Experimental Biology 36*: 227-252.

GORDON, M.S. 1963. Chloride exchanges in rainbow trout (*Salmo gairdneri*) adapted to different salinities. *Biological Bulletin 124*: 45-54.

HENTSCHEL, H..& M. ELGER. 1989. Morphology of glomerular and aglomerular kidneys. In: Kinne, R.K.H., editor. Structure and function of the kidney. *Comparative Physiology*. Basel: Karger. *vol 1*, p 1-72.

HICKMAN JR, C.P. & B.F. TRUMP. 1969. The Kidney. In: Hoar, W.S.; D.J. Randall, editors. *Fish physiology*. New York: Academic Press. *vol. 1*, p 91-239.

IMSLAND, A.K.; S. GUNNARSSON; A. FOSS & S.O. STEFANSSON. 2003. Gill Na^+,K^+-ATPase activity, plasma chloride and osmolality in juvenile turbot (*Scophthalmus maximus*) reared at different temperatures and salinities. *Aquaculture 218*: 671-683.

JOBLING, M. 1995. *Environmental Biology of Fishes*. London: Chapman & Hall.

KELLY, S.P. & N.Y.S. WOO. 1999. The response of sea bream following abrupt hyposmotic exposure. *Journal of Fish Biology 55*: 732-750.

KELLY, S.P.; I.N.K. CHOW & N.Y.S. WOO. 1999. Haloplasticity of black seabream (*Mylio macrocephalus*): hypersaline to freshwater acclimation. *Journal of Experimental Zoology 283*: 226-241.

LAIZ-CARRIÓN, R.; P.M. GUERREIRO; J. FUENTES; A.V.M. CANARIO; M.P.M. DEL RÍO & J.M. MANCERA. 2005a. Branchial osmoregulatory response to salinity in the gilthead sea bream, *Sparus auratus*. *Journal of Experimental Zoology 303A*: 563–576.

LAIZ-CARRIÓN, R.; S. SANGIAO-ALVARELLOS; J.M. GUZMÁN; M.M.P. DEL RÍO; J.L. SOENGAS & J.M. MANCERA. 2005b. Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions: Implications for osmoregulation and energy metabolism. *Aquaculture 250*: 849-861.

LAURENT, P. & S. DUNEL. 1980. Morphology of gill epithelia in fish. *American Journal of Physiology 238*: R147-R159.

PERRY, S.F. 1997. The chloride cell: struture and function in the gills of freshwater fishes. *Annual Review of Physiology 59*: 325-347.

PISAM, M.; G. BOEUF; P. PRUNET & A. RAMBOURG. 1990. Ultrastructural features of mitochondria-rich cells in stenohaline freshwater and seawater fishes. *American Journal of Anatomy 187*: 21-31.

POTTS, W.T.W. & D.H. EVANS. 1967. Sodium and chloride balance in the "killifish" *Fundulus heteroclitus*. *Biological Bulletin 133*: 411-425.

PRODOCIMO, V. & C.A. FREIRE. 2001. Ionic regulation in aglomerular tropical estuarine pufferfishes submitted to seawater dilution. *Journal of Experimental Marine Biology and Ecology 262*: 243-253.

PRODOCIMO, V. & C.A. FREIRE. 2003. Glomeruli and renal tubules are restricted to the cranial kidney of the adult estuarine *Sphoeroides testudineus*. *Journal of Fish Biology 63*: 1258–1265.

PRODOCIMO, V. & C.A. FREIRE. 2004. Estuarine pufferfishes (*Sphoeroides testudineus* and *S. greeleyi*) submitted to seawater dilution during ebb tide: a field experiment. *Marine and Freshwater Behaviour and Physiology 37*: 1-5.

PRODOCIMO, V. & C.A. FREIRE. 2006. The Na^+, K^+, $2Cl^-$ cotransporter of estuarine pufferfishes (*Sphoeroides testudineus* and *S. greeleyi*) in hypo- and hyper-regulation of plasma osmolality. *Comparative Biochemistry and Physiology 142C*: 347-355.

REYNOLDS, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology 17*: 208-212.

SHIRAISHI, K.; T. KANEKO; S. HASEGAWA & T. HIRANO. 1997. Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater. *Cell and Tisssue Research 288***:** 583-590.

VAN DER HEIJDEN, A.J.H.; P.M. Verbost; J. Eygensteyn; J. Li; S.E.W. Bonga & G. Flik. 1997. Mitochondria-rich cells in gills of tilapia (*Oreochromis mossambicus*) adapted to fresh water or seawater: quantification by confocal laser scanning microscopy. *Journal of Experimental Biology 200*: 55-64.

VENDEL, A.L.; H.L. SPACH; S.G. LOPES & C. SANTOS. 2002. Structure and dynamics of fish assemblages in a tidal creek environment. *Brazilian Archives of Biology and Technology 45*: 365-373.

WOO, N.Y.S. & A.C.Y. FUNG. 1981. Studies on the biology of the red sea bream, *Chrysophrys major* – II. Salinity adaptation. *Comparative Biochemistry and Physiology* 69A: 237-242.

WOOD, C.M. & P. LAURENT. 2003. Na^+ versus Cl^- transport in the intact killifish after rapid salinity transfer. *Biochimica et Biophysica Acta* 1618: 106–119.

WOOD, C.M. & W.S. MARSHALL. 1994. Ion balance, acid-base regulation, and chloride cell function in the common "killifish", *Fundulus heteroclitus* — a euryhaline estuarine teleost. *Estuaries 17*: 34-52.

ZADUNAISKY, J.A. 1996. Chloride cells and osmoregulation. *Kidney International 49*: 1563-1567.

Recebido em 15 de junho de 2006.