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Kidney ATPase response in seawater-transferred rainbow trout *(Oncorhynchus mykiss)*. Effect of salinity and fish size

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J. FUENTES, J. L. SOENGAS, M. BUCETA, J. OTERO, P. REY and E. REBOLLEDO. *Kidney ATPase response in seawater-transferred rainbow trout* (Oncorhynchus mykiss). *Effect of salinity and fish size*. J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (4), 231-238, 1996.

Two sizes of domesticated rainbow trout (*Oncorhynchus mykiss*, 40 ± 0.67 and 180 ± 3.9 g) were directly transferred to brackishwater (9 ppt) and seawater (28 ppt). Kidney Na^+-K^+-ATPase and Mg^{2+}-ATPase activities were measured in fresh water, and after long-term seawater adaptation (up to 21 days). Renal Na^+-K^+-ATPase activity increased after saltwater loading in small trout, while large trout displayed an unmodified ATPase activity. The smallest trout showed a low but progressive increase in renal Mg^{2+}-ATPase activity after the transfer to both salinities. However, ATPase activity remained unchanged or significantly decreased in large trout after the transfer to seawater or brackishwater, respectively.

Key words: ATPase activity, Kidney, Rainbow trout , Seawater adaptation.

Euryhaline teleosts can survive in a wide range of salinities by maintaining osmotic and ionic parameters within narrow limits. Different organs like gills, gut and kidney contribute to the process of adaptation to changes in external salinity (14).

The relative contribution of the kidney to osmoregulation varies depending on the species and habitat, and its function is mainly dominated by the glomerular filtration, the tubular reabsorption and secretory processes (31). Glomerular filtration rates (GFR) are higher in freshwater than in marine fishes whereas the urine

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output, expressed as urine formation rate (UFR), is highly more diluted. Most teleosts show a linear relationship between GFR and UFR and, therefore, the extracellular volume regulation depends directly on glomerular filtration (6).

In hypoosmotic environments the kidney is mainly involved in the conservation of monovalent ions and in the elimination of the body water excedents (6). Two tubular characteristics are particularly essential for the efficient performance of this function: 1) a powerful monovalent ion reabsorption mechanism which operates in conjunction with 2) a low tubular permeability to filtered plasma water (20). The transepithelial sodium transport in freshwater teleosts occurs against an electrochemical gradient (29, 30) involving a Na^+-K^+-ATPase placed in the peritubular membrane (11). The kidney function is essential during seawater loading since fish need to alter the pattern of renal excretion to become mainly involved in both the preservation of body water and in the elimination, through tubular secretion, of divalent ions such as magnesium, calcium and sulphate (13, 20, 32). However, little information is available about the mechanism of magnesium transport by the renal tubules, though kinetic studies with isolated and perfused tubules of the winter flounder (*Pseudopleuronectes americanus*) suggest an active transport (7).

The rainbow trout is a freshwater resident species, which possesses however a certain degree of eurihalinity (4). Although decreases in both GFR and UFR, and in the size of renal corpuscles have been described during seawater adaptation in the rainbow trout (6, 10, 32), little attention has been paid to changes in the activities of kidney Na^+-K^+-ATPase and Mg^{2+}-ATPase during the initial stages of acclimation to seawater. JOHNSSON and CLARKE (23) suggested that seawater adaptation in rainbow trout takes place in a size-dependent manner. The main goal of the present study was to examine the initial effect of seawater transfer on kidney ATPase activity in two sizes of domesticated rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Sexually immature non-anadromous rainbow trout (N = 500) (*Oncorhynchus mykiss*) were purchased from a local trout farm (Soutorredondo, Lousame, Galicia). The trout were randomly distributed in 180 l fibreglass tanks, supplied with well aerated, ozonated and physically filtered recirculating tap water. All experiments were carried out between October and January, under natural conditions of photoperiod, water temperature (10-14 °C) and unchanged rearing conditions (dissolved oxygen > 8.5 mg/l; water flow per tank 10 l/min). Food was offered once daily (commercial dry pellets: 1.5 % of body wt/day), and suppressed 24 hours before fish sacrificing.

After three weeks of acclimation, trout of different batches (mean weights in grams: 40 ± 0.67, and 180 ± 3.9; lengths (in cm): 15.78 ± 0.46 and 26.35 ± 0.67) were directly transferred to salinities of 9 and 28 ppt, reached by adding the suitable amount of artificial seasalts (Instant Ocean^R) to the rearing systems. Salinity was tested all along the experiments to ensure salt concentration.

Once in both salinities, trout of both sizes were sampled 1, 4, 7, 15 and 21 days after transfer. Fish were transferred to 25 l backets and anesthetized with a sublethal dose of buffered tricaine methasulphonate (50 ppm, Sigma USA) and killed by decapitation. Although no functional division has been found in rainbow trout

caudal kidney (18), the highest mean density of renal glomerular corpuscles is found in the caudal one-quarter of the kidney (10). The fish were dissected and the caudal one-quarter of the kidney was removed, frozen on dry ice and stored at –80 °C for further ATPase analysis.

Kidney samples for enzyme assays were obtained by homogenizing 150-200 mg of thawed tissue (12 complete strokes at 800 rpm in a teflon homogenizer) in 7 ml of a chilled buffer containing 250 mM sucrose, 1 mM Na_2-EDTA and 20 mM Tris-HCl (pH 7.5).

Kidney homogenates were incubated for ATPase assay at 22 °C for 20 min. All reaction mixtures contained 50 mM Tris-HCl, the convenient proportion of chloride salts and pH adjusted for an optimal activity development. The reaction was started by adding ATP (disodium salt, vanadate free, Sigma) and stopped with 30 % (w/v) trichloroacetic acid.

Na^+-K^+-ATPase activity was determined in a reaction mixture containing 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$ and 5 mM Na_2ATP (pH 7.4) in the presence or absence of 1 mM ouabain (Sigma). Mg^{2+}-ATPase activity was determined in the presence of 5 mM of $MgCl_2$ and 5 mM Na_2ATP (pH 7.4).

The inorganic phosphate (Pi) produced by ATP hydrolysis was measured as described by LE BEL *et al.* (25). Protein measurements were conducted by the method of LOWRY *et al.* (26) using bovine serum albumin (Sigma) as standard. The specific ATPase activities are expressed as μmol Pi min^{-1} g^{-1} protein.

The outcoming data were statistically analysed by using a two-way ANOVA to assess the main effects of size and time spent in salinity. A one-way ANOVA complemented with the multiple comparison test Student-Newman-Keuls, was used to assess differences between means. Normality and homocedasticity were tested by using the Kolmogorov-Smirnov's and Cochran's C tests, respectively. Logarithmic transformations of the data were used to reach normality, if necessary. The significant differences between means were established for $p < 0.05$. Statistical analyses were performed using the SPSS/PC$^+$ statistical package.

Results

During the adaptation trials from freshwater to brackishwater (9 ppt) and to seawater (28 ppt), trouts showed a normal behaviour and swimming performance, and mortality never exceeded 10 %. However, the feeding behaviour was strongly reduced after the transfer to both salinities from the 7th day of experiment onwards. During this period, both freshwater and saltwater transferred trouts were fed with the same ration and no detectable effect of decreased food intake was detected on kidney ATPase when compared with the initial levels in freshwater-adapted trout (one way ANOVA).

Samples from both sizes of freshwater-adapted trout were obtained all along the experiments. No significant modifications were found in freshwater values (one-way ANOVA), and all the data were pooled into one group for further graphical plotting and statistical analyses. The ATPase activity and the significant differences found between treated and control groups are shown in figs. 1 and 2. The significant effects found for trout size and time spent in salinity (after two-way ANOVA) are shown in table I.

Kidney Na^+-K^+-ATPase activities of rainbow trout after the transfer to brackishwater and seawater are shown in fig. 1. The small trout showed higher Na^+-K^+-ATPase activity than the freshwater-adapted trout, while large fish displayed,

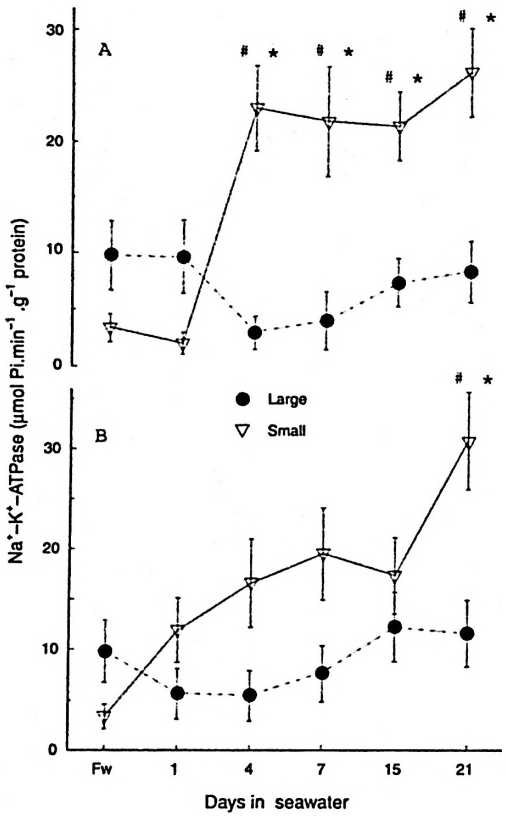

Fig 1. *Kidney Na^+-K^+-ATPase activity in rainbow trout transferred to brackishwater (A) and seawater (B).*
Each point represents the mean ± 1 SEM of 10 to 12 trouts. (*) Significantly different ($p < 0.05$) from freshwater values. (#) Significantly different ($p < 0.05$) between groups at the same sampling time.

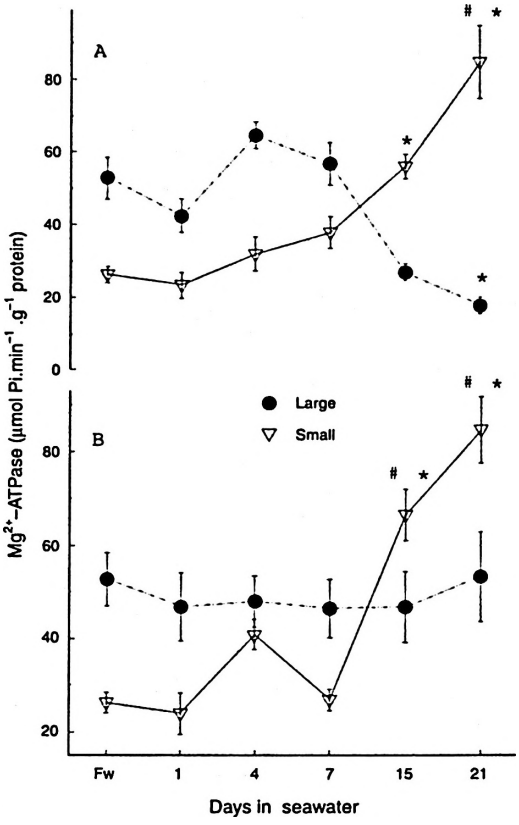

Fig 2. *Kidney Mg^{2+}-ATPase activity in rainbow trout transferred to brackishwater (A) and seawater (B).*
Legend as in fig. 1.

after 21 days of study, unmodified ATPase activity in either brackishwater or seawater. A general significant effect of fish size on kidney Na^+-K^+-ATPase activity (table I) was detected after the transfer to both seawater and brackishwater ($p < 0.001$). The periods spent in each salinity have only a detectable effect after the transfer to seawater. The interaction (size x time spent in salinity) detected in brackishwater showed also an effect of fish size on the dynamics of kidney Na^+-K^+-ATPase activity.

The variations of Mg^{2+}-ATPase activity during the adaptation to brackishwater and seawater, as well as the significant differences observed between treated and control groups are shown in fig. 2. Transfer to brackishwater (fig. 2A) induced a significant twofold and fourfold increase in the activity of Mg^{2+}-ATPase in small trout (40 ± 0.67 g) at 15 and 21 days after transfer, respectively. Conversely, Mg^{2+}-ATPase activity decreased in large fish after 15 days of transfer to brackishwater

reaching values 2.5 times lower than those of freshwater at the end of the experiment. Mg^{2+}-ATPase activity remained unchanged in large trout (180 ± 3.9) after the transfer to seawater, while it increased in small trout (fig. 2B). The present data regarding Mg^{2+}-ATPase activity, show a significant effect of the time spent in salt media as well as an strong interaction with fish size (table I).

Discussion

According to the models of BEYENBACH and DANTZLER (2), and HETSCHELL and ELGER (19), the Na^+-K^+-ATPase placed in the peritubular membrane of the distal tubules drives the potential for the sodium reabsorption from the ultrafiltrate. JAMPOL and EPSTEIN (22) found that kidney Na^+-K^+-ATPase activity in the largemouth bass (*Micropterus dolomieui*), a strictly fresh water fish, was about twice than those of the marine species winter flounder (*Pseudopleuronectes americanus*) and sea raven (*Hemitripterus americanus*). However, renal Na^+-K^+-ATPase activity did not change in seawater adapted yellow eel (*Anguilla rostrata*) (22), Atlantic salmon (*Salmo salar*) (27), or seawater adapted rainbow trout (24). In contrast, the results obtained in the present study showed a clear increase in Na^+-K^+-ATPase activity after saltwater loading in small trout.

Table I. Effects *obtained after two-way analysis of variance of kidney ATPase activity in brackish-water and seawater.*

The main factors considered were fish size and time spent in salinity. The interactions (size x time spent in salinity) were included in the analyses. $^*p < 0.05$; $^{***}p < 0.001$

Source of variation	Brackishwater (9 ppt) F	Seawater (28 ppt) F
Na^+-K^+-ATPase activity		
Size	10.913***	7.185***
Time	1.786	5.119***
Interaction	4.168***	2.438*
Mg^{2+}-ATPase activity		
Size	1.580	0.252
Time	1.022*	7.644***
Interaction	20.576***	4.343***

Seawater teleosts tend to gain salts and to lose water (14). Although seawater fish must ingest the surrounding seawater to balance the osmotic loss of water across the gills, the glomerular species of marine teleost reduce GFRs and urine flows (15). However, those urine flows occurring in glomerular species indicate that some reabsorption of urine does take place, presumably in the collecting duct o tubule, or in the urinary bladder where water permeability is increased over the freshwater conditions (12, 13). Primary urine reabsorption is secondary to the active transport of NaCl mediated by Na^+-K^+-ATPase (33).

In euryhaline species, and during seawater loading, a decrease in GFR has been detected associated with an increase in single nephron glomerular filtration rate (SNGFR) (6). The increase in SNGFR and the permeabilization to water occurring in the luminal surface of the distal segments and collecting tubules (20) may be accounted for by the lack of changes in kidney Na^+-K^+-ATPase activity of euryhaline fish fully adapted to seawater. In the present study, a quick permeabilization to water may be the reason that kidney Na^+- K^+-ATPase did not change in 180 g seawater-adapted trout. If osmoregulatory imbalances occur in the drinking/water absorption in the gut (J. FUENTES, in preparation), the increased enzyme activity observed in small trout would be due to an increased water reabsorption.

Nevertheless, the kidney Na^+-K^+-ATPase activity in seawater could be

related to secretion of NaCl. BROWN *et al.* (6), classified nephrons into three groups depending on their perfusion pattern with ferrocyanide: perfused filtering (PF), not perfused (NP) and not filtering (NF). The amount of these nephrons changed in a different way during seawater adaptation: the % of PF decreased, the % of NP increased 4-fold whereas no changes were detected in the % of NF. CLIFF and BEYENBACH (8, 9) suggested that NP and NF nephrons may be involved in the process of secretion since they observed that the proximal tubules of the NP nephrons in winter flounder secrete fluid, secondary to NaCl transport, into the lumen, in a way similar to that ocurring in killifish (*Fundulus heteroclitus*) (3) and in several freshwater teleosts (15).

The physiological role of NaCl and fluid secretion in marine environments is not clear in winter flounder. In freshwater teleosts NaCl secretion into the urine, possibly through Na^++Cl^- cotransport, may be drawing water into the lumen osmotically, thus increasing urine flow (15). However, the kidney Na^+-K^+-ATPase activity reported in the present study can be related to reabsorption of NaCl rather than to secretion since several features such as body dehydration (21), reabsorption of 70% of the water filtered and diminution of urine flows (6) have been reported after transfer of rainbow trout to seawater.

Even the relatively low salinity of 9 ppt induced an increase in the activity of kidney Na^+-K^+-ATPase in small trout (fig. 1A). Thus, isoosmotic media may induce increases in ATPase activity similar to those occurring in hyperosmotic environments, though the rapid increase found in 9 ppt vs 28 ppt needs further study. Thus, JURSS *et al.* (24) and MORGAN and IWAMA (28) found increases in gill Na^+-K^+-ATPase activity as well as increases in the energetic cost of osmoregulation, respectively, after transfer of small rainbow trout to isoosmotic environments. Gill Na^+-K^+-ATPase activity characteristically increases in salmonids after sea water adaptation (5, 17).

The dynamics of renal Na^+-K^+-ATPase activity after 21 days spent in saline media seems to show that the periods of osmoregulatory adaptation and stabilization described by BATH and EDDY (1) may be different depending on salinity and fish size (table I). The exposure to increased salinity induces a long-term increase in Na^+-K^+-ATPase activity only in the smallest trout. If a good adaptation to saline media is directly associated with an unchanged renal Na^+-K^+-ATPase activity, then small trout neither in isoosmotic media nor in seawater reach total adaptation during changes in environmental salinity.

The smallest trout showed a low but progressive increase in renal Mg^{2+}-ATPase activity after the transfer to both salinities (fig. 2), reaching values 4 times higher than those of freshwater at the end of the experimental period. However, ATPase activity remained unchanged or significantly decreased in large trout after the transfer to seawater or brackishwater, respectively.

The changes in Mg^{2+}-ATPase activity found in the present study may be accounted for by changes in the % of secretory nephrons. The increase in Mg^{2+}-ATPase activity in small trout in isoosmotic and hyperosmotic media may be the result of the progressive increase in the amount of functional NP nephrons, since a significant interaction with time was observed in the present study (table I). On the other hand, an increase in Mg^{2+}-ATPase activity may be necessary to regulate body magnesium, which is critical for the survival of small fish in saline media (16).

The isoosmotic media induces changes in both Mg^{2+}- and Na^+-K^+- ATPase activities similar to those occurring in hyperosmotic environments only in small trout. The decrease in Mg^{2+}-ATPase activity observed in large fish when no changes were observed in Na^+-K^+-ATPase, may be attributed to a different functional position of the enzymes in different populations of secretory and filtering nephrons. Mg^{2+}-ATPase activity in kidney may also change in a dose-dependent way regarding plasma magnesium concentration. The results, taken together, suggest that the acclimation of small trout to increased salinity may be related to a higher energetic cost in kidney.

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J. FUENTES, J. L. SOENGAS, M. BUCETA, J. OTERO, P. REY y E. REBOLLEDO. *Respuesta de las ATPasas renales durante la transferencia de la trucha arco iris al agua de mar. Efecto de la salinidad y del tamaño del pez.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (4), 231-238, 1996.

Ejemplares de trucha arco iris doméstica de dos tamaños (40 y 180 g) se transfieren directamente a agua salobre (9 ‰) y agua de mar 28 ‰). Las actividades Na^+-K^+- y Mg^{2+}-ATPasa se miden en agua dulce y tras adaptación a los medios salinos. La actividad Na^+-K^+- ATPasa renal aumenta después de la carga de sales en las truchas pequeñas, mientras que no se modifica en las de gran tamaño. Las truchas pequeñas muestran un lento, pero progresivo aumento en la actividad Mg^{2+}-ATPasa renal después de la transferencia a ambas salinidades. Sin embargo, la actividad ATPásica no cambia o disminuye significativamente en las truchas grandes después de la transferencia al agua de mar o agua salobre, respectivamente.

Palabras clave: Actividad ATPásica, Riñón, Trucha arco iris, Adaptación al agua de mar.

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