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HCO_3^--ATPase and Ca^{2+} Dependent ATPase Activities in the Gills of the Rainbow Trout after the Transfer to Brackishwater and Seawater

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The effect of seawater and brackishwater exposure on gill HCO_3^--ATPase and Ca^{2+} dependent ATPase activity in rainbow trout *(Oncorhynchus mykiss)* was investigated at different periods of time. HCO_3^--ATPase activity decreased after the transfer to either brackishwater or seawater. Ca^{2+} dependent ATPase activity decreased during the initial period (1 to 4 days) in both salinities and recovered freshwater values from the 7th day onwards. No effect from fish size was detected in both parameters after saltwater transfer. The results are discussed in terms of salinity and long-term saltwater adaptation.

Key words: Rainbow trout, Seawater adaptation, Gill, Calcium, Calcium ATPase, Bicarbonate ATPase.

Euryhaline teleosts survive in a wide range of salinities as long as they are able to maintain osmotic and ionic parameters within narrow limits. The gills are the major site for ion uptake in freshwater, and salt excretion in seawater fish. The processes of uptake and elimination of salts, taking place against an ion-osmotic gradient, are mediated by membrane bound ATPases (4).

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Plasma chloride content in freshwater fish is much more concentrated than in the environment, so that chloride uptake must be the result of an active transport. A counter-gradient transport (not coupled to sodium uptake) is placed in the apical membrane of chloride cells (11) mediating chloride uptake by a chloride/bicarbonate exchange mechanism (29). LIN and RANDALL (19) have suggested that the exchanger, in contrast to previous models, makes a minor contribution to acid-base balance in freshwater-adapted rainbow

trout, and its function would mainly be involved in chloride movements through the gills. The presence of an HCO_3^--ATPase in branchial plasma membranes, stimulated by both bicarbonate and chloride, provides a mechanism that may be involved in the movement of chloride and bicarbonate across fish gills (3). HCO_3^--ATPase activity has also been measured in crude gill homogenates of freshwater adapted trout (31). In addition, LIN and RANDALL (20) described a 30 % N-ethylmaleimide (NEM)-insensitive ATPase activity in crude homogenates of trout gills when measuring NEM-sensitive H^+-ATPase, probably driven by HCO_3^--ATPase which suggest its involvement in Cl^-/HCO_3^- exchange.

On the other hand, the branchial Ca^{2+}-ATPase in freshwater fish is the enzyme involved in calcium active uptake, which takes place exclusively in such tissue (7, 28, 34). The possibility of a Ca^{2+}-ATPase playing a role in branchial calcium transport was first suggested by MA *et al.* (22). The enzyme placed in the basolateral membranes of chloride cells (28) drives the potential for the passive diffusion of calcium through the apical membranes of the cell (7).

According to many authors, gill Ca^{2+}-ATPase activity is modified in response to changes in environmental calcium concentrations, however the results obtained upto date are somehow contradictory. Thus, several authors have described a positive relationship between Ca^{2+}-ATPase activity and the concentration of calcium in the water (32), whereas an inverse correlation between calcium content and Ca^{2+}-ATPase activity has been described by others (10, 26), with no changes found in some cases (5, 22).

The adaptation and further growth of euryhaline teleosts after transfer to seawater depends on the activation of ionic excretion mechanisms (13) and the length-of the crisis and stabilization periods (1) varying between 1-4 days and 7-10 days, respectively. Most studies performed in seawater transferred salmonids have been focused on the activation of Na^+-K^+-ATPase in fish gills, but no clear studies have been performed up to now regarding a precise response of Ca^{2+}-ATPase and calcium HCO_3^-–ATPase activities during the initial period of adaptation to increased environmental salinity. Therefore, the main goal of this work was to elucidate whether the exposure to seawater affects both ATPase activities in the gills of rainbow trout *(Oncorhynchus mykiss).*

Materials and Methods

Sexually immature freshwater-adapted rainbow trout *(Oncorhynchus mykiss),* ranged from 30 to 200 g (n = 400), were maintained in 180 l fibreglass tanks supplied with well-aerated recirculating tap water. Food was delivered once daily (commercial dry pellets; ration equivalent to 1.5 % of body weight/day), and fish were starved 24 hours before sacrificing. The experiments were performed between October and January 1992 under natural photoperiod, water temperature (10-14 °C), and constant rearing conditions (dissolved O_2 > 8.5 mg/l; water pH 7-7.5).

After three weeks of acclimation, trout were directly transferred to 9 and 28 ppt salinity, adjusted by adding the appropriate amount of artificial seasalts (Instant Ocean) to the holding tanks. Salinity was tested and corrected if necessary along the experiment to ensure salt concentrations. Once placed in both salinities, trout were sampled at 1, 4, 7, 15 and 21 days after transfer. Fish were anaesthetized with a sublethal dose of MS-222 (50 ppm, Sigma) in a water tank, and killed by decapitation. Gill arches were excised, rinsed with saline to remove blood and scraped to

obtain branchial samples. Samples were directly frozen on dry ice and kept at –80 °C for further ATPase analysis.

Branchial crude homogenates for ATPase assays were obtained by homogenizing approximately 150 mg of the scraped tissue (12 complete strokes at 600 rpm in a teflon homogenizer, Braun model 853202) in 7 ml of an ice-cold buffer containing 250 mM sucrose, 1 mM Na_2-EDTA and 20 mM Tris-HCl, pH 7.5. HCO_3^-–ATPase activity was determined at 22 °C as the increase in inorganic phosphate hydrolysis after the addition of 30 mM $NaHCO_3$ to an assay mixture containing 50 mM Tris-HCl (pH 8), 2 mM Na_2ATP and 2 mM $MgCl_2$. The Ca^{2+} dependent ATPase activity was determined in an assay mixture containing 5 mM $CaCl_2$ and 5 mM Na_2ATP at 22 °C. Na^+-K^+-ATPase assay was performed according to a previous method (31). Protein measurements were performed by the method of LOWRY *et al.* (21) using bovine serum albumin (Sigma) as standard. The production of inorganic phosphate by ATP hydrolysis, was measured by the method recommended by LE BEL *et al.* (18). The specific ATPase activities are expressed as mmoles $Pi.min^{-1}.g\ protein^{-1}$.

The existence of possible significant differences between means for Ca^{2+} dependent ATPase and HCO_3^-–ATPase activities were assessed using a paired *t*-test (controls vs treated). The normality as well as homocedasticity of the variables were assessed by using Kolmogorov-Smirnov's and Cochran's tests, respectively. Logarithmic transformations were performed if necessary to reach normality. Non-parametric analysis using a Kruskall-Wallis ANOVA on ranks, complemented with the multiple comparisons paired Dunn's test was used for Na^+-K^+-ATPase activity (control vs treated groups). The significance level is specified in tables and figures.

Results

During the trials in both brackishwater and seawater, the fish mortality accumulated never exceeded 10 % of the total number of fish. The experimental series were performed 3 times with different sizes of rainbow trout (i.e. 180 ± 3.9 g, 81 ± 1.78 g and 42 ± 0.67 g), since seawater adaptation in rainbow trout has been described to be size-dependent (15). No significant effect from size (two-way ANOVA) was detected during the transfer to brackishwater or to seawater (table I) in the range of 30-200 g, and therefore data were pooled for further statistical analysis.

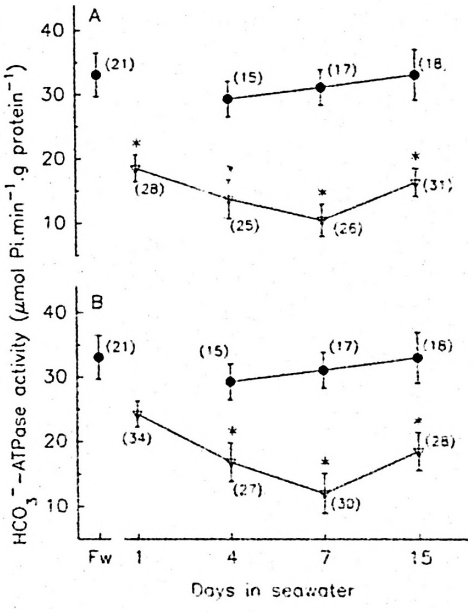

Figure 1. *Gill HCO_3^--ATPase activity in seawater (A) and brackishwater (B) transferred rainbow trout.*

Each point represents the mean ± SEM of the number of fish given in brackets. The asterisks represent significant differences ($p < 0.01$) from freshwater values obtained from paired *t*-tests. Freshwater values are denoted using circles and saltwater values using triangles.

In rainbow trout transferred to either seawater (28 ppt) or brackishwater (9 ppt), branchial HCO_3^-–ATPase activity decreased as long as external salinity was increased (fig. 1, A and B). Although the decrease in HCO_3^-–ATPase activity was slower in brackishwater than in seawater transferred trout (4th day vs 1st day), no significant difference (one-way ANOVA) was found between brackish and seawater experiments. The decrease in HCO_3^-–ATPase activity was salinity-independent in saltwater-adapted trout, and in both treated groups the activity was only 30 to 40 % of the freshwater controls (depending on the sampling). In addition, no significant modifications were found within any of the treatments at different sampling days (one-way ANOVA). Salinity was the main factor affecting HCO_3^-–ATPase activity in our experimental design (table I).

Ca^{2+} dependent ATPase activity showed an initial decrease after the transfer to both seawater (fig. 2A) and brackishwater (fig. 2B). The significant decrease of ATPase activity, lasting 4 days, took place after the transfer to both salinities to the same extent, and no significant differences (one-way ANOVA) were detected between the treated groups in this initial period.

A recovery of Ca^{2+} dependent ATPase activity up to values recorded in freshwater took place after 7 days in both salinities (fig. 2, A and B), lasting until the end of the experimental period.

The two-way ANOVA showed no effect from fish size on the values noticed in brackish or seawater (table I), and the main factor affecting Ca^{2+} dependent ATPase activity in both cases was also the change in salinity.

Na^+-K^+-ATPase activity was also measured at different periods in response to transfer to seawater (fig. 3A) and brackishwater (fig. 3B). Significant increases in Na^+-K^+-ATPase activity were found only at day 1 in both salinities (Kruskal-Wallis non-parametric test). The two-way ANOVA (table I), with salinity and fish size as main factors, showed no significant effect from both factors on Na^+-K^+-ATPase activity in saltwater transferred

Table I. *Significant effects of salinity and fish size on gill ATPase activity after the transfer to either brackishwater or seawater* (two-way ANOVA).
Logarithmic transformations were performed to reach normality if necessary. Significant effects were considered for $p < 0.05$ unless any other statement.

		Brackishwater		Seawater	
	Source of variation	F	p	F	p
Na^+-K^+-ATPase	Salinity	1.059	n.s.	0.534	n.s.
	Size	2.047	n.s.	0.350	n.s.
	Salinity vs size	0.972	n.s.	0.407	n.s.
Ca^{2+}-ATPase	Salinity	5.104	<0.001	2.436	<0.05
	Size	0.290	n.s.	2.287	n.s.
	Salinity vs size	0.320	n.s.	0.757	n.s.
HCO_3^--ATPase	Salinity	8.129	<0.05	9.560	<0.01
	Size	2.800	n.s.	2.320	n.s.
	Salinity vs size	2.731	n.s.	2.838	n.s.

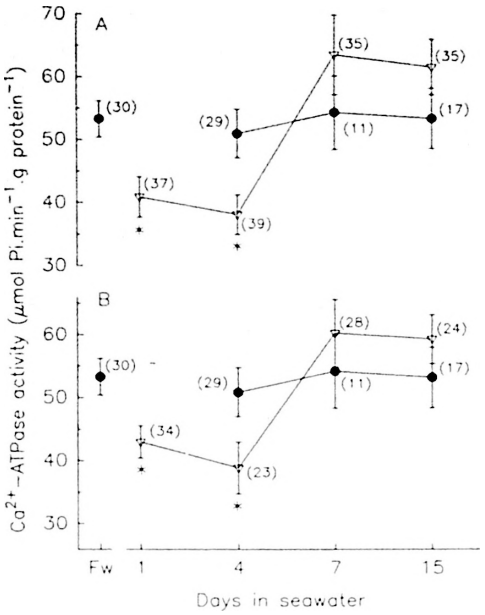

Figure 2. *Gill Ca^{2+} dependent ATPase activity in seawater (A) and brackishwater (B) transferred rainbow trout.*
Legend as in fig. 1.

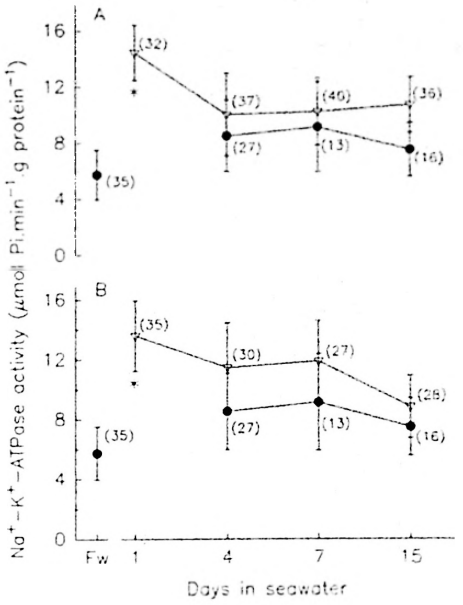

Figure 3. *Gill Na^+-K^+-ATPase activity in seawater (A) and brackishwater (B) transferred rainbow trout.*
Each point represents the mean ± SEM of the number of fish given in brackets. The asterisks represent significant differences ($p < 0.01$) from freshwater values obtained from Kruskal-Wallis nonparametric test. Freshwater values are denoted using circles and saltwater values using triangles.

trout. In spite of this result, Na^+-K^+-ATPase activity was consistently higher in both salinities than in the freshwater controls.

Discussion

A bicarbonate-dependent ATPase has been characterized in trout gills (17). HCO_3^-–ATPase is stimulated by both bicarbonate and chloride, and can be related to chloride uptake through gill epithelia in freshwater-adapted trout (3). Several studies indicate that the kinetic characteristics of HCO_3^- ATPase are not modified in response to seawater adaptation in the trout (17), *Anguilla japonica* (12) or Atlantic eel (25). However, Ip *et al.* (14) found an increase in gill HCO_3^-–ATPase activity when external salinity was lowered from 36 to 3.6 ppt in the mudskipper *Boleophtalmus boddaerti.* In addition, a negative correlation with Na^+-K^+-ATPase activity was also found after the transfer to low salinity environments.

In this study HCO_3^-–ATPase activity decreased in response to the transfer to both saline media, 9 and 28 ppt (fig. 1), showing significant differences from freshwater fish, in agreement to the resuls obtained by Ip *et al.* (14). However, Na^+-K^+-ATPase dynamics obtained in our study did not show the characteristical trend of a well seawater-adapted fish (13). Thus, increases in gill Na^+-K^+-ATPase

activity in seawater after 4 days have been described in Atlantic salmon (24), coho salmon (13) and Atlantic eel (6). The increase and mainteinance of gill Na^+-K^+-ATPase activity enable the fish to get rid of the excess of NaCl generated by the increased drinking rates in response to seawater (2, 33).

Despite of the low gill Na^+-K^+-ATPase activity found in our study, the fish mortality accumulated never exceeded 10 %. The lowered HCO_3^-–ATPase activity involved in choride uptake in freshwater-adapted trout, could have a contribution to the regulation of monovalent ion levels in rainbow trout after direct transfer to highly saline media when gill Na^+-K^+-ATPase activity remains low. However, an activation of gill Na^+-K^+-ATPase has been found in rainbow trout transferred gradually to seawater (J. FUENTES, in preparation).

Ca^{2+} dependent ATPase dynamics obtained after the transfer of rainbow trout to saltwater (fig. 2) showed an initial decrease (1-4 days) followed by a further recovery up to the values in freshwater or even higher (7-15 days). The response of Ca^{2+}-ATPase to changed external salinity often differs. FENWICK (6) reported lowered Ca^{2+}-ATPase activities in seawater transferred *Anguila anguila,* MA *et al.* (22) described unchanged ATPase activities in seawater-adapted rainbow trout, whereas HO and CHAN (12) found a consistent increase of the activity in seawater adapted *Anguila japonica.*

Two different Ca^{2+}-ATPases have been described in fish gills (5), with low and high affinity to calcium (8, 9). Both high and low Ca^{2+}-ATPases affinities are found in gill epithelia independently of external calcium concentration (23), and both can be measured in an incubation medium in the presence of high calcium concentration and in the absence of magnesium, which inhibits the high affinity Ca^{2+}-ATPase (23). In freshwater teleosts the active calcium uptake from the medium is essential to replace the loss of calcium induced in a hypoosmotic environment. FLIK *et al.* (7) proposed that the active calcium uptake in freshwater-adapted fish through gill epithelia takes place by means of the high affinity Ca^{2+}-ATPase and denied a role for the Ca^{2+}-ATPase low affinity. However, NAON and MAYER-GOSTAN (25) suggested that the low affinity Ca^{2+}-ATPase could be involved in the cellular calcium movements when the permeability of the apical membranes is increased.

The decrease of Ca^{2+} dependent ATPase activity detected in this work during the initial 4 days in seawater, might account for the inhibitory effect of increased external concentrations of both magnesium and calcium on the high affinity Ca^{2+}-ATPase activity (23, 26). The further increase in activity up to freshwater values could depend on the Ca^{2+}-ATPase low affinity, being therefore a consequence of the loss of inperviousness induced by the cytologic rearrangement of gill epithelium during seawater exposure (16, 30). In this work, both high and low affinity Ca^{2+} dependent ATPase activities are measured in the same incubation mixture used. Nevertheless, unchanged gill Na^+-K^+-ATPase activity indicates that the epithelial rearrangement described in seawater transferred salmonids from the 3rd or 4th day onwards, could not be fully developed, as the increase in gill Na^+-K^+-ATPase activity is found after proliferation of both chloride and accessory cells, and the loss of apical imperviousness in gill epithelia (13, 27, 30).

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J. FUENTES, J. L. SOENGAS y E. REBOLLEDO. *Actividades HCO_3^--ATPasa y ATPasa Ca^{2+} dependiente en las branquias de la trucha arco iris después de la transferencia al agua salobre y al agua de mar.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (2), 93-100, 1995.

Se estudia el efecto de la exposición al agua de mar y al agua salobre sobre las actividades HCO_3^--ATPasa y ATPasa Ca^{2+} dependiente en las branquias de la trucha arco iris. La actividad HCO_3^--ATPasa disminuye en respuesta a la transferencia a ambas salinidades. La actividad ATPasa Ca^{2+} dependiente, disminuye en respuesta a ambas salinidades durante el período inicial (1-4 días) y recupera valores de agua dulce a partir del día 7 días en agua salada. No se detecta efecto sobre el tamaño de las truchas en los dos parímetros analizados, en respuesta a la salinidad. Los resultados obtenidos se discuten en términos de salinidad y adaptación a largo plazo al agua salada.

Palabras clave: Trucha arco iris, Adaptación al agua de mar, Branquias, Calcio, Calcio ATPasa, Bicarbonato ATPasa.

References

1. Bath, R. N. and Eddy, F. B. (1979): *J. Comp. Physiol.*, 134, 351-357.
2. Boeuf, G. (1993): In "Fish Ecophysiology" (J. C. Rankin and F. B. Jensen, eds). Chapman and Hall, London. pp. 105-135.
3. Bornancin, M., De Renzis, G. and Naon, R. (1980): *Am. J. Physiol.*, 238, R251-R259.
4. De Renzis, G. and Bornancin, M. (1984): In "Fish Physiology" (W. S. Hoar and D. J. Randall, eds). Academic Press, New York. Vol. XB, pp. 65-104.
5. Doneen, B. A. (1981): *J. Comp. Physiol.*, 145, 51-61.
6. Fenwick, J. C. (1979): *Comp. Biochem. Physiol.*, 62B, 67-70.
7. Flik, G., Wendelaar-Bonga, S. E. and Fenwick, J. C. (1983): *Comp. Biochem. Physiol.*, 67B, 745-754
8. Flik, G., Wendelaar-Bonga, S. E. and Fenwick, J. C. (1984): *Comp. Biochem. Physiol.*, 76B, 521-524
9. Flik, G., Wendelaar-Bonga, S. E. and Fenwick, J. C. (1984): *Comp. Biochem. Physiol.*, 79B, 9-16.
10. Flik, G. and Perry, S. F. (1989): *J. Endocrinol.*, 120, 75-82.
11. Goss, G. G., Laurent, P. and Perry, S. F. (1992): *Cell Tiss. Res.*, 268, 539-552.
12. Ho, S.M. and Chan, D. K. O. (1980): *Comp. Biochem. Physiol.*, 67B, 639-645.
13. Hoar, W.S. (1988): In "Fish Physiology" (W. S. Hoar and D. J. Randall, eds). Academic Press, New York. Vol. XIB.
14. Ip, Y. K., Lee, C. G. L., Low, W. P. and Lam, T. J. (1991): *Fish. Physiol. Biochem.*, 9, 63-68.
15. Johnsson, J. and Clarke, W. C. (1988): *Aquaculture*, 71, 247-263.
16. Karnaky, K. J. Jr. (1986): *Amer. Zool.*, 26, 209-224.
17. Kestetler, T. H. and Kirschner, L. B. (1974): *Comp. Biochem. Physiol.*, 48B, 581-589.
18. Le Bel, D., Poirier, C. G. and Beaduoir, A. R. (1978): *Anal. Biochem.*, 85, 86-89.
19. Lin, H. and Randall, D. J. (1991): *J. exp. Biol.*, 161, 119-134.
20. Lin, H. and Randall, D. J. (1993): *J. exp. Biol.*, 180, 163-174.
21. Lowry, O. H., Rosenbrough, N. J., Farr, A. C. and Randall, R. J. (1951): *J. Biol. Chem.*, 193, 265-275.
22. Ma, S.W.Y., Shauri, Y. Messer H.H. and Copp, D.H. (1974): *Biochim. Biophys. Acta*, 345, 243-251.
23. Mayer-Gostan, N. and Naon, R. (1992): *Fish Physiol. Biochem.*, 10, 75-89.
24. McCormick, S. D., Moyes, C. D. and Ballantyne, J. S. (1989): *Fish Physiol. Biochem.*, 6, 243-254.
25. Naon, R. and Mayer-Gostan, N. (1989): *Am. J. Physiol.*, 256, R313-R322.
26. Parker, D. B., McKeown, B. A. and McDonald, T. S. (1985): *Comp. Biochem. Physiol.*, 81A, 149-156.
27. Payan, P., Girard, J. P. and Mayer-Gostan, N. (1984): In "Fish Physiology". (W. S. Hoar and D. J. Randall, eds). Academic Press, New York. Vol. X, pp 39-64.
28. Perry, S. F. and Flik, G. (1988): *Am. J. Physiol.*, 254, R491-R498.

29. Perry, S. F. and Randall, D. J. (1981): *J. exp. Zool.*, 215, 225-228.
30. Pisam, M., Boeuf, G., Prunet, P. and Rambourg, A. (1990): *Am. J. Anat.*, 187, 21-31.
31. Rey, P., Rozas, G., Andres, M. D., Aldegunde, M. and Rebolledo, E. (1990): *J. interdiscip. Cycle Res.*, 21, 65-74.
32. Shepard, K. L. (1981): *J. exp. Biol.*, 90, 115-121.
33. Usher, M. L., Talbot, C. and Eddy, F. B. (1988): *Aquaculture*, 73, 237-246.
34. Wendelaar-Bonga, S. E. and Flik, G. (1982): In "Comparative Endocrinology of Calcium Regulation" (C. Oguro and P. K. T. Pang, eds). Japan Scientific Societies Press, Tokyo. pp 21-26.