Turkish Journal of Veterinary & Animal Sciences

Volume 29 | Number 6 Article 7

1-1-2005

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Turk J Vet Anim Sci
29 (2005) 1259-1266

Research Article

Effects of Salinity on the Osmoregulatory Functions of the Gills in Nile Tilapia (*Oreochromis niloticus*)

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Received: 17.09.2004

Abstract: Changes in branchial Na^+-K^+-ATPase activity, and the number and size of chloride cells resulting from the transfer of fish into seawater were investigated in tilapia (*Oreochromis niloticus*) (22.03 ± 0.91 g), which were transferred to full strength seawater (36‰) directly and for 14 days. Whole mortality occurred when the tilapia were transferred into seawater directly. That is, no acclimation was allowed. Branchial chloride cell numbers decreased after seawater exposure, whereas a gradual increase was observed in chloride cell sizes. However, the chloride cells of seawater-adapted individuals showed a 2-fold increase in size ($P < 0.05$). Initially 5‰ and 10‰ salinity resulted in lowered branchial Na^+-K^+-ATPase activity but then this activity increased and the highest activity was at 25‰ salinity ($P < 0.01$). This study demonstrated the effects of high salinity through direct and gradual acclimations on branchial Na^+-K^+-ATPase activity and chloride cell abundance.

Key Words: *Oreochromis niloticus*, salinity, gill, osmoregulation

Tuzluluğun Nil Tilapyası'nda (*Oreochromis niloticus*) Solungaç Osmoregülasyon Fonksiyonlarına Etkileri

Özet: Deniz suyuna (‰36) doğrudan ve 14. günde transfer edilen tilapyalarda (*Oreochromis niloticus*) (22,03 ± 0,91 g), çevresel tuzluluğa bağlı olarak solungaç Na^+-K^+-ATPaz enzim aktivitesinde, klorit hücre sayısı ve boyutlarında meydana gelen değişimler incelenmiştir. Doğrudan deniz suyuna transfer edilen balıklarda % 100 ölüm olmuştur. Deniz suyuna kademeli transferden sonra solungaç klorit hücre sayısı azalırken, klorit hücre boyutlarında artış kaydedilmiştir. Deniz suyundaki tilapyaların solungaç klorit hücrelerinde yaklaşık iki katı bir büyüme görülmüştür ($P < 0,05$). Solungaç Na^+-K^+-ATPaz aktivitesi ‰ 5 ve ‰ 10 tuzluluk oranlarında düşmüş, fakat daha sonra artmış ve ‰ 25 tuzlulukta en yüksek değerine ulaşmıştır ($P < 0,01$). Bu çalışma, doğrudan ve kademeli adaptasyon sonucu yüksek tuzluluğun solungaç Na^+-K^+-ATPaz enzim aktivitesi ve klorit hücre yoğunluğu üzerine etkilerini ortaya koymaktadır.

Anahtar Sözcükler: *Oreochromis niloticus*, tuzluluk, solungaç, osmoregülasyon

Introduction

Tilapia are important species, especially in tropical aquaculture, and show varying degrees of tolerance to salinity. Euryhaline tilapia are suitable biological models for studying the osmoregulatory mechanisms in teleost fish. The adaptive capacity of tilapia to different salinities depends on the integrated osmoregulatory function of numerous organs, mainly the gills, digestive tract and kidney (1). The gills of teleost fish play an important role in ion regulation (2,3). The adaptation of tilapia to saline water involves some functional changes in gill epithelium chloride cells (CCs) and Na^+-K^+-ATPase activities. The mitochondria-rich CCs are found to be sparsely distributed on the filament, in the interlamellar regions, and at the bases of lamellae (4,5). The CCs have been identified as the only elements of the gill epithelium undergoing clear modifications in euryhaline fish during adaptation to different salinities (1). These cells are the main location of the gill Na^+-K^+-ATPase (3,6). Increased salinity results in the augmentation of Na^+-K^+-ATPase activity as well as morphological changes in CCs (3,7,8).

The aim of the present study was to determine the effects of high salinity on gill Na^+-K^+-ATPase activity and CC abundance in *Oreochromis niloticus*.

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Materials and Methods

Materials

The experiment was carried out at Ege University Fisheries Faculty, Aquaculture Department, İzmir, Turkey. The 108 fish used were Nile tilapia (*Oreochromis niloticus*), raised in freshwater, weighing 22.03 ± 0.91 g and kept in a aquaria 0.6 kg/0.16 m^3 with a 12L:12D photoperiod. Water temperature was controlled at 24 ± 1.5 °C. Composition of the external media is given in Table 1.

Gradual and direct transfer methods were applied for the acclimatization of *O. niloticus* to 36‰ salinity. Experimental fish were gradually acclimatized to 36‰ for 14 days at a rate of 5‰ every 2 days. The other groups were transferred to 36‰ directly. Some fish remained in freshwater (FW) as a control group. Four individuals were sampled for gill analysis in every adaptation phase 48 h after transfer. Feeding was not undertaken during the trial period. Measurements were obtained in triplicate.

Quantitative and Morphometric Analysis of Gill Chloride Cells

After anesthetizing with 0.3 ml phenoxyethanol/l for 1 min, the fish were killed and the gills from FW and seawater (SW) adapted individuals were immediately excised. The right gill of each fish was fixed in buffered formaldehyde (100 ml formaldehyde – 40%, 4 g $NaH_2PO_4 \cdot H_2O$, 6 g $NaHPO_4$, 900 ml distilled water). The left gill was used for Na^+-K^+-ATPase analysis. Sections of fixed specimens (5 µm) were cut and stained with Alcian blue for light microscopic investigation (9,10). The number of CCs and their sizes were estimated in 4 fish sampled from each adaptive condition. CCs were counted on sections of selected areas of 10 inter-lamellar regions and then the mean CC density was calculated. CC size was estimated by measuring the sectional area of the cell using an ocular micrometer (1).

Determination of Branchial Na^+-K^+-ATPase Activity

Branchial Na^+-K^+-ATPase activity was determined according to the modified methods described by Canli and Stagg (11) and Ay et al. (2). The fish were anesthetized and killed immediately. The left gill was removed and kept at -30 °C until assayed. Frozen filaments were weighed (200 mg) and homogenized in 2 ml of buffer containing 250 mmol sucrose, 100 mmol imidazol - pH 7.8, and 5 mmol EDTA (Sigma). Homogenization of the filaments was conducted at +4 °C using a homogenizer (IKA Labortechnik, Homogenizer T8.10). Homogenates were centrifuged at 1000 g for 15 min (at +4 °C). ATPase assays were carried out with supernatants within 1 h. ATPase activity was measured by the determination of the inorganic phosphate (P_i) liberated from the hydrolysis of ATP. All assays were carried out in triplicate. The final assay concentrations of the chemicals used here were 135 mmol Tris-HCl (pH 7.4), 100 mmol NaCl, 10 mmol KCl, 6 mmol $MgCl_2$, 0.1 mmol EDTA, 1.5 mmol ouabain, and 6 mmol ATP. Buffer solution (1600 ml) was pre-incubated at 37 ºC for 5 min and then the reaction was started by adding 200 ml of homogenate and 200 ml of ATP. The incubation lasted 30 min and the incubation medium was shaken at 100 rev/min during this time. The reaction was stopped after 30 min by placing the samples on ice and adding a cirrasol acid molybdate mixture. Inorganic phosphate was measured by the determination of the soluble yellow complex of cirrasol acid molybdate at 390 nm (Jenway 6305 UV/Vis spectrophotometer). Samples were compared with standards of KH_2PO_4 phosphate content. Protein content was determined according to the method described by Lowry et al. (12) and bovine serum albumin was used as a standard. Na^+-K^+-ATPase activity was calculated as the difference between the inorganic phosphate liberated in the absence and presence of ouabain, expressed as micromoles of inorganic phosphate per milligram of protein per hour.

Table 1. Water chemistry variables (mean ± S.E., n = 5) for seawater (SW) and freshwater (FW) conditions.

Experimental Conditions	Salinity (g l^{-1})	Ca^{+2} (mg l^{-1})	Mg^{+2} (mg l^{-1})	HCO_3 (mg l^{-1})	Total hardness (mg l^{-1})	Dissolved Oxygen (mg l^{-1})	pH
SW	36.5 ± 0.21	462.22 ± 39.91	1497.09 ± 16.55	165.77 ± 22.11	7306.67 ± 93.33	4.26 ± 0.06	8.12 ±0.09
FW	0.45 ± 0.05	170.97 ± 7.05	77.82 ± 4.86	287.6 ± 29.48	746.67 ± 37.12	4.44 ± 0.08	7.46 ± 0.02

Statistical Analysis

All the means of data were expressed with their standard errors. Analysis of data was carried out using SPSS. One-way ANOVA was used to examine differences among the experimental groups and means were analyzed by the LSD test. Statistically significant differences were expressed as $P < 0.05$. The relationships between Na^+-K^+-ATPase activity and CC were tested by regression and correlation analyses.

Results

Branchial Chloride Cell Number and Size

The CCs of SW-adapted fish showed an approximately 2-fold increase in size ($P < 0.05$). Sectional areas of the CCs were found to be lowest for FW fish and SW fish transferred directly. Following gradual adaptation to SW, the sectional area of CCs showed a significant increase and the size was largest in fish acclimated to 36‰ ($P < 0.05$) (Table 2) (Figures 1-4). While the number of branchial CCs was highest in fish acclimated to 15‰, the lowest CC density was found at 36‰ salinity ($P < 0.05$) (Table 2).

The number of branchial CCs was negatively correlated with salinity ($r = -0.5310$; $P < 0.01$), but CC size was positively correlated with environmental salinity ($r = 0.8518$; $P < 0.01$).

Branchial Na^+-K^+-ATPase Enzyme Activity

Branchial Na^+-K^+-ATPase activity was highest in 25‰ and lowest in 10‰ acclimated fish (Table 2). The results of regression analysis showed that branchial Na^+-K^+-ATPase activity had a negative correlation with salinity up to 10‰ ($r = -0.8679$; $P < 0.01$). After this level, however, activity of Na^+-K^+-ATPase showed a positive relationship with salinity up to 25‰ ($r = 0.7956$; $P < 0.01$). Na^+-K^+-ATPase activity was significantly elevated over FW values at 25‰, but returned to FW levels at the end of acclimation.

According to the results, Na^+-K^+-ATPase activity is positively correlated with CC size ($r = 0.5279$; $P < 0.01$) (Figure 5), whereas there is no correlation between number of CCs and Na^+-K^+-ATPase activity ($r = -0.2958$; $P > 0.05$) (Figure 6).

Discussion

In this study, the physiological changes in the gills of *O. niloticus* due to increased salinity were determined during the acclimation process. Branchial Na^+-K^+-ATPase activity had a negative correlation with salinity up to 10‰ ($r = -0.8679$; $P < 0.01$). However, after this level, a highly significant positive correlation was found between Na^+-K^+-ATPase and salinity up to 25‰ ($r = 0.7956$; $P < 0.01$). Vonck et al. (13) suggested that both FW and 75% or more SW appear to require enhanced ion

Table 2. Branchial Na^+-K^+-ATPase activity (μmol P_i mg protein^{-1} h^{-1}), Chloride cell number (CC per filament) and size (μm^2) in the various acclimations. (FW: Freshwater, DA: Direct acclimation).

Acclimation phases	Time after transfer (h)	Sectional area of chloride cells	Number of chloride cells	Branchial Na^+-K^+-ATPase Activity
FW	0	72.78 ± 3.29 ^{a,b}	87.78 ± 8.17	425.14 ± 49 ^{a,d}
5‰	48	86.29 ± 4.04 ^{b,c}	194.68 ± 13.54 ^{A}	168.93 ± 9.16 ^{b,e,f}
10‰	96	82.88 ± 2.57 ^{b,c}	150 ± 26.76 ^{b}	128.29 ± 6.15 ^{e,f}
15‰	144	92.36 ± 4.87 ^{c,d}	195.43 ± 8.17 ^{a}	273.11 ± 24.97 ^{a,e,f}
20‰	192	103.89 ± 4.99 ^{d,e}	180.20 ± 10.75 ^{a,b}	417.95 ± 80.74 ^{a,d}
25‰	240	118.36 ± 5.37 ^{e,f}	45.11 ± 9.59 ^{c}	539.10 ± 105.34 ^{c,d}
30‰	288	123.92 ± 20.86 ^{f}	46.03 ± 11.32 ^{c}	273.47 ± 52.47 ^{a,e,f}
36‰	336	151.06 ± 41.48	41.38 ± 8.99 ^{c}	451.13 ± 101.31 ^{a,c,d}
DA	3-4	60.8 ± 11.48 ^{a}	44.74 ± 2.77 ^{c}	314.14 ± 14.04 ^{a,b}

*All data are expressed as mean values ± S.E. (n = 4).

*Within the same columns, values with different superscripts are significantly different ($P < 0.05$).

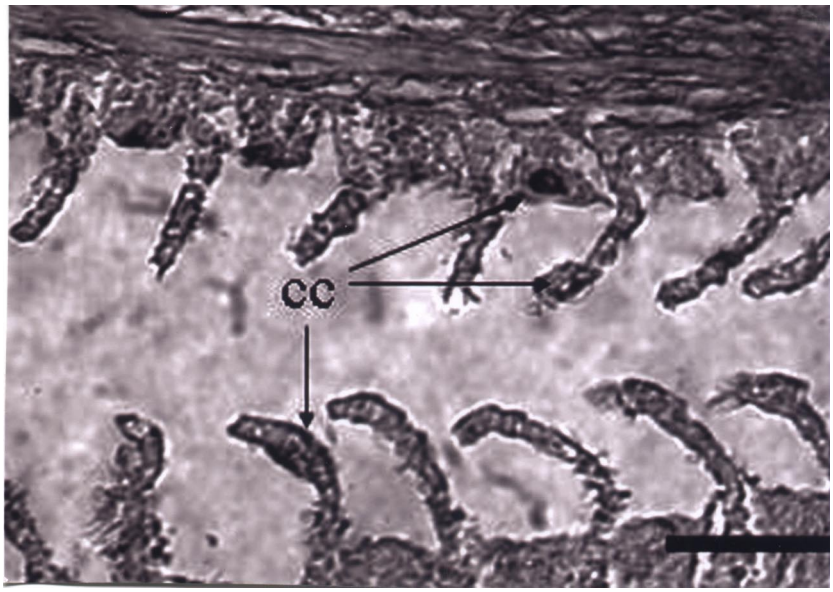

Figure 1. Branchial chloride cells in fish in the freshwater phase (Buffered formaldehyde, Alcian blue, x400). CCs: Chloride cells, Bar = 50 μm.

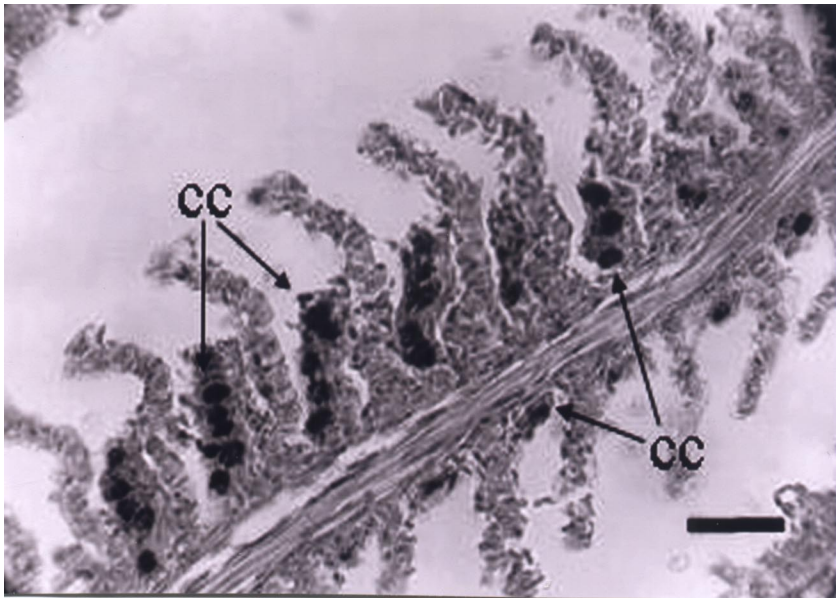

Figure 2. Branchial chloride cells in fish acclimated to 5‰ salinity (Buffered formaldehyde, Alcian blue, x400). CCs: Chloride cells, Bar = 50 μm.

regulatory capacity. According to our results, the lowest Na^+-K^+-ATPase activity in fish was observed at 5-10‰ salinity, which is iso-osmotic to the fish (Table 2). The metabolic cost of osmoregulation is reduced in brackish water, because the blood-medium osmotic gradient is minimal (7,14,15). Results from previous studies indicate that an iso-osmotic environment causes a reduction in gill Na^+-K^+-ATPase activity, whereas this activity is elevated in a hypo- or hyper-osmotic environment (13,16-18).

The highest branchial Na^+-K^+-ATPase activity was measured at 25 ‰ salinity. A significant decrease occurred at 30‰ salinity ($P < 0.05$). Bashamohiden and

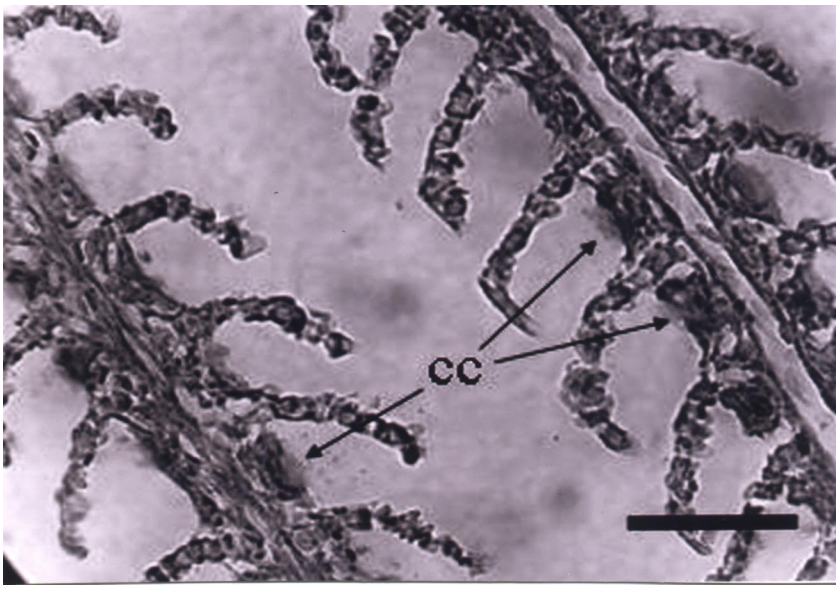

Figure 3. Branchial chloride cells in fish acclimated to 36‰ salinity (Buffered formaldehyde, Alcian blue, x400). CCs: Chloride cells, Bar = 50 µm.

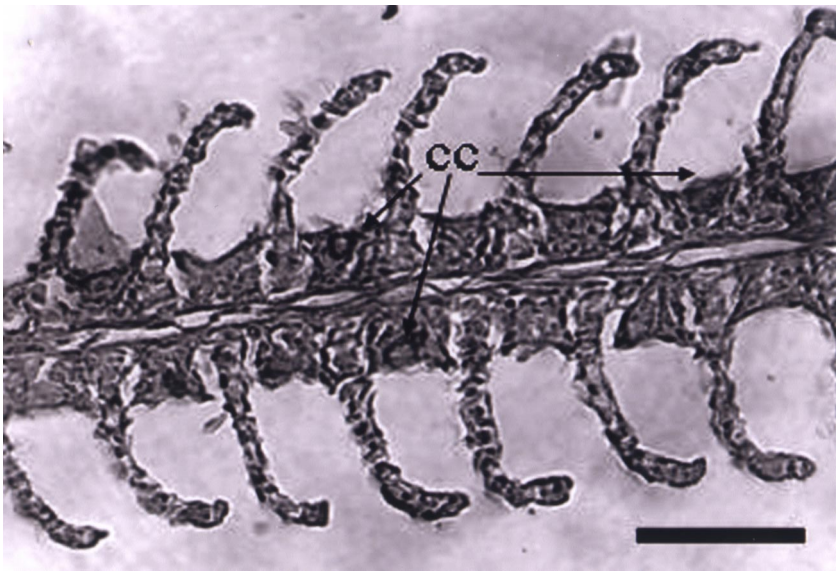

Figure 4. Branchial chloride cells in fish transferred directly to 36‰ salinity (Buffered formaldehyde, Alcian blue, x400). CCs: Chloride cells, Bar = 70 µm.

Parvatheeswararao (19) reported that the osmoregulation decreased at 50% SW and increased at 75% SW. A decrease once again occurred at 100% SW salinity. The authors suggested that the prior adaptation of the fish to 75% SW with high energy cost facilitated its subsequent adaptation to 100% SW. Results from the present study indicate that there was a pre-adaptation at 25‰ salinity to subsequent higher salinities. Hwang et al. (8) revealed that pre-acclimatization to 20‰ salinity allows *O. mossambicus* to have sufficient time to regulate osmoregulatory mechanisms for subsequent exposure to 30‰ salinity. This concept may explain why gradually

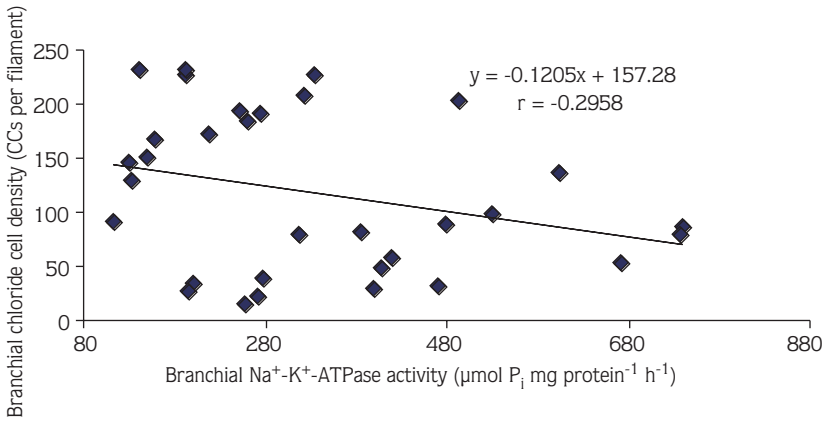

Figure 5. Relationship between branchial Na^+-K^+-ATPase enzyme activity and number of chloride cells.

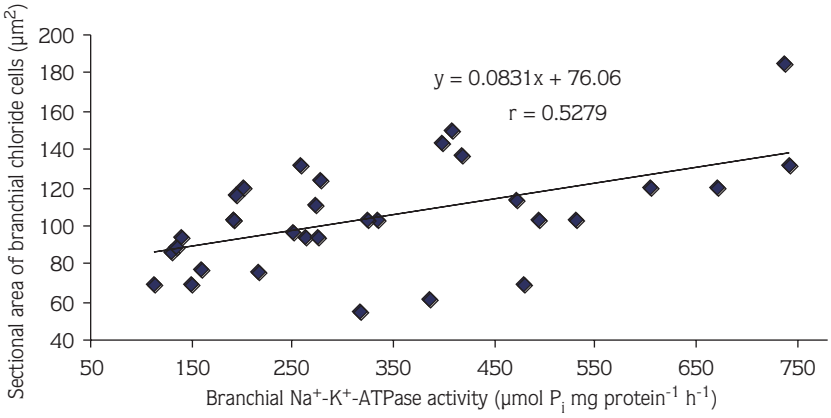

Figure 6. Relationship between branchial Na^+-K^+-ATPase enzyme activity and area of chloride cells.

acclimated fish can perform better than their directly acclimated counterparts (7).

O. niloticus died approximately 4 h after being transferred to 36‰ salinity directly. After direct transfer, Na^+-K^+-ATPase activity was lower than FW values ($P > 0.05$). Weng et al. (3) reported that *O. mossambicus* died 4 h after direct transfer from FW to 35‰ seawater. The authors suggested that direct transfer from FW to hyper-saline conditions cause severe dehydration, which might impair the function of Na^+-K^+-ATPase activity and this impairment may be the reason for mortality.

Following gradual acclimation to 36‰ salinity, the sectional area of CCs showed a significant increase ($P < 0.05$) and CC size was positively correlated with environmental salinity ($r = 0.8518$; $P < 0.01$). Similar increases in the size of CCs during SW acclimation have been reported for *O. niloticus* and *O. mossambicus* by Cioni et al. (1). Previous studies reported that acclimation of fish from FW to SW directly causes severe dehydration (3,8). The results of the present study concerning the lowest sectional area of CCs in fish acclimated to 36‰ salinity directly may suggest that decreasing size of CCs was associated with severe dehydration due to direct transfer.

Cioni et al. (1) observed that the number of CCs did not increase in SW-adapted *O. niloticus* and *O. mossambicus*. In SW-adapted *O. mossambicus* CCs showed an increase in number, which was revealed by

Nolan et al. (17) and Lee et al. (18). However, Brown (20) showed that the number of CCs was greater in FW-adapted trout than SW-adapted trout. We found that the correlation between CC density and environmental salinity was negative ($r = -0.5310$; $P < 0.01$). CCs in FW fish were localized to the filament with very few on the lamellae. However, with increasing salinity CCs were found only on interlamellar regions. The reduction in the number of lamellar CCs following the transfer to SW suggest that lamellar CCs become obsolete during SW adaptation and degenerate (5,21). This process may be one of the survival strategies of fish to increasing salinity (21,22).

CCs contain the bulk of the branchial Na^+-K^+-ATPase, which is located in their extensive tubular membrane system (3,6,16). The present study showed that following SW acclimation, Na^+-K^+-ATPase activity was positively correlated with CC size ($r = 0.5279$; $P < 0.01$). This relationship is explained by the amplification of the basolateral tubular system, which might be related to increased Na^+-K^+-ATPase activity (1). However, there was no correlation between number of CCs and Na^+-K^+-ATPase activity ($r = -0.2958$; $P > 0.05$). A positive correlation between Na^+-K^+-ATPase activity and CC density was reported for *O. mossambicus* (13,17,18) and for *Dasyatis sabina* (23). On the other hand, while some studies reported increased numbers of CCs, others reported no change in CCs during Na^+-K^+-ATPase activity for *Mugil cephalus* (24) and for *O. mossambicus* (25). These results demonstrated an uncoupling of the relationship between CCs density and Na^+-K^+-ATPase activity (26).

In conclusion, *O. niloticus* could tolerate high salinity (36‰) only by gradual acclimation; otherwise mortality occurred within 4 h of direct transfer. It was suggested that gradual acclimation was more effective than direct transfer in stimulating the osmoregulatory mechanisms. Further studies are needed to understand the combined effects of acclimation methods and salinity on the osmoregulatory organs of tilapia. Moreover, interactions from other physiological factors, water qualities, and stresses should also be taken into consideration.

Acknowledgments

This work was supported by a grant from the Scientific and Technical Research Council of Turkey (TÜBİTAK), grant number VHAG-1702.

References

1. Cioni, C., De Merich, D., Cataldi, E., Cataudella, S.: Fine structure of chloride cells in freshwater- and seawater-adapted *Oreochromis niloticus* (Linnaeus) and *Oreochromis mossambicus* (Peters). J. Fish Biol., 1991; 39: 197-209.
2. Ay, Ö., Kalay, M., Tamer, L., Canlı, M.: Copper and lead accumulation in tissues of a freshwater fish *Tilapia zillii* and its effects on the branchial Na^+,K^+-ATPase activity. Bull. Environ. Contam. Toxicol., 1999; 62: 160-168.
3. Weng, C.F., Chiang, C.C., Gong, H.Y., Chen, M.H.C., Lin, C.J.F., Huang, W.T., Cheng, C.Y., Hwang, P.P., Wu, J.L.: Acute changes in gill Na^+-K^+-ATPase and creatine kinase in response to salinity changes in the euryhaline teleost, Tilapia *(Oreochromis mossambicus)*. Physiol. Biochem Zool., 2002; 75: 29-36.
4. Greco, A.M., Fenwick, J.C., Perry, S.F.: The effects of soft-water acclimation on gill structure in the rainbow trout *Oncorhynchus mykiss*. Cell Tissue Res., 1996; 285: 75-82.
5. Perry, S.F.: The chloride cell: Structure and function in the gills of freshwater fishes. Annu. Rev. Physiol., 1997; 59: 325-347.
6. McCormick, S.D.: Methods for nonlethal gill biopsy and measurements of Na^+-K^+-ATPase activity. Can. J. Fish. Aquatic Sci., 1993; 50: 656-658.
7. Suresh, A.V., Lin, C.K.: Tilapia culture in saline waters: a review. Aquaculture, 1992; 106: 201-226.
8. Hwang, P.P., Sun, C.M., Wu, S.M.: Changes of plasma osmolarity chloride concentration and gill Na^+-K^+-ATPase activity in tilapia *O. mossambicus* during seawater acclimation. Mar. Biol., 1989; 100: 295-299.
9. Hinton D.E.: Histological Techniques. In: Schreck, C.B., Moyle, P.B., Eds. Methods for Fish Biology. Am. Fish. Soc., Maryland, 1990; 191-209.
10. Powell, M.D., Parsons, H.J., Nowak, B.F.: Physiological effects of freshwater bathing of Atlantic salmon (*Salmo salar*) as a treatment for amoebic gill disease. Aquaculture. 2001; 199: 259-266.
11. Canli M., Stagg R.M.: The effects of in vivo exposure to cadmium, copper and zinc on the activities of gill ATPases in the Norway lobster, *Nephrops norvegicus*. Arch. Environ. Contam. Toxicol., 1996; 31: 494-501.
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurements with the Folin phenol reagent. J. Biol. Chem. 1951; 193: 265-275.

13. Vonck, A.P.M.A., Wendelaar Bonga, S.E., Flik, G.: Sodium and calcium balance in Mozambique tilapia, *Oreochromis mossambicus*, raised at different salinities. Comp. Biochem. Physiol. A: Mol. Integr. Physiol., 1998; 119: 441-449.

14. Morgan, J.D., Sakamoto, T., Grau, E.G., Iwama, G.K.: Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp. Biochem. Physiol. A: Physiol., 1997; 117: 391-398.

15. Fontainhas-Fernandes, A., Monteiro, M., Gomes, E., Reis-Henriques, M.A., Coimbra, J.: Effect of dietary sodium chloride acclimation on growth and plasma thyroid hormones in tilapia *Oreochromis niloticus* (L.) in relation to sex. Aquacult. Res., 2000; 31: 507-517.

16. Avella, M., Berhaut, J., Bornancin, M.: Salinity tolerance of two tropical fishes, *O. aureus* and *O. niloticus*, Biochemical and morphological changes in the gill epithelium. J. Fish. Biol. 1993; 42: 243-254.

17. Nolan, D.T., Op't Veld, R.L.J.M., Balm, P.H.M., Wendelaar Bonga, S.E.: Ambient salinity modulates the response of the tilapia, *Oreochromis niloticus* (Peters), to net confinement. Aquaculture, 1999; 177: 297-309.

18. Lee, T.H., Hwang, P.P., Shieh, Y.E., Lin, C.H.: The relationship between 'deep-hole' mitochondria-rich cells and salinity adaptation in the euryhaline teleost, *Oreochromis mossambicus*. Fish Physiol. Biochem., 2000; 23: 133-140.

19. Bashamohiden, M., Parvatheeswararao, V.: Adaptations to osmotic stress in the freshwater euryhaline teleost, *Tilapia mossambica*. Zool. Anz., Jena., 1976; 197: 47-56.

20. Brown, P.: Gill chloride cell surface area is greater in freshwater adapted adult sea trout (*Salmo trutta*, L.) than those adapted to sea water. J. Fish Biol., 1992; 40: 481-484.

21. Hartl, M.G.J., Hutchinson, S., Hawkins, L.E., Grand, D.J.: Environmental levels of sediment-associated tri-*n*-butyltin chloride (TBTCl) and ionic regulation in flounders during seawater adaptation. Mar. Biol., 2001; 138: 1121-1130.

22. Madsen, S.S., McCormick, S.D., Young, G., Enderson, J.S., Nishioka, R.S., Bern, H.A.: Physiology of seawater acclimation in the striped bass, *Morone saxatilis* (Walbaum). Fish Physiol. Biochem., 1994; 13: 1-11.

23. Piermarini, P.M., Evans, D.H.: Effects of environmental salinity on Na^+/K^+-ATPase in the gills and rectal gland of a euryhaline Elasmobranch (*Dasyatis sabina*). J. Exp. Biol., 2000; 203: 2957-2966.

24. Ciccotti, E., Marino, G., Pucci, P., Cataldi, E., Cataudella, S.: Acclimation trial of *Mugil cephalus* juveniles to fresh-water - morphological and biochemical aspects. Env. Biol. Fish., 1994; 43: 163-170.

25. Van Der Heijden, A.J.H., Verbost, P.M., Eygenstein, J., Li, J., Wendelaar-Bonga, S.E., Flik, G.: Mitochondria-rich cells in gills of tilapia (*O. mossambicus*) adapted to fresh water or sea water: quantification by confocal laser scanning microscopy. J. Exp. Biol., 1997; 200: 55-64.

26. Deane, E.E., Kelly, S.P., Woo, N.Y.S.: Hypercortisolemia does not affect the branchial osmoregulatory responses of the marine teleost *Sparus sabra*. Life Sci., 2000; 66: 1435-1444.