

**CHANGES IN GILL ION TRANSPORT PROTEIN EXPRESSION
ASSOCIATED WITH ENVIRONMENTAL SALINITY. A TALE FROM
THE COHO SALMON (*ONCORHYNCHUS KISUTCH*)**

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Introduction

The ionic regulatory needs of teleost fishes living in fresh water and sea water are diametrically opposed so animals moving between these environments are presented with a physiologically challenging situation. These fishes must realign their transepithelial ion transport mechanisms to successfully adapt (ion uptake in freshwater and ion elimination in seawater). A number of salmonid species make these migrations and have thus been a popular group to study from both academic and commercial perspectives. Ion transport proteins (ATPases, symporters, antiporters and channels) and paracellular pathways form the basis of transepithelial ion movements. In the present paper, we will focus on the distribution of Na⁺,K⁺-ATPase, Cl⁻/HCO₃⁻ anion exchanger (AE), and Na⁺/H⁺ exchanger in the gills (and gut) of juvenile coho salmon (*Oncorhynchus kisutch*) which have been raised in fresh water or transferred to sea water. Of interest is the fate of the freshwater chloride cell (Cl⁻ uptake utilizing an apical AE; Goss et al. 1995) in fish transferred to sea water and NHE 2-like protein which appears to label the accessory cell type which is associated with NaCl elimination. (Wilson et al. 2000)

Materials and Methods

Juvenile coho salmon (*O. kisutch*) raised in freshwater or acclimated to seawater for 6 weeks were obtained from the Department of Fisheries and Oceans West Vancouver facility. Six animals were sampled from each group and gill and intestinal tissue fixed in Bouin's solution and embedded in paraffin. Ion transport proteins were localized using indirect immunofluorescence techniques employing non-homologous antibodies (Table 1).

Table 1. List of antibodies employed and their sources

Antibody	Antigen	Host	Source
Na ⁺ ,K ⁺ -ATPase	α-subunit	mouse	DSHB*
Na ⁺ /H ⁺ Exchanger	NHE-2 fusion protein	rabbit	Hoogerwerf et al. 96
Anion Exchanger	trout erythrocyte AE1	rabbit	Cameron et al. 96

*Developmental Studies Hybridoma Bank, University of Iowa, USA

Results and Discussion

Anion Exchanger (AE)

In freshwater coho salmon, the branchial epithelium has a typical teleostean population of mitochondria-rich (MR) cells which show high levels of Na⁺,K⁺-ATPase immunoreactivity (Fig1B). A sub-population of these MR cells in the lamellar epithelium show apical immunoreactivity for the AE1 (arrows; Fig1A) in addition to erythrocytes, which show strong immunoreactivity (Fig1A,D). The AE labeled freshwater MR cells are presumably active in freshwater Cl⁻ uptake (= Chloride Cells; apical Cl⁻/HCO₃⁻ anion exchanger; Goss et al. 1995, Wilson et al. 2000).

In coho salmon that have been acclimated to sea water, there is an absence of AE1 apical immunoreactivity (Fig1D) associated with MR cells (identified by high Na⁺,K⁺-ATPase immunoreactivity) (Fig1E). Predictably, the freshwater Cl⁻ uptake cells are lost in the seawater acclimated fishes. Apical AE1 immunoreactivity is, however, observed in enterocytes of seawater acclimated animals (data not shown). These intestinal cells are presumably involved in the HCO₃⁻ elimination reported by R.W. Wilson et al. 1996.

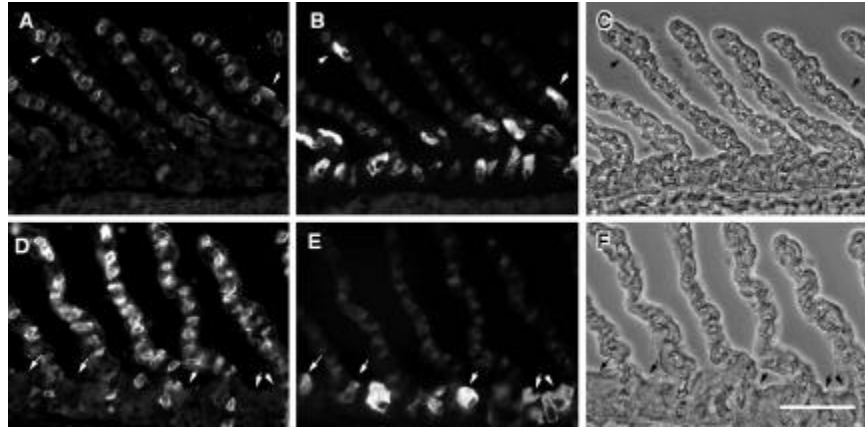


Figure 1 Immunohistochemistry of double labeled sections from the gills of coho salmon adapted to either freshwater (**ABC**) or seawater (**DEF**) showing the distributions of the band 3-like anion exchanger (AE1;**A,D**) and Na^+, K^+ -ATPase (**B,E**). The corresponding phase contrast images are shown for orientation (**C** and **F**, respectively). Scale bar = 50 μm .

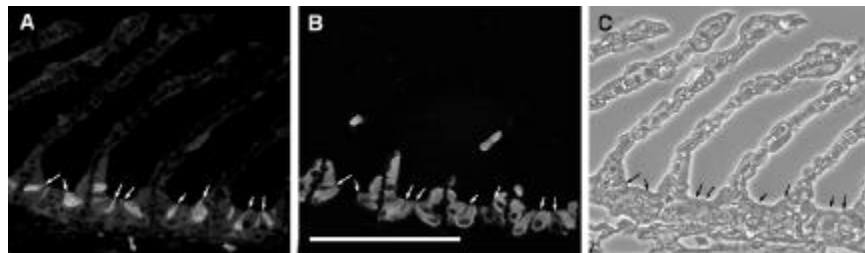


Figure 2 Double labeled section of the seawater adapted coho gill epithelium using a rabbit polyclonal antibody (597) against the NHE-2 (*arrows*; **A**) and mouse monoclonal antibody against the α subunit of Na^+, K^+ -ATPase (**B**). The corresponding phase contrast image is shown in (**C**). Scale Bar= 50 μm

Na^+/H^+ Exchanger (NHE)

NHE 2-like immunoreactivity is associated with cells in the interlamellar spaces that do not have high Na^+, K^+ -ATPase immunoreactivity (Fig2). These NHE-2 labeled cells appear to be accessory cells on the basis of their morphology and location. Although typically associated with NaCl elimination in seawater teleost fishes, these cells are also found in the freshwater fish sampled. These

results are consistent with the literature (Pisam and Rambourg 1991). However, the functional significance of the accessory cell labeling is not clear, although, in any case, the NHE-2 antibody may prove to be a useful marker for the accessory cell type in future studies.

Acknowledgements

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