$Na^+/K^+/2Cl^-$ COTRANSPORTER AND Na^+ K^+-ATPASE mRNA EXPRESSION IN THE SOUTH AMERICAN RAINBOW CRAB *CHASMAGNATHUS GRANULATUS* AFTER ACCLIMATION TO LOW AND HIGH SALINITY

Carlos Luquet[1], Mihaela Senek[2] and David W. Towle[3]

[1]University of Buenos Aires – Conicet, Pab. II Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina. [2]College of the Atlantic, 105 Eden Street Bar Harbor, ME 04609.
[3]Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672.

Electrophysiological evidence suggests that the posterior gills of the euryhaline crab *Chasmagnathus granulatus* take up ions from a dilute medium through basolateral Na^+ K^+-ATPase and apical $Na^+/K^+/2Cl^-$ cotransporter (NKCC) (Luquet et al., J. Exp. Biol. 205:71-77, 2002). However, the participation of the latter transporter could not be directly studied because of the impermeability of the cuticle to the inhibitor. In this study, we amplified, partially sequenced, and measured the abundance of Na^+ K^+-ATPase and NKCC mRNA in gill 3 (representative of anterior gills) and gill 7 (representative of posterior gills) of *C. granulatus* transferred from seawater to low salinity (2 ppt). Poly-A mRNA was reverse transcribed using oligo-dT and SuperScript II reverse transcriptase. cDNA was amplified by PCR using Sigma Red Taq DNA polymerase. Amplification products were extracted from electrophoresis gels using a Qiagen MinElute gel extraction kit and prepared for direct sequencing. We obtained partial sequences of 678 and 762 base pairs for *C. granulatus* Na^+ K^+-ATPase and NKCC respectively (Acc. Nos. AF548369 and AF548368) from which specific primers were designed. To measure mRNA, cDNA was amplified in the presence of SYBR Green dye using Qiagen Quantitect chemistry and the Stratagene MX4000 Multiplex Quantitative PCR System. A dilution series demonstrated a linear relationship between threshold cycle (C_t) and $\log_{10}$ of template availability. Transfer of crabs from 30 to 2 ppt salinity was accompanied by a significant increase in the abundance of Na^+ K^+-ATPase mRNA in gills, particularly in the posterior ones (Fig. 1A) starting at 24 hour after transfer. NKCC mRNA sharply decreased after the first 6 hours and then gradually increased in posterior gills, while no change was observed in anterior gills (Fig. 1B).

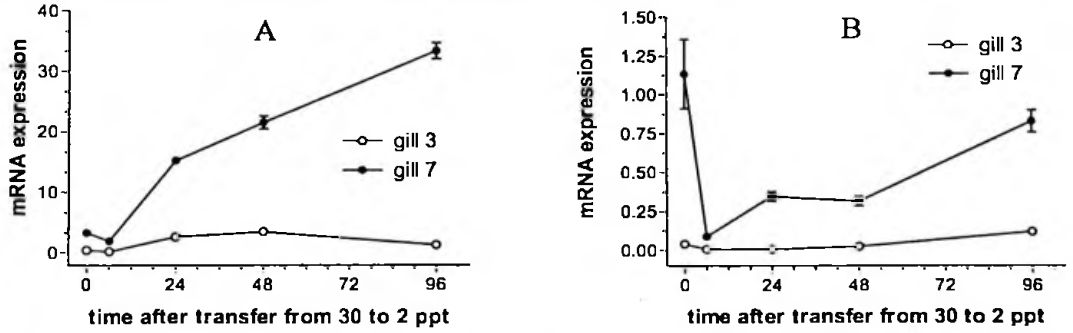

Fig. 1. mRNA expression in gills 3 and 7 of *Chasmagnathus granulatus* transferred from 30 to 2 ppt salinity measured by real-time PCR and SYBR green binding, normalized to posterior gill at 30 ppt. A: Na^+ K^+-ATPase, B: $Na^+/K^+/2Cl^-$ cotransporter. Time is given in hours.

Supported by MDIBL New Investigator Award to C. Luquet, NSF (IBN-0196423 and DBI-0100394), NCRR Biomedical Research Infrastructure Network (1P20RR16463-01), and the University of Buenos Aires (UBACYT X222).