$NA^+/K^+/2CL^-$ COTRANSPORTER mRNA EXPRESSION IN THE BLUE CRAB *CALLINECTES SAPIDUS* MEASURED BY REAL-TIME QUANTITATIVE PCR

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Euryhaline crabs osmoregulate in low salinities by taking up NaCl across the gill epithelium, driven by basolateral Na^++K^+-ATPase. Among the candidate apical transporters that may participate in this process is the $Na^+/K^+/2Cl^-$ cotransporter (NKCC), which has been amplified and sequenced from ion-transporting posterior gills of the blue crab *Callinectes sapidus* (Towle, Am. Zool. 38:114A, 1998; Accession Number AF190129). In the present study, we measured the abundance of NKCC mRNA in a variety of tissues and asked whether acclimation salinity might induce enhanced transcription of NKCC mRNA in posterior gills. Poly-A mRNA in 2 µg of total RNA was reverse transcribed using oligo-dT and SuperScript II reverse transcriptase in a final volume of 20 µl. Cotransporter cDNA in 1-µl aliquots 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 (Fig. 1).

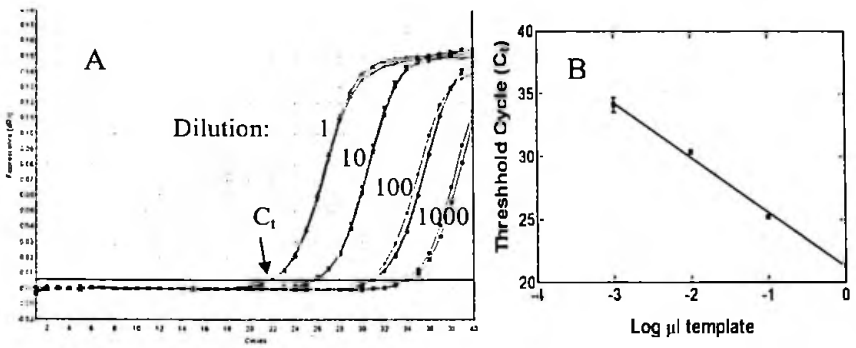

Fig. 1. Standardization of multiplex quantitative PCR system with a template dilution series (A) showing linear relationship between threshold cycle (C_t, shown for undiluted template) and $\log_{10}$ of template abundance (B).

Analysis of NKCC mRNA abundance in total RNA extracts of tissues from crabs acclimated to 35 ppt salinity revealed the highest expression in antennal gland, with intermediate expression levels in gills and hepatopancreas (Fig. 2). Heart, hypodermis, and skeletal muscle showed negligible levels of NKCC mRNA. Transfer of crabs from 35 to 5 ppt salinity was accompanied by a significant increase in the abundance of NKCC mRNA in gills, particularly in posterior gills. Supported by the National Science Foundation (DBI-0100394).

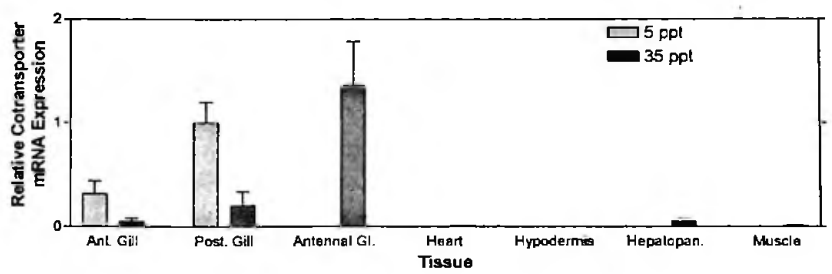

Fig. 2. NKCC mRNA expression in tissues of *Callinectes sapidus* acclimated to 35 (all tissues) or 5 ppt (gills only shown) measured by real-time PCR and SYBR green binding, normalized to posterior gill at 5 ppt.