Quantitative expression of carbonic anhydrase mRNA and protein-specific activity in the gills of the euryhaline green crab, *Carcinus maenas*, during low salinity acclimation

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The enzyme carbonic anhydrase (CA), a central molecular component of the physiological mechanism of active ion transport in the gills of euryhaline crustaceans, has been shown to be induced by about 8 fold during acclimation to low salinity[1]. In the green crab, this induction takes place over 48-96 hr after transfer, a time course that is consistent with the synthesis of new enzyme. Recent evidence, using semi-quantitatve PCR, showed that CA mRNA in the posterior, ion transporting gills, increases at 24 hr after transfer to low salinity, and protein-specific CA activity begins to increase at 48 hr post-transfer and continues to increase through 96 hr[2]. These results strongly suggest that CA induction, in response to low salinity exposure, is under transcriptional control. The advent of real time, quantitative PCR (qPCR) allows this hypothesis to be tested directly.

Green crabs were collected from Frenchman's Bay, maintained at 32 ppt seawater, and transferred directly to 11 ppt. Before transfer, and at various times after, subsets of crabs were killed and the gills dissected out for analysis. Anterior, respiratory gills (G3) were used as control tissue, and posterior, ion-transporting gills (G7) served as the experimental tissue. G3 and G7 from the right side of each crab were assayed for CA activity immediately upon dissection, and the corresponding gills from the left side of each crab were used for total RNA extraction. The RNA was reverse transcribed, and gene-specific primers for CA were used with the cDNA template for qPCR (Stratagene MX4000).

Critical cycle (Ct) values for anterior gills of crabs acclimated to 32 ppt averaged about 25, and they did not change over the time course of low salinity adaptation (Fig. 1). For posterior gills (G7), Ct values at 32 ppt were also about 25, but at 24 hr post-transfer, these values had dropped to 22, and they remained low throughout the 7-day experimental time course (Fig. 1).

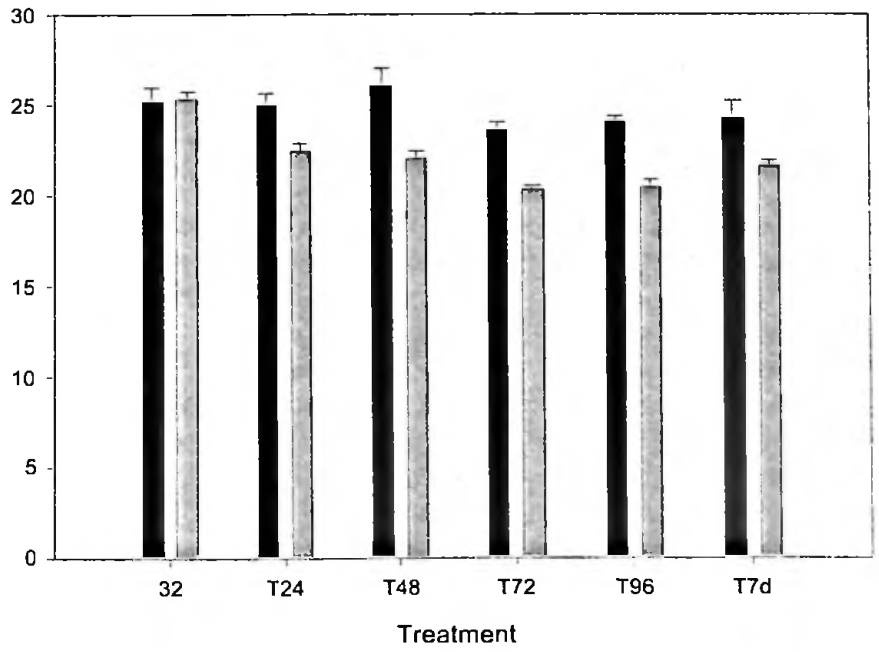

Figure 1. Critical cycle number for anterior (G3)(dark bars) and posterior (G7)(light bars) gills of green crabs acclimated to 32 ppt and transferred to 11 ppt for various times. Mean ± SEM (N=3-6).

By 72 hr post-transfer, Ct values were approximately 20. This represents an approximate 6-fold increase in CA mRNA expression at 24 hr after low salinity transfer, and a 10-fold increase by 72 hr.

CA activity in G3 in crabs acclimated to 32 ppt was low and did not change over the time course of low salinity acclimation (Fig. 2). CA activity in G7 was also low in crabs at 32 ppt, but this value doubled at 48 hr post-transfer and continued to increase throughout the 7-day time course (Fig. 2).

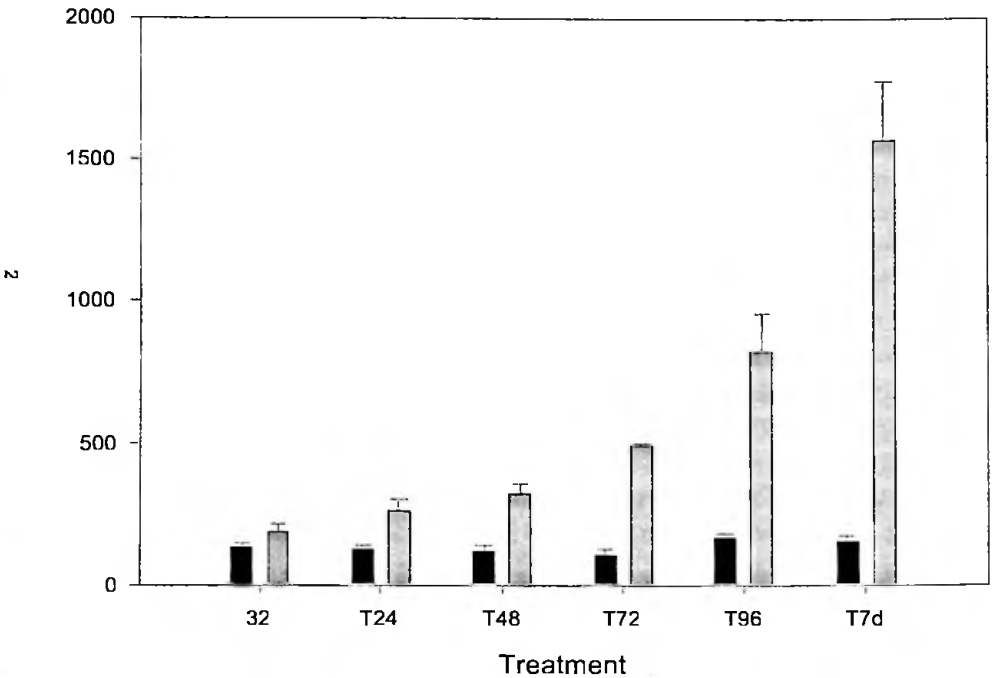

Figure 2. Carbonic anhydrase activity in anterior (G3)(dark bars) and posterior (G7)(light bars) gills of green crabs acclimated to 32 ppt salinity and transferred to 11 ppt. Mean ± SEM (N=5-8).

These results show more clearly that salinity-mediated CA induction occurs at the transcriptional level. CA mRNA expression is stimulated first, and the induction of protein-specific CA activity follows immediately thereafter. Further experimentation will show how this process is regulated.

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2. Henry, R.P., Gehnrich, S., Weihrauch, D., and Towle, D.W. Salinity-mediated carbonic anhydrase induction in the gills of the euryhaline green crab, *Carcinus maenas*. Comp. Biochem. Physiol. 136A:243-258. 2003.