Quantitative expression of branchial carbonic anhydrase in the euryhaline green crab, *Carcinus maenas*, in response to stepwise dilutions in salinity

Raymond P. Henry[1,2], Kim L. Thomason[1,2], & David W. Towle[2]

[1]Department of Biological Sciences
Auburn University, Auburn, AL 36849
[2]Mount Desert Island Biological Laboratory
Salisbury Cove, ME 04672

The enzyme carbonic anhydrase (CA) has been shown to be a central component in the molecular suite of adaptations to low salinity in euryhaline crustaceans. The cytoplasmic isoform is believed to support general cation and anion transport by providing counterions in the form of H^+ and HCO_3^- through the catalyzed hydration of respiratory CO_2.[1] Cytoplasmic CA activity is highly salinity-sensitive, being induced by six fold when green crabs are transferred from high (32 ppt) to low (10 ppt) salinity.[2] CA induction is believed to be under transcriptional regulation, as increases in CA mRNA expression were shown to immediately precede increases in CA protein-specific activity during the acute phase of low salinity adaptation.[3] The overwhelming majority of studies on CA induction have been done at only one low salinity value, 10 ppt. It is not known, therefore, whether CA induction is regulated with any degree of precision by differences in the magnitude of salinity reductions and whether any such putative regulation occurs at the transcriptional or post-translational level.

In order to test the effects of intermediate salinity reductions on CA activity and expression, green crabs, acclimated to 32 ppt, were directly transferred to one of the following salinities: 25, 20, 15, or 10 ppt. Crabs were allowed to acclimate for 7 days, the time needed for fully acclimated levels of CA activity to be reached after low salinity exposure. At that time, crabs were anesthetized in crushed ice, hemolymph samples were taken from the infrabranchial sinus at the base of the walking legs, and crabs were killed by exsanguination. Anterior, respiratory gills (G4) were used as control tissue, and posterior, ion transporting gills (G8) served as the experimental tissue. G4 and G8 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 determination of mRNA expression by quantitative PCR (Stratagene MX 4000). The sample with the highest level of CA

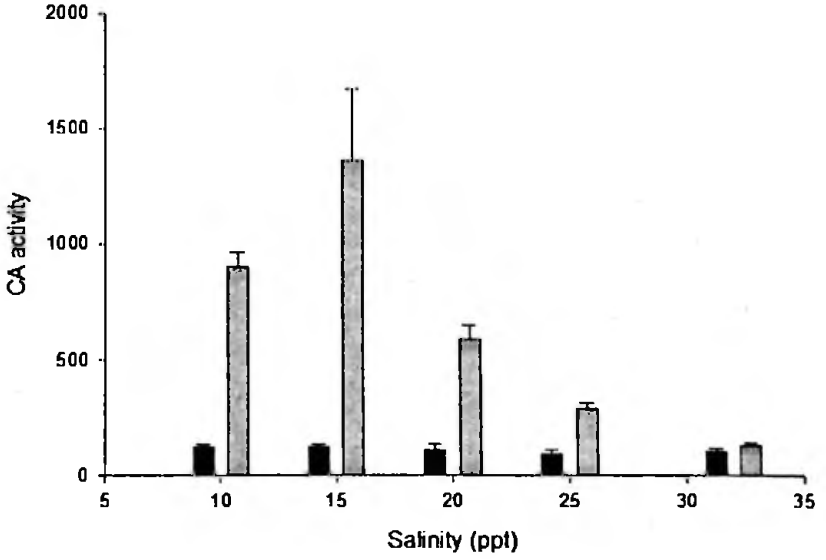

Figure 1. CA activity (μmol CO_2 mg protein^{-1} min^{-1}) in anterior (G4, black bars) and posterior (G8, gray bars) of green crabs vs acclimation salinity. Mean ± SEM (N = 5-6).

activity was used to construct an internal standard curve against with other samples were compared.

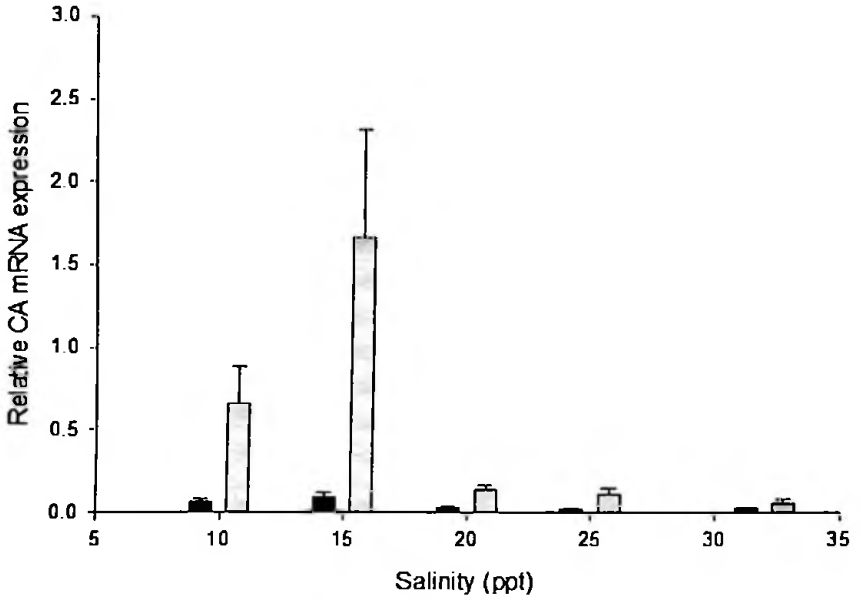

Figure 2. CA mRNA relative expression in anterior (G4, black bars) and posterior (G8, gray bars) of green crabs vs acclimation salinity. Mean ± SEM. (N = 5-6).

Anterior gills did not show any increase in CA activity in response to low salinity and thus served as a control tissue (Fig. 1). CA activity in posterior gills, however, increased progressively with decreasing salinity from 32 to 15 ppt, at which there was an approximate 8 fold induction. Interestingly, CA activity then decreased in crabs transferred directly to 10 ppt. This is in contrast to another, more euryhaline crustacean, *Callinectes sapidus*, in which CA activity in the posterior gills continues to increase from 35 to 5 ppt.[4] *C. maenas* is less euryhaline than *C. sapidus*, being found in nature down to only 8 ppt, as opposed to 0 ppt for *C. sapidus*.

CA mRNA expression followed the same pattern as that seen for CA activity. There were no significant changes in mRNA expression in anterior gills (Fig. 2), but mRNA expression in posterior gills increased with decreasing salinity. As with CA activity, CA mRNA expression peaked at 15 ppt and then decreased in crabs acclimated to 10 ppt.

The general pattern appears to be that acclimation salinity controls the level of CA mRNA expression, and that, in turn, determines the level of CA activity. CA activity appears to be dependent on the synthesis of different levels of the enzyme. It is also interesting to note that the maximum level of CA expression occurs at or near 15 ppt. Below that value, the induction mechanism appears to become less responsive to any further decrease in salinity. It may be, therefore, that the lower limit of salinity tolerance is set by the value at which the induction mechanism becomes refractory and can no longer increase the expression of the critical transport proteins. Green crabs are considered to be moderately euryhaline and are rarely found in nature in salinities below 10 ppt, so it is possible that these crabs were close to their physiological limit. It will be interesting to compare the pattern of CA expression in a more euryhaline species, such as *Callinectes sapidus*, which can survive down to 0 ppt.

Supported by NSF IBN 02-30005 and by an award from the Thomas H. Maren Foundation.

1. **Henry, R.P.** Multiple functions of gill carbonic anhydrase. *J. Exp. Zool.* 248:19-24. 1988.

2. **Henry, R.P., Garrelts, E.E., McCarty, M.M., and Towle, D.W.** Differential induction of branchial carbonic anhydrase and Na^+/K^+ ATPase in the euryhaline crab, *Carcinus maenas*, in response to low salinity exposure. *J. Exp. Zool.* 292:595-603. 2002.
3. **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.
4. **Henry, R.P., and S.A. Watts.** Early carbonic anhydrase induction in the gills of the blue crab, *Callinectes sapidus*, during low salinity acclimation is independent of ornithine decarboxylase activity. *J. Exp. Zool.* 289:350-358. 2001.