Hsp70 mRNA expression in the South American rainbow crab *Chasmagnathus granulatus* after acclimation to low and high salinity

Carlos M. Luquet[1], Griselda Genovese[2] and David W. Towle[3]

[1]CONICET-University of Comahue, Pasaje de la Paz 235, 8370 San Martín de los Andes, Neuquén, Argentina
[2]CONICET-University of Buenos Aires, Pab. II Ciudad Universitaria, 1428, Buenos Aires, Argentina.
[3]Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672.

Chasmagnathus granulatus is a highly euryhaline crab with strong capacity of hyper- and hypo-regulation. This species is normally exposed to rapid changes in ambient salinity, due to the dilution-concentration effect of the tides in its estuarine habitat. Since *C. granulatus* is a semiterrestrial species, it is often exposed for short periods to freshwater or to concentrated seawater by entering into rain pools or tide pools concentrated by evaporation. On the other hand, in many estuarine environments like the Rio de la Plata estuary, seasonal changes in freshwater runoff and persistent winds may force salinity to remain higher or lower than average for days or even weeks, submitting the crabs to long-term osmotic stress. The heat shock protein Hsp70 is likely involved in tolerance of osmotic stress. Induction of this protein in response to salinity changes has been recently reported to start in the gills of a related species, *Pachygrapsus marmoratus* between 4 and 24 hours of acclimation[1].

In order to study the stress response of *C. granulatus* to prolonged acclimation to different salinities, we acclimated the crabs to seawater (30 ‰) for at least 15 days and then submitted them to olygohaline or concentrated medium (2 or 45 ‰ salinity) for 1, 2, 7 and 15 days. The reverse acclimation protocol was also followed. We measured the abundance of Hsp70 mRNA in anterior and posterior gills by means of real time PCR. Poly-A mRNA was reverse transcribed using oligo-dT and SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA). cDNA was amplified by PCR using Sigma (St Lois MO) Red Taq DNA polymerase and specific primers designed from partial sequences for *C. granulatus* Hsp70, previously obtained in our laboratory. To measure mRNA, cDNA was amplified in the presence of SYBR Green dye using Qiagen Quantitect chemistry (Valencia CA) and the Stratagene MX4000 Multiplex Quantitative PCR System (LaJolla CA). A dilution series demonstrated a linear relationship between threshold cycle (C_t) and $\log_{10}$ of template availability. For each sample, cDNA was reverse transcribed from 0.1 μg total RNA, thus normalizing to total RNA (Bustin, 2002). 3 to 5 individual samples from each time and gill group were analyzed in triplicate. Results were compared by two way ANOVA and *post hoc* comparisons, taking time of acclimation and gill group (anterior *vs.* posterior) as factors.

Transference of crabs from 30 to 2 ‰ salinity was accompanied by a 3 fold increase in the abundance of Hsp70 transcript, both in anterior and posterior gills at day 1, $p < 0.05$ for time (Fig. 1A). This increased abundance remained after 7 days in posterior gills but decreased partially in anterior gills. At day 15 Hsp70 transcript decreased to a value about 2 fold higher than that of seawater acclimated crabs, although this difference was not significant. Acclimation from 2 to 30 ‰ salinity did not induce any change in Hsp70 expression after the first day. Then there was a sharp decrease to very low values at day 7 and a recovery to normal seawater values at day 15 ($p < 0.05$ for time).

Transference to 45 ‰ induced a two-fold increase of Hsp70 mRNA expression at day 1, both in anterior and posterior gills ($p < 0.05$). In posterior gills, transcript abundance remained high after 7 days and suffered a further increase at day 15. On the other hand, anterior gills showed a marked reduction at day 7 and reached values lower than those of seawater crabs at day 15 (fig. 1B). Consistently, transference from 45 to 30 ‰ induced a reduction in Hsp70 expression at day 1 in posterior gills. At days 7 and 15, Hsp70 expression showed similarly low values.

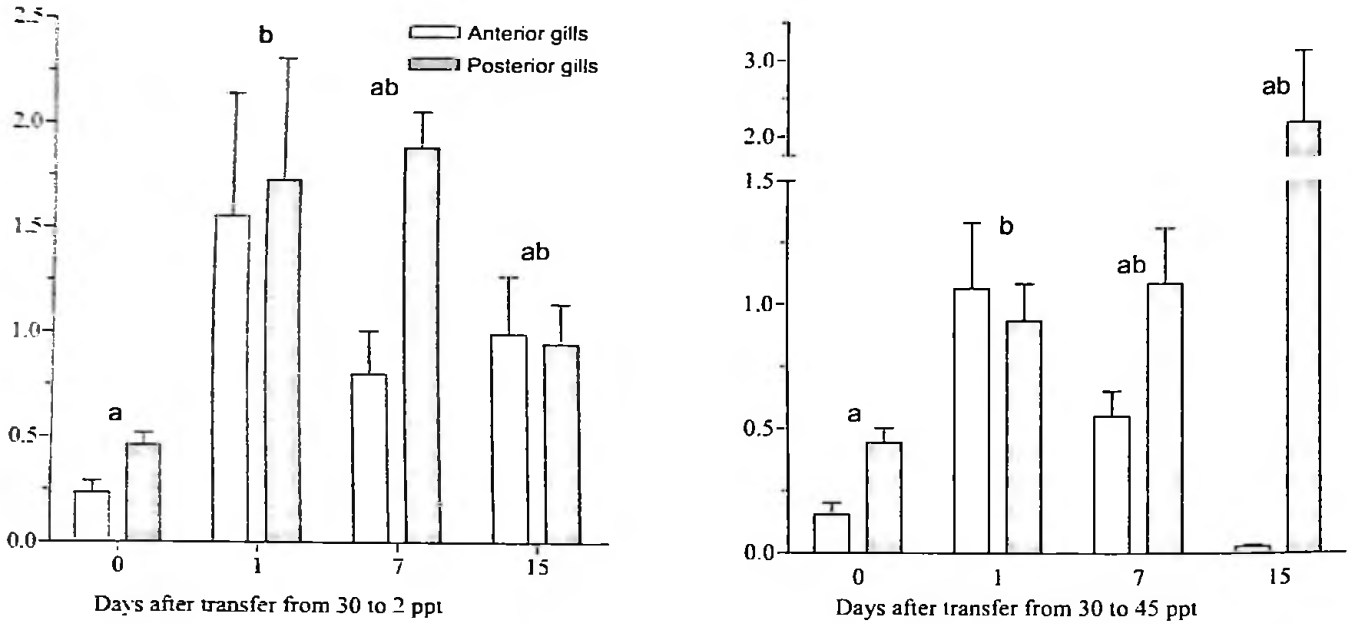

Fig. 1. mRNA expression (mean ± SE. N = 3-5) in anterior and posterior gills of *C. granulatus* transferred from 30 ‰ to 2 ‰ (A) or 45 ‰ (B) salinity, measured by real-time PCR and SYBR green binding, normalized to posterior gill at 2 ‰. Different letters indicate significant differences among acclimation times.

Transcripts for Hsp70 in *C. granulatus* are induced by acclimation to both low and high salinity. In accordance with a previous report on *P. marmoratus*[1], there are no significant differences between anterior and posterior gills of animals acclimated to seawater. We do not find any significant differences among gills of crabs acclimated for long-term to low salinity either. The increased Hsp70 expression at day 1 at low salinity in both anterior and posterior gills is also coincident with the results of the above cited paper[1] and correlates with our previous report on induction of Na^+K^+-ATPase _-subunit. At day 2 the abundance of transcripts for Na^+K^+-ATPase continues growing in posterior gills but recovers initial values in anterior gills[2]. Posterior gills reach steady-state values of ion transporters expression[2] and also low salinity ultrastructural features[3] after one week of acclimation. The high levels of Hsp70 transcript after 7 days reflect that posterior but not anterior gills are still under osmotic stress.

Upon transference to high salinity, Na^+K^+-ATPase _-subunit transcripts are only increased in posterior gills after 4 days. In contrast, induction of Hsp70 occurs both in anterior and posterior gills at day 1. As acclimation proceeds, transcript abundance decreases in anterior gills but remains high in posterior ones.

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