# Quantitative analysis of hsp70 mRNA expression under salinity stress in the euryhaline shore crab *Pachygrapsus marmoratus*

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Marbled rock crabs (*Pachygrapsus marmoratus*) inhabit coastal regions of the Mediterranean Sea where they may be subjected to osmotic stress due to large and rapid fluctuations in seawater salinity. We have previously shown that the expression of genes encoding ion transport proteins in the gills of this species is responsive to salinity change[2,3]. Based on work in other laboratories on the heat shock response to osmotic stress[4], we hypothesized that extremes of salinity might also induce changes in the expression of heat shock proteins (hsps) in the gills of *P. marmoratus*. We were particularly interested in the possible differentiation of gill function as noted with ion transporter expression.

Using degenerate oligonucleotide primers based on hsp70 sequences in several aquatic species, we successfully identified and sequenced hsp70 cDNA from *P. marmoratus*. Following amplification and sequencing of an initial hsp70 fragment, species-specific primers were used with 5'-RACE (Clontech) and 3'-RACE (Invitrogen) protocols, yielding a 2,189-bp cDNA encoding a 650-amino-acid protein that showed high homology to proteins identified as hsp70 in other arthropods (Fig. 1).

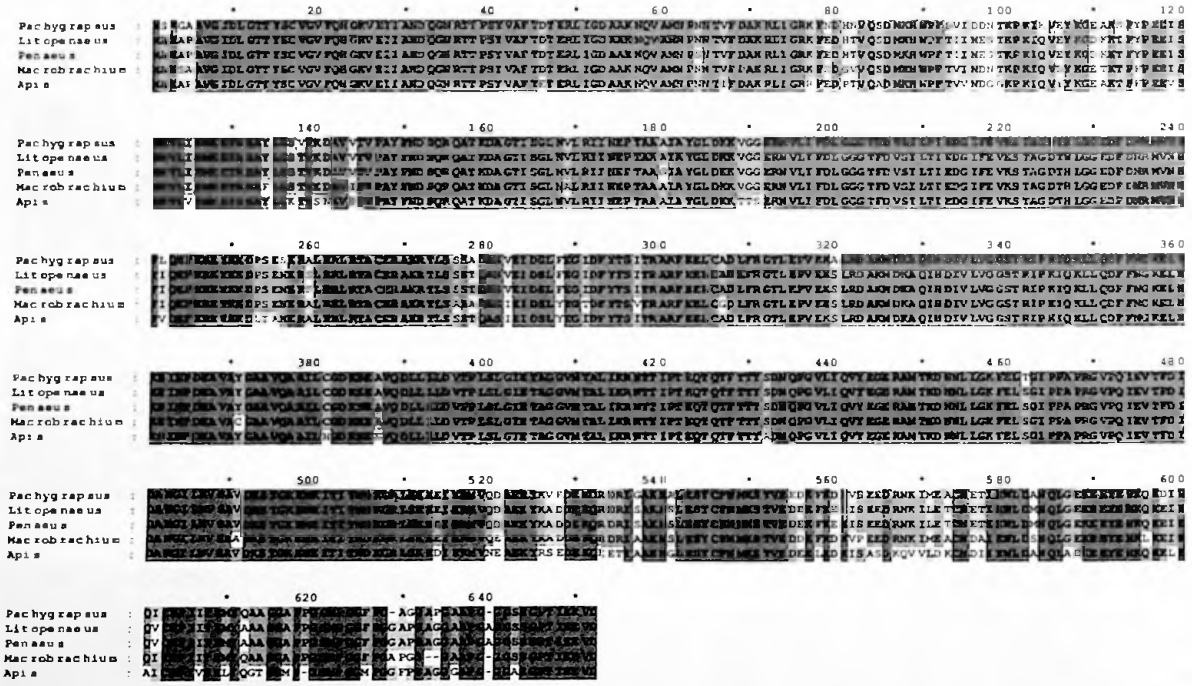


Fig. 1. Multiple alignment of hsp70 amino acid sequences from four crustacean species and honeybee. *Pachygrapsus marmoratus* (present study), *Litopenaeus vanname* (Acc. No. AAT46566), *Penaeus monodon* (Acc. No. AAQ05768), *Macrobrachium rosenbergii* (Acc. No. AAS45710), and *Apis mellifera* (Acc. No. XP_392933).

Species-specific primers and real-time quantitative PCR with SYBR green were used to measure the relative expression of hsp70 mRNA in triplicate samples of the equivalent of 0.1 µg of total RNA prepared from gills of *P. marmoratus* sampled over a time course following transfer from 32 ppt

seawater to either 10 ppt or 45 ppt seawater. In the lower salinity, *P. marmoratus* effectively hyper-osmoregulates its hemolymph via increased ion uptake across the gills, mediated at least in part by induction of ion transporter gene transcription. In the higher salinity, the crab is a hypo-osmoregulator, most likely by enhanced salt excretion across the gills[1].

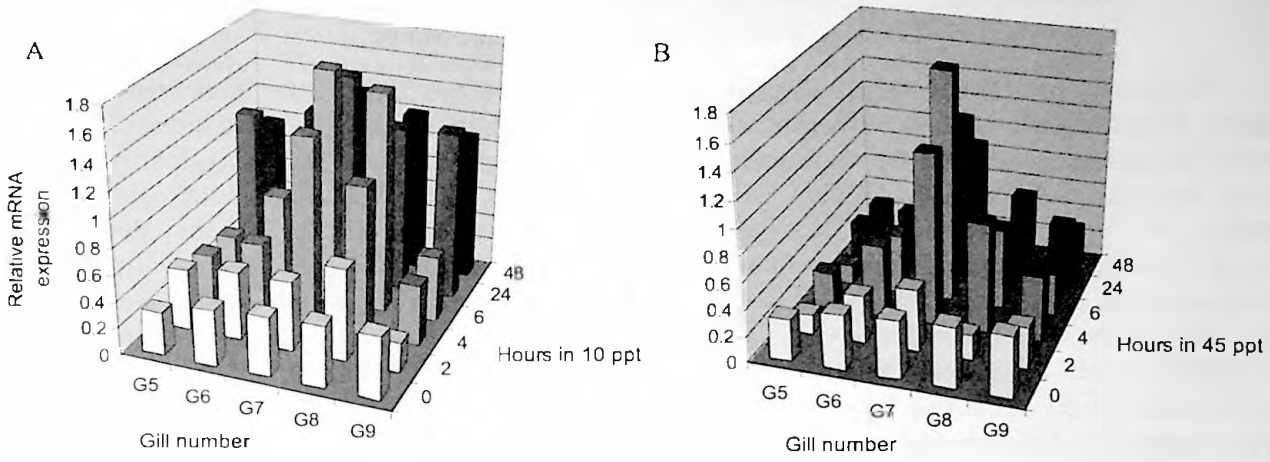


Fig. 2. Relative mRNA expression of hsp70, determined by quantitative PCR, in gills of *Pachygrapus marmoratus* pooled from at least three animals per sampling interval, in relation to gill 9 after 48 hours of exposure of crabs to 10 ppt seawater.

Our analysis showed that the expression of arginine kinase (AK), a putative housekeeping gene, was at nearly equal levels at all times (data not shown). Moreover, hsp70 mRNA was expressed equally under control conditions (zero time) in anterior (G5, G6) and posterior (G7, G8, G9) gills. Following transfer to low salinity, hsp70 mRNA expression increased in all gills (Fig. 2A). The degree of increase was about 2-fold in G5, G6 and G9 at 24 and 48 h. However, in G7 and G8 hsp70 mRNA started to increase by 3 to 4-fold within the first 6 h and then slightly decreased by 48 h. These data suggest that response to osmotic stress experienced by the gill tissue may lead to enhanced hsp70 expression in all gills, with a more rapid response in the two posterior gills that are believed to be most involved in ion uptake[2,3].

Following transfer to high salinity, G5, G6, and G9 did not show any significant change in hsp70 mRNA throughout the study period. However, G8 showed a 2-fold increase within 4 h and by 48 h decreased gradually to the level observed under control conditions. In G7, on the other hand, hsp70 mRNA expression increased about 3-fold within 4 h and about 4-fold in 6 h, then slightly decreased at 24 and 48 h to a level that was still twice the amount expressed in controls. These data, coupled with our observation of a dramatic induction of ion transporter gene expression in G7 under similar conditions[2,3], suggests that G7 plays an important role in the response to hypersaline conditions and may indeed be primarily responsible for salt excretion during hyperosmotic stress.

Supported by NSF Grant Number IBN-0340622 to DWT.

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[2]Spanings-Pierrot, C., and D.W. Towle. Expression of $Na^+$,$K^+$-ATPase mRNA in gills of the euryhaline crab *Pachygrapsus marmoratus* adapted to low and high salinity. *Bulletin MDIBL* 42: 44-46, 2003.

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