Methods

Select transcripts were selected for qPCR analysis on individual oyster samples from each location (n=15) to corroborate RNA-Seq characterization. Specific transcripts of interest were selected from GigasDatabase based on their role in stress response. For these samples, 1 ug of total RNA from each individual was reverse transcribed using M-MLV Reverse Transcriptase (Promega) and oligo dT primers (Promega), according to the manufacturer's protocol. Quantitative PCR was performed using 1uL of cDNA in a 25uL reaction containing 1x of Immomix Master Mix (1.5mM MgCl2; Bioline), 0.2uM forward primer, 0.2uM reverse primer and 2uM SYTO13 (Invitrogen). Thermal cycling and fluorescence detection was performed using an Opticon 2 (Bio-Rad). Cycling parameters were as follows: 95C for 10 min; 40 cycles of: 95C for 15s, 55C for 15s, 72c for 30s; 72C for 10 min. Fluorescence readings were taken two times per cycle, once after annealing and once after extension. Immediately after cycling, a melting curve protocol was run to verify that a single product was generated in each reaction. RNA samples were analyzed to ensure absence of DNA carryover. All samples were run in duplicate. Table 1 contains a list of primers used, primer sequences, and respective GenBank accession numbers for each target gene.

Quantitative PCR analysis was performed using Real-time PCR Miner (Zhao and Fernald 2005). Quantification was performed by calculating the relative mRNA concentration (R0) for each gene for each individual. Briefly, this was calculated using the following equation: R0= 1/(1+E)^Ct, where E is the average gene efficiency and Ct is the cycle number at threshold. The R0 for each gene was normalized to a control (elongation factor 1 (*ef1*)) R0 from each individual. Using the normalized R0, fold increase over the minimum R0 value for each gene was calculated for all individuals. Two-tailed Student's t-tests were carried out to determine significant differences in expression ($p \leq 0.05$) (SPSS version 16).