*Larval oysters exposed to V. tubiashii at two different temperatures*

The experimental protocol will be briefly summarized here since it was discussed in more detail in the previous report. Larval *C. gigas* were acclimated to either 25C or 12C for 24 hours before being exposed to *V. tubiashii* for 3 days. Based on Neutral Red staining, there was significant increased mortality of the larvae at 25C compared to 12C. Analysis of differential gene expression was attempted but could not be done due to poor quality of samples taken.

*Ocean acidification effects on development*

Wild-collected adult *C. gigas* were strip spawned and gametes (separate eggs and sperm) were pooled. Fertilizations occurred in water equilibrated to two different pCO2: 380 ppm (current levels) and 840 ppm (near end-of-century estimation). Fertilization times were staggered and there were 3 fertilization times per treatment with 3 replicates per time (9 replicates per treatment). Developing embryos were sampled at times post-fertilization that correspond to important stages in development: 1 h, 2h, 5h, 17h, and 24h. We collected data on the proportion of larvae that had reached cleavage, hatching, etc. for the different time points. We found that the larvae developing in the 840 ppm treatment were slower to develop than larvae at 380 ppm.

*Ocean acidification effects on* C. gigas *development, calcification, and gene expression*

In collaboration with the Northwest Fisheries Science Center (NOAA, NWFSC) we reared larvae until 48 hours post-fertilization at 4 different pCO2 treatments: 280 ppm (pre-industrial levels), 380 ppm (current day), 750 ppm (projected mid-century), 2000 ppm (pessimistic end-of-century). There were 6 replicates per treatment. As described above, adult wild-collected oysters were strip spawned and larvae were monitored in the different treatments for ability to reach developmental milestones, percent fertilization, morphological abnormalities, and swimming activity. At 24 hours post-fertilization larvae were also assessed for percent calcification using polarized light. Also at 24 hours post-fertilization we took samples of larvae from 3 larval chambers per treatment to store for gene expression analysis. We found that *C. gigas* larvae in this experiment had the greatest success of calcification at 380 ppm and the worst at 2000 ppm. Calcification at 280 and 750 ppm were worse than 380, but the majority of the larvae were fully calcified. There was also the greatest proportion of larvae with normal morphology at 380 ppm and the most with abnormal morphology at 2000 ppm (with 280 an 750 ppm being intermediate). Gene expression analysis at *hsp70* showed significant greater expression at 750 ppm when compared with 380 ppm, but decreased expression at 2000 compared to 750 (although still greater than at 380 ppm). We hypothesize that the larval stress response reaches a threshold in its ability to react to this particular stressor at water that is more acidified than 750 ppm. There was also increased expression of *hsp70* at 280 ppm compared to 380 ppm.

*CO2 data from Dabob Bay*

Based on preliminary data that we have seen from Dabob Bay, WA, there were no significant upwelling events leading to acidified water this past summer (2010).