SK Accomplishments to date

Larval stress trials

Two experimental set-ups (totes), each holding 8 larval chambers, were designed and built. The two totes allow for different temperature treatments. Each larval chamber has a supply of air and water so that CO2 can be controlled and systems can be flow-through for better larval survival.

Determined how many larvae are needed to do a usable RNA extraction for experiments by extracting different numbers of larvae, n= 50 to n=1000. Best yields came from n~1000 larvae for a RNA extraction.

Trial 1: low pH

Accomplished a 3-day trial of oyster larvae under elevated pCO2 (low pH) conditions. Increased pCO2 for 4 of the larval chambers, keeping 4 as controls. Collected samples daily for RNA extractions (gene expression). Observed behavior/mortality under the microscope. Completed a second low pH trial for 6 days and collected the same data.

Trial 2: *Vibrio tubiashii* exposure

Exposed *C. gigas* larvae (4 treatment, 4 control) to heat-killed *Vibrio tubiashii*. Larvae were exposed for 4 days and re-inoculated each day when water was changed. Data/samples were collected for mortality estimates, size/growth data, and for later gene expression analysis.

Trial 3: *V. tubiashii* + temperature

Sixteen larval chambers were used: 8 were at 12 degrees C and 8 were at 25 degrees C. Within each temperature treatment 4 chambers were inoculated with *V. tubiashii* (live). Data were collected daily for mortality, growth and RNA extractions. Samples were also collected for *V. tubiashii* physiology analysis (see details below). RNA has been extracted from all the of *C. gigas* larval samples and we are in the process of analyzing the gene expression data.

Trial 4: *V. tubiashii* + low pH

This trial is currently underway. There are 8 larval chambers containing *C. gigas* – 4 are maintained at ambient pCO2/pH (~400 ppm) and 4 are maintained at elevated pCO2 (~800 ppm). Two larval chambers within each CO2 treatment were inoculated with *V. tubiashii*. Air containing the treatment CO2 concentration is constantly bubbled into the larval chambers to maintain the correct pH throughout the experiment. There are also 2 *V. tubiashii* control containers without *C. gigas* for each of the CO2 treatments. Daily water chemistry data are taken: pCO2, pH, temperature, and salinity. For the first 48 hours samples were taken of the *V. tubiashii* for gene expression analysis. Since the beginning of the experiment (now 5 days ago) *C. gigas* larvae have been counted and assessed for mortality in each of the chambers and a sample has been taken for gene expression analysis.

Microbial community sampling

Since the spring, water samples have been collected weekly from 3 water sources within the hatchery (Taylor Shellfish in Quilcene, WA) at 3 different steps in the larval rearing process. Each sample is filtered on filter paper to collect all the microbes that are in the water. The microbial community in the hatchery at these three locations will be assessed for diversity using ARISAs (automated ribosomal intergenic spacer analysis) and possibly sequenced to determine individual players. These data will create a baseline for day-to-day fluctuations in the hatchery and, if a disease outbreak should occur, could provide tools to create an early-warning system for monitoring.

Staring in June, weekly water samples were collected in Dabob Bay, WA, from which the water for the hatchery is taken. These samples are treated similarly to the internal hatchery sampling described above.

Wild *C. gigas* larvae sampling

Beginning in July, larvae were collected from Dabob Bay. These larvae will be used for gene expression analysis and also to determine larval abundance in the Bay. Temperature and pH are also routinely monitored in the Bay. A better understanding of how environmental properties – temperature, pH, microbes – affect oyster larvae will be provided by this sampling effort.

*Vibrio tubiashii* characterization

The molecular quantification using a quantitative polymerase chain reaction (qPCR) assay developed from the metalloprotease gene, v*tpA,* has been tested and optimized. A standard curve was developed from bacterial culture to test the accuracy and efficiency of the assay. Specificity of the primers for *vtpA*, published in , was tested against wild-type isolates of *Vibrio tubiashii* strains collected in the Pacific Northwest. All seven strains tested showed peaks at the appropriate melting curve on qPCR for all samples except strain RE68, which exhibited two peaks in the melting curve analysis. This suggests two different sized products were generated during the reaction. All other samples showed a single melting curve peak at the same temperature, indicating that the primers amplify the *vtpA* gene in these *V. tubiashii* strains.

Three 72-hour baseline growth curves at 12°, 18° and 25°C for *V. tubiashii* have been completed. To statistically compare growth, a modified Gompertz growth model will be fitted to each curve. F-tests will compare curve parameters between temperatures. All samples banked from the viable cell count growth curves have been DNA extracted and are currently being analyzed by qPCR, using the metalloprotease gene (*vtpA*) to estimate the accuracy of viable cell counts compared to qPCR. This analysis will determine if the qPCR assay is an option for future abundance estimation.

Currently, *V. tubiashii* partial pressure carbon dioxide (pCO2) challenges are underway. Three different pCO2 levels – 380, 840 and 2000 parts per million are being used to test *V. tubaishii* growth and physiological response. The optimized qPCR assay using the *VtpA* gene will be used to gain abundance data at intermittent time points during the trial. A subset of samples will be used to access the physiological response of *V. tubiashii* reverse transcription qPCR to quantify gene expression of putative virulence factors including a transcriptional protein, VtpR, metalloprotease, chitinase and sigma factors related to environmental stress.