Write-up of oyster pilot experiment, Sept 2010

CO2 Treatments: 280, 380, 750, 2000 ppm

Temp: 20C

Day 0:

Collecting gonads: Emma dissected out the gonads from 10 oysters collected at Big Beef Creek, sexed the gametes, and put all eggs and sperm into two separate 30ml beakers.

Fertilizing eggs: Fertilization for each replicate in each treatment occurred separately. For each replicate, we filled a 30ml beaker with water of the proper pH and sealed the beaker with saran wrap and a rubber band. Eggs and sperm were aliquotted equally among all replicates, one replicate at a time for each treatment (e.g., the A replicate for all treatments was fertilized first, then the B treatment etc). We waited about 10 minutes between fertilizations to allow time to look at development in each replicate at the same time after fertilization. These 30ml beakers sat out at room temperature. We waited XXX (amount of time, maybe 30 min?) after fertilization before putting the eggs&sperm for each replicate into their static container and in their water baths (boxes).

Monitoring development: The progression of development (2, 4, 16+) cells was monitored at ~45min and ~4 hrs using a compound scope and depression slides. Emma and Elene monitored a test beaker for development to know when it first started occurring. They didn’t examine all of the replicates until they saw development in the test beaker. Three or four aliquots were examined for each replicate. Emma and Elene have these data.

Filling jars: We filled the replicate containers with water by timing how long it took to fill the jar and then overflowing the jar for the same amount of time. The first 24hrs of the experiment took place in static jars (no water/gas exchange).

System pH: We collected water samples for spec pH at 10am. There is some question if the pipes in the system were flushed enough prior to sample collection. If some pH levels seem off, this could be why.

Day 1:

Monitoring development: Emma and Elene used compound scopes to look at the progression of development in each replicate. They pulled 5 ml from each replicate and then subsampled this sample 3 or 4 times. They should have used a new pipet tip for each jar.

Sampling: Emma collected animals from the A, C, and E replicates for RNA by scraping animals from sieve during the cleaning process.

pH: I used a 20um sieve and autopipeter to sample the water in the static chambers for spec pH (two replicates for each treatment). Next time, I should use a new pipet tip for each jar and Vortex the sieve between samples. I also measured the pH of the water being delivered to the chambers once they were put online.

Moving animals to new jars: To move animals from the static jars to flowing jars, we used a sieve mounted on the top of a cut jar resting in a Tupperware. We used water from the box to put a small layer of water in the Tupperware under the sieve. This was likely a mistake as this water could have had ciliates and other pathogens in it. Basically, we used the water that outflowed from all replicates for cleaning – not a good idea. We rinsed each jar onto the sieve with a squirt bottle or hose from the system. We then put the sieve in a beaker of clean, treated water to try to flush out ciliates. We used the water in this beaker twice, and that was likely a mistake. We might want to use a squirt bottle over the sieve to wash out the pathogens, instead of just the gentle rinsing. We rinsed the sieve and Tupperware in fresh water between samples. Next time we need to fill the water in the squirt bottle from the delivery tank, not the water bath.

Day 2:

Monitoring development: Emma and Elene used compound scopes to look at the progression of development in each replicate and the normal/mutant/dead ratio. They pulled 5 ml from each replicate and then subsampled this sample 3 or 4 times. Next time, they should use a new pipet tip for each jar.

Density and calcification: I put 1ml of sample from each replicate on a Sedgewick Rafter slide, and used a dissecting scope with double polarized light to count the number of larvae on each slide and the number that were uncalcified/partially calcified/calcified. I should have probably counted 3 samples from each replicate. I slowed down the larvae with a couple drops of ethanol. This likely messed with my density counts.

Vibrio study: Elene took the F replicates to UW for a Vibrio challenge.

Calcification data:



pink = calcified

green = partially calcified

red = uncalcified

Density data (each column is a replicate):

Sampling: I think that Emma collected samples for RNA on this day, too.

Cleaning: I think that we cleaned the samples again in the same way that I described above.

Day 3:

We did no sampling, observing, or cleaning of data on this day. This was probably a mistake. Cleaning the chambers on this day could have prevented the ciliates outbreak.

Feeding: Sarah set up the feeding system. She took about 1.5ml of frozen algae paste, washed it on a sieve, resuspended it in a Nalgene, and used the peristaltic pump to add it to the intake water in the jars. I think that it took about 20 min to feed each treatment. She has the details on the dynamics of feeding.

Chemistry: I took spec pH measurements of the water coming from the system and of two replicates prior to and 2hr 10min after the algae feeding was completed.

System problem: Mike had a problem with the system that caused low pH water to infiltrate the reservoir, the 280 and 380 systems, and, to some degree, the 280 and 380 replicates. The pump that delivered water to the 380 replicates failed overnight, causing the replicated to be static for an unknown amount of time.

Day 4:

Sampling: Emma and Zac reported that the larvae were mostly gone or in poor condition due to ciliates. I found some ok larvae in all treatments, but not enough to keep the experiment going. Interestingly, there were some live larvae that were still partially calcified in the 2000ppm water.

Vibrio animals: Elene reported that all her animals were dead due to ciliates.

Chemistry: I took samples for spec pH from two of the 380 replicates that were static overnight and from each treatment system.

Ended the experiment due to the ciliates outbreak.

Notes for next time: I think that density estimates should be taken on Day 1 and, if the density is over 10/ml, the replicates split to achieve proper density. Density should be standardized across replicates after Day 0.

It seems like the aliquot of eggs/sperm for each replicate was too large, as some replicates had way too many larvae.

We need a better system for moving animals/cleaning out pathogens when larvae are transferred to new jars.

Thoughts: It seems like ciliates might have had a harder time growing in the 2000ppm tank. Maybe this could be a study in and of itself!

Needs: Shallow box/tray to use when putting animals from chambers onto sieves. Shorter beaker to clean ciliates.

pH data over the experiment: