**Pinto Abalone Experiment**

This describes the exposure and collection methods for an experiment in which Pinto abalone were exposed to ambient and 750 ppm CO2 for ~24. They were then sampled for SOLiD sequencing. The experiment took place in the Friedman quarantine room from 9/28/11 to 9/30/11. The seawater temperature was 14 ºC. Salinity was 32 ppt (measured with refractometer).

**9/26/11**

* Pinto abalone spawned at Mukilteo facility. The parents were from Greenhouse B, Tank 14 (F1s from 2007), most of which looked ripe. All eggs came from one cross. Fertilization success was 81%. (see Josh Bouma for further spawning details)

**9/28/11**

* Water source was the Seattle Aquarium.
* Began bubbling 2000ppm CO2 and ambient in the evening, but it ran out overnight. (see 9/29/11 for more details).
* Seawater temperature was 14.0 ± 0.3 ºC.

**9/29/11**

* Arrived in the morning to find that the 2000ppm CO2 had run out.
* 10:30 am - Began bubbling 750ppm CO2 in large 4+ L column. Assumption was that the seawater had been partially equilibrated. I turned the 750ppm CO2 up high (so it was bubbling a lot) to get it to equilibrate in less time.
* Brent picked up larvae from Muk and brought them back to UW.
* 3:00pm –
  + Filled 4, mayo (larval) jars with 4 L ambient water and 4, mayo (larval) jars with 4 L 750ppm CO2 seawater, which had continual bubbling of the appropriate gas and were held in a 14 ºC water bath.
  + Counted and distributed larvae to jars as follows:
    - All larvae were taken from the same 300 mL of larvae in seawater.
      * Estimated larval density:
      * Gently invert jar 5 times, aliquot 150 ul into three separate 3ml wells, and repeat 2 more times
      * Count live, dead, and total larvae in each well
        + Counts were:

|  |  |  |  |
| --- | --- | --- | --- |
| Well # | live | dead | total |
| 1 | 94 | 7 | 101 |
| 2 | 108 | 5 | 113 |
| 3 | 89 | 2 | 91 |
| averages |  | 4.6 | 101.67 |

* + - * Approximate number of larvae was 67,780
      * I brought the total volume of larvae up to 400mL, homogenized (as above), and distributed 50 mL to each replicate jar.
      * This resulted in an estimated 8,472 larvae/ rep jar
* 6:30-10:00pm – took TA/DIC samples and spec pH
  + 6:00 - Took TA/DIC samples according to PMEL protocol. Ambient samples A1 and A2 and 750 ppm CO2 samples 1 and 2 to be dropped off at PMEL on 12/20/11, along with some of Elene’s samples. Ambient samples A3 and A4 and 750 ppm CO2 samples 3 and 4 to be analyzed by Emma and Carolyn (given to Emma on 12/19/11).
  + 7-10:00pm – Took spec pH samples. See data sheet “9-29-11\_Pinto-Ab-larvae-spec pH worksheet.xls”

**9/30/11** – Sampling for survival and taking whole population for RNA

* 1 pm - Took spec pH samples from each larval jar. See data sheet “9-30-11\_Pinto-Ab-larvae-spec pH worksheet.xls”
* 4pm - Take samples for counting live/dead from each rep jar:
  + Filter all larvae from jar through 70 um screen
  + Put all larvae into a 50 ml conical tube (with total volume of 50 ml)
  + Gently invert tube 5 times, aliquot 200ul into a 3 ml well; repeat two more times
  + Count live/dead on inverted dissecting scope
  + See data sheet “9-30-11\_Pinto-Ab-larvae-LIVE-DEAD\_counts.xlsx” for counts
* 6 pm – Take samples for RNA
  + Use larvae already filtered for live/dead counts (as described above)
  + Filter onto 70 um screen.
  + Place larvae into a 1 ml cryovial and place into liquid nitrogen

See Sam for RNA extraction methods.

Note: Anything marked as A# was ambient. Anything with just a number was 750ppm CO2. I made sure to mark this in all the associated datasheet, but I thought I’d put it here just in case…