SAMPLING PROCEDURE FOR FRIDAY HARBOR JANUARY AND FEBRUARY, 2012

1. Histology, gene expression, protein expression: 2 oysters from each container (n = 16 from each *p*CO2 treatment)
   1. Measure each oyster and record measurements on data sheet before shucking
   2. Histology
      1. Label histology cassette with date and sample number
      2. Take a cross section that includes gill, DG, and heart as well as a piece of adductor and put in the labeled cassette, then in a jar of Davidson’s for 24 hours (30 cassettes per jar). After 24 hours transfer to 70% EtOH.
      3. Be sure to record corresponding gene and protein expression samples.
   3. Gene and Protein expression
      1. Cut 2 separate pieces of gill tissue and put in 2 separate 1.5 mL screw cap tubes (1 each for gene and protein). Record on sample sheet.
      2. Be sure to record corresponding histology sample.
2. Heat shock: 4 oysters from each container (n = 32 from each *p*CO2 treatment)
   1. LHT: 1 oyster from each container (n = 8) will be exposed to 43°C for 1 hour (excluding 10 minute warm up at the same temperature). These oysters will be returned to ambient conditions for 1 week after which survivors will be sampled for gene and protein expression (see I.c.).
   2. SLT only: 1 oyster from each container (n = 8) will be exposed to 38°C for 1 hour and then immediately sampled for gene and protein expression (see I.c.).
   3. SLT + LHT: 1 oyster form each container will be exposed to 38°C for 1 hour and then immediately exposed to 43°C for 1 hour. These oysters will be returned to ambient conditions for 1 week after which survivors will be sampled for gene and protein expression (see I.c.).
   4. SLT + LHT: 1 oyster from each container will be exposed to 38°C for 1 hour, returned to ambient conditions for 1 week, then exposed to 43°C for 1 hour and returned to ambient conditions for 1 week after which survivors will be sampled for gene and protein expression (see I.c.).