<u>TEMPERATURE PROTOCOLS</u>

<u>South Carolina (L. Coen et al.)</u>
(For intertidal or subtidal logging; rev. 2/14/05)

Rationale for temperature data collection

To better assess conditions experienced by intertidal organisms such as oyster reefs during their emergence we have been attaching paired (subtidal and intertidal) temperature recorders adjacent to our study site reefs. Initial analyses of these data have clearly shown that the temperature ranges experienced on the intertidal reefs are considerably wider (e.g., our SC values from -7.5^{o}C to 49.9^{o}C) than those encountered by subtidal organisms (0.1^{o}C to 36°C) over the same timeframe (see salinity protocol for similar range). Daily ranges are even more interesting and of relevance for oyster impacts. Such differences in exposure to temperature extremes are likely to have profound effects on intertidal oysters, compared with subtidal oysters found elsewhere, particularly with regard to such aspects of oyster condition and health as reproductive periodicity, disease susceptibility, and responses to anthropogenic stressors. Environmental factors (e.g., elevated salinity and temperature) have already been shown to increase oyster susceptibility to MSX and Dermo (Burrell et al. 1984; Barber et al. 1988; Gibbons and Chu 1989; Crosby and Roberts 1990; Littlewood and Ford 1990, Sindermann 1990, Austin et al. 1993, Ewart and Ford 1993). Additionally, southeastern intertidal *Crassostrea* races in macrotidal areas (> 2 m) may be physiologically predisposed, through selection, to counter the effects of long periods of exposure and starvation, whereas subtidal populations/races may be more adversely affected by such environmental extremes

The two most important disease-forcing factors most often cited in the literature are temperature and salinity (e.g., see DermoWatch website in the Gulf of Mexico run by Drs. Ray and Soniat, http://www.dermowatch.org/). The web site (Kortright et al. 2002, Soniat et al. 2002) calculates a time to a critical level of disease using temperature and salinity. This is an estimate of the time that it would take the parasite to reach a critical level, assuming no change in temperature and salinity. Mean salinities at our long-term study sites have remained around 29-30 ppt based on Hydrolab deployments (Coen et al. 1999).

Oyster diseases in northeastern areas have decimated oyster production due to *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) (reviewed in Sindermann 1990, Ewart and Ford 1993). Disease virulence usually increases with age, size and exposure duration (Sindermann 1990). We have already initiated an experimental program to look at oyster disease (MSX, Dermo) epidemiology among seasons and sites as a direct stress indicator of habitat "quality" and "health". We can establish prevalence, infection, location and intensity (Ford and Haskin 1982) to track oyster diseases at our sites. Previously, a relationship has been established between environmental stress (i.e. temperature, salinity) and Dermo and MSX in the northeast (see reviews by Ford and Haskin 1982, Haskin and Ford 1982, Sindermann 1990). The seasonal epidemiology of these diseases (especially MSX)

in South Carolina was previously unclear (cf. O'Beirn et al. 1994 for Georgia, Bobo et al 1997).

Using Onset Sensors
(http://www.onsetcomp.com/)

Measurement time: use whatever interval works for your needs and allows for sufficient deployment duration (we have used 12 or 48 minute intervals for our SC work, usually deployed for a maximum of two months so if the sensor is lost that is the most data one might lose. There is sufficient memory to allow 6-9 months or more). We use clear or white submersible cases for some of the Onset temperature sensors such as the HOBO® H8 Temperature Data Loggers or the 8K StowAway Temperature logger (XTI08-39+122) with internal sensors good from -39° to 122°C (see below). It has in the case a range of temperatures from -20° to 70°C (-4° to 158°F) (unclear). Also available are submersible cases with an attached 1-foot thermistor cable and the sensor potted to the case wall. Cases cost $39-$60 each (see below). We typically have lined the clear cases with white paper to make them opaque, which deflects any direct sunlight to the sensors causing increased inaccurate temperatures. We have used for years an intertidal and a subtidal sensor in tandem to record water vs. air temperatures that oysters actually encounter (Coen et al. 1999). We have also used the sensors for measuring temperatures within shell piles related to shell quarantine studies (Bushek et al. 2004). Note that as expected, sensors do not mimic exactly the intertidal environments without additional modificiations (e.g., see Fitzhenry et al. 2004).

After each use, the sensor cases should be cleaned (using a wet cloth) and the o-rings greased with silicone grease provided with the cases from Onset. Do not use fingernails to remove the o-rings, rather squeeze with your fingers to avoid cutting the o-ring. Before deploying, place 3 desiccant bags from Onset in each case (need to be dried in an oven and then placed in a desiccator). Screw together case halves so that they are tight, but not overly tight (they should just pinch the o-ring). Cover the cases with nylon stockings so that they are not fouled with barnacles and are easy to clean when retrieved.

Newer case-less sensors (HOBO® Water Temp Pro line, H20-001) are also available from Onset. The Temp Pro has a measurement range of between 0° to 50°C (32° to 122°F) in water and they are rated to 120 m (400 feet). These cost

~$110 each. The Temp Pro has a six-year factory replaceable battery with typical use (see below). Temperature extremes will reduce battery life. Data on the Temp Pro can be downloaded in the field onto a Palm using the Communications Port on the logger and the infrared (IR) port on the Palm.
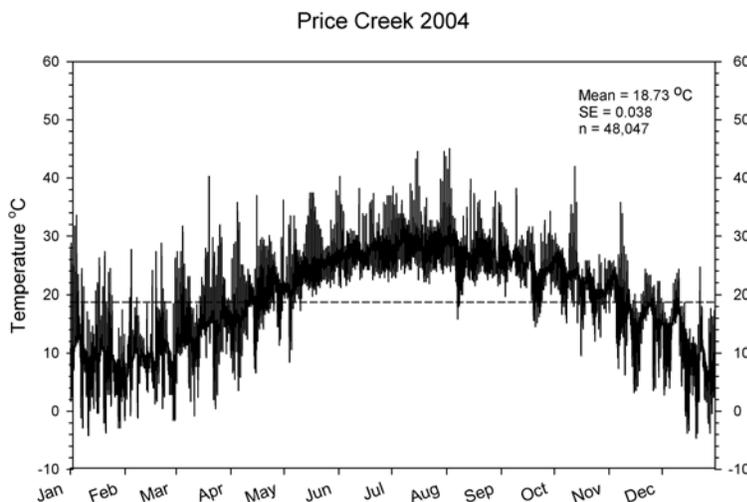
Other sensors that have been used include the ibutton® (above) which is a computer chip enclosed in a 16 mm stainless steel can (http://www.maxim-ic.com/products/ibutton/products/ibuttons.cfm#temperature). These are essentially disposable temperature sensors. Dr. Brian Helmuth at USC has used these extensively in his intertidal research (e.g., Helmuth 1998, Helmuth et al. 2002,).

Onset® Graphing Protocol

Use BoxCar Pro software to download the sensors (you only need one copy for all uses). Open Onset data (.dtf) file in BoxCar Pro 4.0 or later version (the current Version 4.3 costs ~$95) and export as an Excel text file. Once txt files have been saved and opened in Excel, complete steps 1 thru 3 of the Text Import Wizard: 1) Select Delimited data, 2) Select Tab & Space Delimiters; 3) Format Column Data as follows, in Data Preview: a) highlight "Date" column, select **Date:MDY** in data format, b) highlight "Time" column, select **Text**, c) highlight "Temperature" column, select **General**; and d) highlight (*C) column, select **Do Not Import Column**.

	A	B	C	D
1	Date	Time		Temperature
2	12/16/2002	14:00:00.0	=(A2+B2)	23.64
3	12/16/2002	14:48:00.0	12/16/2002 14:48:00	21.19
4	12/16/2002	15:36:00.0	12/16/2002 15:36:00	16.99
5	12/16/2002	16:24:00.0	12/16/2002 16:24:00	10.99

Date, time and temperature data are then imported into Excel as three separate columns. Then Insert a column after the "Time" column and format as: **mm/dd/yyyy hh:mm:ss** (choose from custom category). Finally, in cell 2 of the inserted column, insert the function **=(A2+B2)**. This combines date and time data into a single column. Copy this function for each cell in the entire dataset. (See layout of combined dataset below).

The combined Date/Time and Temperature data can now

Price Creek 2004

Mean = 18.73 °C
SE = 0.038
n = 48,047

Intertidal temperature measurements recorded using StowAway Data Loggers by Onset.
Deployed in white potted cases, plugged.

be graphed in Excel or copied into SigmaPlot for graphing. The new versions of BoxCar Pro have nice simple graphing and stats included. Before graphing, dataset tails should be "cleaned" by verifying the actual dates and times dataloggers were launched and retrieved (if necessary, delete any end data points which are not accurate). We use Sigmaplot to graph data, Excel works also example is shown below.

Relevant Literature

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South Carolina (Hadley & Hodges)
(SCORE Temperature Protocol)

Value of the Metric

     Water temperature influences: dissolved oxygen concentrations; the rate of
plant photosynthesis; the metabolic rates of aquatic organisms; and the timing of
reproduction and migration in many species. Many of the other water quality
parameters you will monitor are affected by temperature. Intertidal oysters go
through exposure and submergence each day and may experience extreme
temperature variance as a result. For this reason it is useful to measure air as well
as water temperatures. Water temperature will also affect associated reef fauna.

Materials

     Pocket-size non-mercury thermometer in plastic shield (e.g. TH27, Aquatic
Ecosystems $5.00)

Test Procedure

     Always take the air temperature reading first (while the thermometer is dry).
Allow time for the thermometer to reach the outdoor temperature (probably at least 5
minutes). During this time the thermometer should not be in the sun, nor in the wind.
When reading the temperature, hold the thermometer at the top or your body heat

will warm it. Record the air temperature in degrees Celsius. Procure a water sample with a plastic beaker from approximately 1 foot below the surface. Place the thermometer in the water for two minutes or until the reading stabilizes. Read the thermometer while it is still submersed. Record the water temperature in degrees Celsius. Rinse the thermometer with fresh water and dry with a paper towel or cloth before storing.