TEMPERATURE PROTOCOLS

South Carolina (L. Coen et al.)
(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^{o}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.

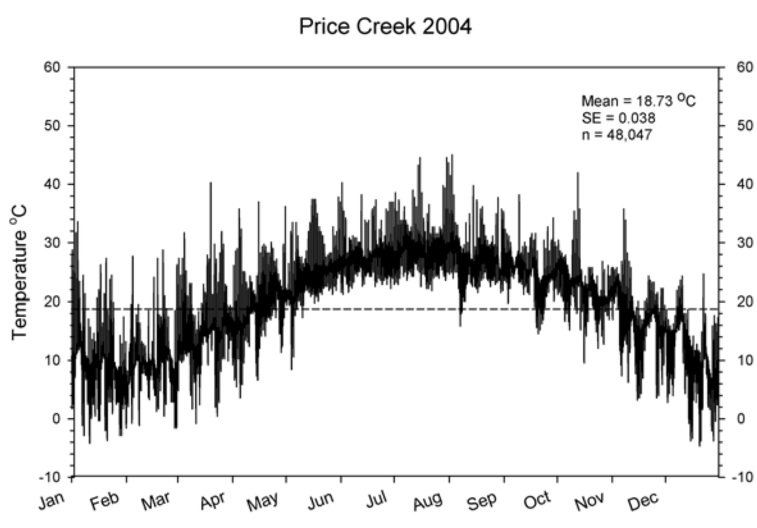
Other sensors that have been used include the ibutton® (above) which is a computer chip enclosed in a 16 mm stainless steel can (http://www.maxim-ic.com/products/ibutton/products/ibuttons.cfm#temperature). These are essentially disposable temperature sensors. Dr. Brian Helmuth at USC has used these extensively 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).



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

The combined Date/Time and Temperature data can now 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

Austin, H., D.S. Haven and M.S. Moustafa, 1993. The relationship between trends in a condition index of the American oyster, *Crassostrea virginica,* and environmental parameters in three Virginia estuaries. Estuaries 16:362-374.

Barber, B.J., S.E. Ford and H.H. Haskin, 1988. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition index and relative fecundity. J. Shellfish Res. 7:25-32.

Burrell, V.G., M.Y. Bobo and J.J. Manzi, 1984. A comparison of seasonal incidence and intensity of *Perkinsus marinus* between subtidal and intertidal oyster populations in South Carolina. J. World Mariculture Soc. 15:301-309.

Bobo, M.Y., D.L. Richardson, L.D. Coen, V.G. Burrell, 1997. A Report on the Protozoan Pathogens *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) in South Carolina Shellfish Populations. SCDNR-MRD-MRRI Technical Report #86, 50 pp.

Bushek, D., D. Richardson, M.Y. Bobo, and L.D. Coen, 2004. Short-term shell pile quarantine reduces the abundance of *Perkinsus marinus* remaining in tissues attached to oyster shell. J. Shellfish Res. 23:369-373.

Coen, L.D., D.M. Knott, E.L. Wenner, N.H. Hadley, A.H. Ringwood, 1999. Intertidal Oyster Reef Studies in South Carolina: Design, Sampling and Experimental Focus for Evaluating Habitat Value and Function. Pages 131-156, In: M.W. Luckenbach, R. Mann, J.A. Wesson (eds.), Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. Virginia Institute of Marine Science Press, Gloucester Point, VA.

Crosby, M.P. and C.F. Roberts, 1990. Seasonal infection intensity cycle of the parasite *Perkinus marinus* (and an absence of *Haplosporidium* spp.) in oysters from a South Carolina salt marsh. Diseases of Aquatic Organisms 9:149-155.

Ewart, J.W. and Ford, S.E., 1993. History and impact of MSX and Dermo diseases on oyster stocks in the Northeast region. NRAC Fact Sheet 200, Northeastern Regional Aquaculture Center, U. Mass. Dartmouth.

Fitzhenry, T., P.M. Halpin and B. Helmuth, 2004. Testing the effects of wave exposure, site, and behavior on intertidal mussel body temperatures: Applications and limits of temperature logger design. Mar. Biol. 145:339-349.

Ford, S.E. and H.H. Haskin, 1982. History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. J. Invertebr. Pathol. 40:118-141.

Gibbons, M.C. and Fu-Lin E. Chu, 1989. Does tidal zonation affect the intensity and incidence of *Perkinsus marinus* in juvenile American oysters in Virginia? J. Shellfish Res. 7:572.

Haskin, H.H. and S.E. Ford, 1982. *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: a host-parasite relationship along a salinity gradient. J. Invertebr. Pathol. 40:388-405.

Helmuth B.S.T., 1998. Intertidal mussel microclimates: Predicting the body temperature of a sessile invertebrate. Ecological Monographs, 68:29-52.

Helmuth, B., C.D.G. Harley, P. Halpin, M. O'Donnell, G.E. Hofmann and C. Blanchette. 2002 . Climate change and latitudinal patterns of intertidal thermal stress Science 298:1015-1017.

Kortright, E.V., T.M. Soniat, and S.M. Ray. 2002. Web model estimates oyster parasite time-to-critical levels. Sea Technol. 43:43-46

Littlewood, D.T.J. and S.E. Ford, 1990. Physiological responses to acute temperature elevation in oysters, *Crassostrea virginica* (Gmelin, 1791), parasitized by *Haplosporidium nelsoni* (MSX) (Haskin, Stauber, and Mackin, 1966). J. Shellfish Res. 9:159-163.

O'Beirn, F.X., C.C. Dean, and R.L. Walker, 1994. Prevalence of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica* in relation to tidal placement in a Georgia tidal creek. Northeast Gulf Science 13:79-87.

Sindermann, C.J., 1990. Principal diseases of marine fish and shellfish: diseases of marine shellfish. 2nd Ed., Vol. 2. Academic Press, San Diego, 516 pp.

Soniat, T.M., E.V. Kortright, and S.M. Ray. 2002. DermoWatch: a web-based approach for monitoring the oyster parasite *Perkinsus marinus (Dermocystidium marinum)* J. Shellfish Res. 21:389

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.