

SOP 4 – Spatial Water-Quality Monitoring with the YSI Sonde

Version 1.01 (June, 2012)

The following table lists all changes that have been made to this Standard Operating Procedure since the original publication date. Any recommended or required changes added to the log must be complete and concise and promptly brought to the attention of the Project Leader. The Project Leader will review and incorporate all changes, officially complete the revision history log, and change the date and version number on the title page. For complete instructions, please refer to Instructions for Recording Revisions in SOP 13.

Revision History Log:

New Version #	Previous Version #	Revision Date	Author (full name, title, affiliation)	Location in Document and Description of Change	Reason for Change
1.01	1.0	6/1/2012	James M. Caldwell, Water Quality Specialist, USGS Maine Water Science Center	Updated all references to YSI dissolved oxygen probes with information specific to new optical sensor 6600 EDS calibration: inserted instruction to change time-averaging for chlorophyll probe from default (12 sec) to 30 sec	Manufacturer's improvements Manufacturer's recommendation

Background and Familiarization with Instruments

This SOP describes operation of the YSI 6600 multi-parameter water-quality monitor (sonde) for spatial surveys of water-quality parameters at stations throughout each of the NCBN parks during a four-week late-summer index period. Data are collected using the YSI 6600 deployed in discrete sampling mode, and logged to the YSI650MDS. The SOP describes a standard procedure on how to prepare, calibrate, program and upload data from these instruments. It also provides detailed methods for operating the instrument in the field. The sonde chosen for continuous monitoring is the YSI 6600EDS (Extended Deployment System) with additional modifications to accept input from two LiCor PAR sensors. The 6600EDS model may also be used for the spatial survey; however, the EDS wiper and PAR input capabilities are not needed for this SOP. The advantage of using the 6600EDS is that sondes can then be swapped between tasks for spatial and continuous monitoring. The disadvantage is the additional cost of the EDS and PAR capabilities, which are not required for this SOP. Both sondes calibrate identically.

Either sonde should be connected to a YSI 650MDS Multiparameter Display System using a field cable of appropriate length for the individual park.

It is outside the scope of this SOP to review all of the information and methods that are required to operate the YSI 6600 or 6600EDS sonde and the 650MDS and to make effective and accurate measurements. It is imperative that both field and laboratory personnel familiarize themselves with the entire YSI 6-Series Environmental Monitoring Systems Operations Manual, the Multiparameter Display System Operations Manual, and the Technical Notes and Technical Documents available from YSI at their web site and attached herein. The purpose of this SOP is to standardize instrument handling, maintenance, calibration, and use for all the NCBN parks. It provides step-by-step instructions as they pertain specifically to this monitoring protocol; however, it does not alone provide enough guidance to ensure consistent and accurate measurements. YSI, Inc. offers courses on instrument calibration and maintenance. We strongly advise that all NER personnel involved in instrument calibration receive annual training from YSI.

YSI, Inc. offers three different depth models of the 6600 (or 6600EDS): shallow (9 m), medium (61 m), and deep (200 m). Although all three models have the same resolution (0.001 m), the accuracy of the shallow water model (± 2 cm) is considerably better than that of the medium depth model (± 12 cm). The shallow model should be selected for parks where depths are not expected to exceed 9 m; the medium depth unit should be selected for the remaining parks. In either case, the sonde should be non-vented for pressure, and equipped with the following sensors:

- YSI 6562 Rapid Pulse Dissolved Oxygen Probe or YSI 6150 ROX Optical Dissolved Oxygen Probe
- YSI 6136 Turbidity Probe
- YSI 6560 Conductivity & Temperature Probe

Note that a pH sensor is not included in the above list. The 6600 and 6600EDS are routinely shipped with a pH sensor, but it should be removed from the order for a purchase credit.

YSI, Inc. also offers several options on the 650MDS. This SOP calls for the “650-04” configuration which includes the 1.5MB high-memory option and an integrated barometer. The rechargeable battery pack is not recommended. In addition to the sonde, sensors, and display system described above, this SOP calls for the following equipment and supplies:

- personal computer with RS232-compliant serial port (or USB to serial converter) and running the current version of YSI EcoWatch software
- YSI 6-Series Environmental Monitoring Systems Operations Manual, and YSI Technical Notes and Technical Documents attached herein
- YSI 650 MDS Multiparameter display System Operations Manual
- YSI 6067B Dry Calibration Cable
- YSI 655174 PC Interface Cable for 650 MDS
- YSI 5775 Oxygen Probe Service Kit* (membranes and O-rings for 6562 probe)
- YSI 6144 Wiper Pad Kit*

- Thermometer – NBS traceable with a tolerance of ± 0.2 °C
 - C-size alkaline batteries: 8 for the 6600 sonde and 4 for the 650MDS (do not substitute rechargeable batteries or battery packs)
 - Deionized water (ASTM type III, Laboratory Grade, or better)
 - Conductivity standard between 10 and 50 mS/cm (YSI # 3163 and 3169 or equivalent)
 - Turbidity standard of AMCO-AEPA-1® Microspheres (YSI # 6073)
 - 1 Large ring stand or other home-made bracket that will allow for the 6600EDS to be suspended from its bail and hang so that the face of the turbidity sensor will be no less than 7.6 cm (3 in) from the bottom of the calibration cup sitting on the bench beneath it.
 - Laboratory glassware for preparation and dilution of standards
 - Field Cable: YSI 6091-25 ft (for shallow model sonde) or 6092-50 ft (for medium depth sonde)
 - YSI 6115 GPS Cable
 - GPS unit: WAAS compliant with output capabilities using the NMEA 0183 protocol
 - If the GPS output is not from a DE-9 connector (9-pin D-Subminiature) you will also need a DE-9 adaptor
 - 5-gal plastic bucket or insulated cooler box (for transporting sonde)
 - Spatial Survey YSI Sonde Calibration Logs (Appendix B)
- * These are expendable items provided in limited quantity with the original purchase of YSI 6600EDS sondes.

Pre-Deployment Preparation and Calibration Methods

Preparation and programming of the YSI 650MDS data logger & display

The YSI 650MDS serves several functions. It provides a user interface to the sonde while in the field, it logs data from the sonde during hydrocasts, and it provides a real-time display of the output from each of the sonde sensors. At the beginning of each field season, install four fresh C-size alkaline batteries according to the directions in the 650MDS Operations Manual. Pay particular attention to the installation of the battery cover O-ring. These batteries are likely to last for the duration of the index period, but you should monitor their charge level (see thermometer bar at bottom right corner of display) and replace them as needed. Note that should the sonde batteries get low, it is possible to power the sonde from the batteries in the 650MDS (“power sonde” option under the <System Setup> menu). It is also prudent to bring a spare set of batteries into the field.

Before the instrument can be deployed, it must be properly programmed. There are three menu sections that you will need to program: <File>, <Logging Setup> and <System Setup>.

650MDS <File> menu: Check to confirm that there are no remaining data (.dat) files stored in the 650MDS memory. All files should previously have been removed as part of the SOP for

downloading data to computer, so the presence of any data files will indicate a failure to follow the SOP. Proceed with caution! Download any files to a specially labeled directory and annotate the calibration log to this effect. Use YSI EcoWatch software to open the data files and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm that they too are readable using EcoWatch. After these steps have been completed, select <Delete all files> from the 650MDS <File> menu.

650MDS <Logging Setup> menu: From the main menu in the 650MDS, go to <Logging Setup> and program as follows:

- i. Set the sampling interval to one second (= 00:00:01)
- ii. Enable “Use site list”
- iii. Enable “Store Barometer”
- iv. Enable “Store Lat and Long”
- v. Enable “Store site number”
- vi. Select <Edit Site List> and create a new file for each of the 30 spatial elements of the sampling design. File names are limited to eight characters.
 - a. As a standardized naming convention, each file name should start with the four-character park code followed by the two-numeral hexagon number.
 - b. For discrete measurements made at the continuous logging station (quality control data for continuous monitoring), substitute “LS” for the hexagon number.
 - c. The 7th character is used to record the site visit number. For probability stations, the site number should never increment beyond 1, but trend stations and quality control data collected at the logging station will increment with each visit.
 - d. The 8th character is reserved for those very few cases where parks with existing monitoring programs have more than one useful trend station within a single hexagon. In this case, a letter code is added to distinguish them.
 - e. Using the site list, create four site numbers for each filename (station). “Site number” zero will be used for all sub-surface measurements, site number 1 will be used for mid-depth, site number 2 will be used for near bottom, and site number 9 will be used for logging quality control data related to the dissolved oxygen calibration.
 - f. The “Site Name” field is for your convenience only. It is not included in the data file, but you may use it as a reminder of the depth locations for the four “site numbers.”

650MDS <System Setup> menu: From the main menu in the 650MDS, go to <System Setup> and program as follows:

- i. Set the correct date and LOCAL TIME. For all anticipated use of the SOP, this will be Eastern Daylight Savings Time. Select the “4 digit year” option.
- ii. Make certain that the “Power sonde” and “Comma radix” options are NOT selected.

- iii. Set the “Shut off time” to 0 minutes. This will keep the unit on until manually shut off.

This menu also provides for user calibration of the barometer. It is recommended that the barometer be checked annually for drift. Recalibration is a delicate procedure. Carefully follow the guidance in the 650MDS Operations Manual and be certain that you are calibrating against barometric pressure that has not been corrected for altitude (the National Weather Service generally reports barometric pressure corrected to sea level). If in doubt, return the unit to YSI for a factory recalibration.

Preparation and calibration of the YSI 6600 (6600EDS) sonde

General notes:

During the calibration procedure, never accept any calibrations that have produced a warning message. You must instead determine the cause of the problem, correct it, and recalibrate following this SOP and the instrument manual.

Each field season, batteries, dissolved oxygen membranes, and wiper pads for the turbidity, chlorophyll and ROX optical dissolved oxygen probes must all be replaced with new ones. Pay particular attention to YSI guidance on appropriate clearances for wiper assemblies since excessively tight installation could slow down or stall the motor on the turbidity and chlorophyll sensors. All of these components should last for the duration of the index period, except perhaps for the dissolved oxygen membrane which is easily damaged.

Since dissolved oxygen membranes on the 6562 rapid pulse dissolved oxygen sensors are unstable for the first 3 to 6 hours after they are installed, it is important to check their condition and performance in advance of any planned field work. This SOP calls for the DO membrane to be examined (and replaced if necessary) and the sonde calibrated 6-12 hours before deployment. After this stabilization period, just the DO sensor is recalibrated, and the sonde is ready for deployment. This step is not required if using the 6150 ROX optical dissolved oxygen sensor.

Except where explicitly noted in the SOP, standards should NOT be used for more than one calibration. They may, however, be saved and used to pre-rinse the sensors and the calibration cup prior to putting them into fresh standard solutions. Rather than measure calibration solutions with graduated cylinders each time they are dispensed, you may choose to mark the outside of the calibration cup at the appropriate levels for each probe. Dispensing standards directly into the calibration cup will also help prevent contamination from dirty glassware. For many of the calibration steps, reaching a stable calibration point requires the probe and calibration solution to thermally equilibrate. This occurs more quickly if both the sonde and all the calibration solutions are at laboratory temperature. Be sure to prepare deionized water in advance and store an ample supply in a carboy.

The 6600 or 6600EDS should be calibrated while hanging from a ring stand or custom-mounted hook. Height is critical for proper calibration of the turbidity sensor. The bottom face of the 6136 Turbidity Probe should be set so there is at least 7.6 cm of clearance between it and the inside bottom of the calibration cup (when unthreaded from the sonde body). This is the distance between the sensor face and the inside end of the sensor guard, and will provide the best reproduction of conditions when calibrating the turbidity sensor. Closer distances will result in excessive backscatter from the bottom of the calibration cup. The conductivity probe can be calibrated upside down or horizontally to conserve calibration solution, but it is CRITICAL that the conductivity ports, the recessed area on side of sensor, and the thermistor are all entirely submerged when calibrating. If you are calibrating both YSI 6600 and 6600EDS sondes, note that the sonde bodies are different and require different hanging brackets or heights.

The wiper assemblies on the turbidity, chlorophyll and ROX optical dissolved oxygen probes must “park” 180° away from the optical windows on these probes. Actuating the wipers while in air can sometimes cause them to park in different locations. At several points in the calibration

procedure you are prompted to confirm that the wipers are parking properly. If they fail to do so, troubleshoot using guidance in the YSI 6-Series Operations Manual.

Taking the above notes into account, the following calibration procedure should be completed in the order it is presented.

Instrument Preparation:

Before the instrument can be deployed, it must be calibrated and several maintenance steps performed. Unlike for continuous monitoring, it is not necessary to paint the bulkhead, probe guard, and individual sensors with marine antifouling paint. Assemble the individual sensors onto the sonde per instructions in the manual using extra care not to cross-thread the sensors when installing them onto the softer PVC sonde bulkhead. As a final check prior to calibration, visually inspect the sonde for any abnormalities.

Install eight fresh C-size alkaline batteries according to the directions in the YSI Operations Manual. Pay particular attention to the installation of the bottom O-ring, as this is the one that keeps the battery compartment from leaking. It must be properly seated in its groove on the bottom of the battery lid, clean and free from contaminants, and lubricated with a VERY light film of clean silicone grease. The O-ring on the side of the battery lid seals out contaminants and fouling material and should also be cleaned and lubricated with a light film of clean silicone grease. Also note that over tightening the battery lid screws may distort the lid and cause the battery compartment to flood.

Standard solutions:

Only turbidity standard and deionized water are required for this SOP (no chlorophyll dye standard). For details on deionized water and turbidity standard requirements see the section on standard solutions within SOP - 3). Rhodamine WT dye standard is not required because chlorophyll is determined solely through extractive analysis on discrete grab samples for this SOP.

Dissolved Oxygen:

6562 Rapid Pulse Dissolved Oxygen Membrane Probe:

- i. Inspect the DO probe anodes. If they are darkened or gray in color, recondition using the 6035 reconditioning kit.
- ii. Inspect the DO membrane. It should be undamaged, tightly stretched, wrinkle free, and there should be no air or gas bubbles under it. If necessary, install a new membrane following guidance in the YSI 6-Series Operations Manual and Technical Notes.
- iii. Hang the sonde from the ring stand or custom support bracket so that the turbidity probe is on the left, and the chlorophyll probe on the right. Put approximately 0.5 cm of water in the bottom of the calibration cup and loosely attach it to the sonde (engaging several threads). Attach the Dry Calibration Cable between the sonde and computer, launch EcoWatch software, and establish communication with the sonde.
- iv. Go to the sonde <Report> menu and enable the <DO Charge> option.

- v. Go to the sonde <Advanced> <Setup> menu and disable the “RS-232 auto-sleep” function.
- vi. If you have just installed a new membrane, go to the “<Run> menu and start the sonde in the <Discrete Sample> mode with a 4-second sample interval. Allow the sonde to run for 10 minutes to “burn in” the new membrane.
- vii. Perform a High-to-Low Transmission Test: Start the sonde in <Discrete Sample> mode with a 4 second sample interval. Disregard the first two DO % Saturation samples, and then observe the next 10 samples. The DO % Saturation values must start high and drop with each 4-second reading. It does not matter if the readings do not reach 100%, but there must be a high to low trend. If the recorded values start low then climb upward, the sensor has a problem and must be reconditioned or replaced. Record the pass-fail status of the test on the appropriate calibration log. Escape from the <Run> mode.
- viii. Wait at least 5 minutes in the idle (not <Run>) mode before proceeding. You must also wait at least 10 minutes from the time the calibration cup (with 0.5 cm water in bottom) was attached to the sonde body. Once these minimum times have been reached, calibrate the DO sensor using the <% Saturation> method. If the sonde is properly programmed (auto-sleep off) then you will be prompted to accept the calibration once the values have stabilized (display will update with each reading). NOTE that this is different from the method used for continuous monitoring where the calibration is accepted automatically following a 60-second sensor warm-up. Be sure to check and enter the current barometric pressure. Note that laboratory barometer readings are usually “true” values of air pressure and can be used “as is” for oxygen calibration. The barometer on the 650MDS (if properly calibrated) also reports “true” barometric pressure and can be used “as is.” Pressure reported by the National Weather Service, however, is usually “corrected” to sea level and cannot be used in raw form. See the YSI Operations Manual for correction methods. Record the DO Charge on the appropriate calibration log. It should be 50 ± 25 .
- ix. When the calibration has been accepted, check the “DO gain” for the sensor and record it on the calibration log. This is found under the sonde’s <Advanced Menu> under the <Cal Constants> option. The target value for gain is 1.0, with an acceptable range from 0.7 to 1.4.

Dissolved Oxygen:

6150 ROX Optical Dissolved Oxygen Probe

- i. The calibration for this sensor is best performed in air-saturated water which requires an aquarium pump, an air-stone and a large enough vessel to immerse the sonde vertically into the air-saturated water. The water must be sparged for at least one hour prior to calibration.
- ii. With the sensor guard in position place the sonde into the container of air-saturated water which is being continuously sparged with air.
- iii. Wait for at least 10 minutes to allow the temperature and oxygen pressure to equilibrate before proceeding to the next step.
- iv. Calibrate the sensor by selecting ODOsat % and then 1-point to access the DO calibration.

- v. Enter the current barometric pressure in mm of Hg.
- vi. Press enter, all the current values of the enabled sensors will appear.
- vii. When the ODOsat % reading has stabilized press enter.
- viii. When the calibration has been accepted, record the final reading and the “ODO gain” for the sensor on the spatial survey YSI sonde calibration log. The ODO gain is found in the ADVANCED/CAL CONSTANTS menu. The acceptable range for the ODO gain is 0.85 to 1.15.
- ix. If the ODO gain reads outside of the acceptable range or zero, perform an UNCAL to reset the ODO gain to the factory default and recalibrate.

Conductivity:

- i. Calibration of the conductivity probe is vulnerable to contamination. Before performing the calibration, the calibration cup and all the surfaces of all the probes must be triple rinsed with a small amount of conductivity standard. Used standard from previous execution of this SOP is acceptable.
- ii. Pour approximately 50 ml of conductivity standard (within the range of 10 to 50 mS/cm) into a clean and dry calibration cup and fully attach it to the sonde body. Swirl, tip, and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution (do not re-use rinse standards). Repeat this step two more times.
- iii. You will need approximately 370 ml of conductivity standard to calibrate this probe. This volume can be reduced to approximately 340 ml if the sonde is held upside down while performing the calibration, and to as little as 125 ml if the sonde is held horizontally. It is CRITICAL, however, that the conductivity ports, the recessed area on side of sensor, and the thermistor are all entirely submerged when calibrating, and horizontal calibration is particularly prone to air entrapment.
- iv. Pour the appropriate volume of FRESH conductivity standard into the calibration cup and attach it to the sonde body.
- v. Insure that the conductivity probe is completely submerged in the standard. The hole in the side of the probe must be under the surface of the solution and not have any trapped bubbles in the opening. Tap the sonde and/or calibration cup to release entrapped air bubbles.
- vi. Initiate calibration of the probe by selecting the <specific conductance> option and entering the value for the conductivity standard (Be sure to enter the conductivity of the standard in mS/cm, not $\mu\text{S/cm}$).
- vii. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde, the conductivity standard, and the deionized water from previous steps are not all at the same laboratory temperature). Once a stable reading has been maintained for approximately 30 seconds, accept the calibration (press <enter>) and record it on the spatial survey YSI sonde calibration log the last value reported prior to doing so. With an NBS-traceable thermometer, verify the accuracy of the sonde temperature reading and record it on the calibration log.

- viii. When the calibration has been accepted, check the “Conductivity Cell Constant” for the sensor and record it on the appropriate calibration log. This is found under the sonde’s <Advanced Menu> under the <Cal Constants> option. The target value for this probe is 5.0 ± 0.45 . Numbers outside this range usually indicate a problem in the calibration process or a contaminated standard. Never override a calibration error message and investigate any “Out of Range” report.

Turbidity:

- i. The sonde and calibration cup must be clean or contamination of the zero NTU standard will occur. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip, and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this rinse water and repeat two more times.
- ii. Fill the calibration cup with at least 370 ml of fresh deionized water and set it on the bench below the sonde. Do not attach it to the sonde body. The distance between the face of the turbidity probe and the inside bottom of the calibration cup should be no less than 7.6 cm.
- iii. Select the <2 point> method from turbidity calibration menu, and enter zero <0> as the value for the first point. Start the calibration routine and actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180° away from the optical window on the probe face.
- iv. Wait for a stable reading to be maintained for approximately 30 seconds, then press <enter> to set the calibration. The reading on-screen should now be approximately 0.0. On the appropriate calibration log, record the last value displayed before pressing <enter>.
- v. Pour approximately 50 ml of turbidity standard (used is acceptable) into a clean and dry calibration cup and attach it to the sonde body. Swirl, tip, and gently shake the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.
- vi. Fill the calibration cup with at least 370 ml of fresh turbidity standard and set it on the bench below the sonde. Do not attach it to the sonde body.
- vii. Continue the calibration routine entering 126 NTU for the second standard. (NOTE: the recommended AMCO-AEPA-1® standard has a different value depending upon the sensor. It is 126 NTU for the YSI 6136.) Actuate the wipers to remove any air bubbles from the face of the probe (press<3>). Confirm that the wiper is parked 180° away from the optical window on the probe face.
- viii. Allow the sonde to run until a stable reading is maintained for approximately 30 seconds, then press <enter> to set the calibration. On the appropriate calibration log, record the last value displayed before pressing <enter>.
- ix. When the calibration has been accepted, check the “Turbidity Offset” for the sensor and record it on the appropriate calibration log. This is found under the sonde’s <Advanced Menu> under the <Cal Constants> option.

- x. Rinse probes with deionized water, put approximately 0.5 cm of water in the bottom of the calibration cup and loosely attach it to the sonde (engaging several threads).

Pressure/Depth calibration:

- i. From the <Calibrate> menu, select <Pressure-Abs>.
- ii. Input 0.00 (or a known sensor offset in feet if working far from sea level).
- iii. Wait until no significant change occurs for approximately 30 seconds, then press <enter> to confirm the calibration.

Sonde programming and miscellaneous preparations:

When preparing the sonde for discrete sampling, realize that all data will be logged directly to the YSI 650MDS data logger and display. Methods for programming the 650MDS are given earlier in this SOP under “Preparation and programming of the YSI 650MDS data logger & display”.

- i. Program the 6600 or 6600EDS sonde with the correct date and LOCAL TIME even though the 650MDS date and time will be logged with the data. For all anticipated use of this SOP, this will be Eastern Daylight Savings Time.
- ii. Enable the reporting of dissolved oxygen as percent of saturation using the “%local” convention (non-standard but allows for 100% of saturation at all barometric pressures). “DOsat %Local” is used only for quality assurance purposes and not for general reporting. This feature may not appear on the <Report> menu of a new sonde or after a firmware upgrade. To activate it, exit the menu structure of the sonde to the command line prompt (“#”) using a computer and dry calibration cable. The “#” sign appears after you press Esc from the Main menu and answer “Y” to the question “Exit menu (Y/N)”?. Once the “#” prompt is shown on the display, type “dolocal 1” and press Enter. The screen will show “OK” to indicate that the action was successful and return another “#” sign. Type “menu” to re-enter the sonde software menu structure. After taking the above action the parameter “DOsat %Local” will be present in the <Report> menu.
- iii. Enable the following report parameter in the <Report> menu: temperature, specific conductance, salinity, DO concentration, DO saturation %, DO saturation % local, DO charge, depth, turbidity, barometric pressure, and battery voltage.
- iv. Set the sampling interval to 1 second. This option is set in the <Run> menu under the <Discrete Sample> option.
- v. Set the data filter for ALL sensors to 12 seconds. This will provide some averaging even for the DO sensor which pulse samples every 4 seconds. This option is set in the <Advanced> menu under the <Data filter> option.
- vi. Confirm that there are no remaining data (.dat) files stored in the sonde’s memory.

If this sonde was previously used for continuous monitoring, all files should previously have been removed as part of the SOP for downloading data to computer. Since this SOP calls for data to be logged to the 650MDS, the presence of any data files in the sonde’s memory will indicate a failure to follow the SOPs of this or other protocols. Proceed with caution! Download

any files to a specially labeled directory and annotate the appropriate calibration log to this effect. Use YSI EcoWatch software to open the data files and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm that they too are readable using EcoWatch. After these steps have been completed, select <Delete all files> from the sonde's <File> menu.

Final lab calibration of dissolved oxygen and preparing the sonde for field work:

- i. Just prior to bringing the sonde into the field, perform a final lab calibration of the dissolved oxygen probe. To do this, go to the <calibrate> menu and repeat the last 2 steps (steps vii and viii) of the instructions for calibrating the 6562 rapid pulse dissolved oxygen sensor. If using a 6150 ROX optical dissolved oxygen sensor this step is not necessary but a quick check that the sensor is reading 100% saturation in air-saturated water is recommended.
- ii. Terminate communication with the computer, disconnect the dry calibration cable, and install the field cable. Attach the strain relieve connector between the field cable and the bail on the sonde.
- iii. Attach the other end of the field cable to the YSI-6115 GPS cable, and the other end of the GPS cable to the 650MDS. Attach the strain relieve connector between the field cable and strain relief lanyard on the 650MDS.
- iv. Attach the GPS unit to the YSI-6115 GPS cable (using a DE-9 adaptor if needed) and temporarily power up the 650MDS and GPS to confirm communication between them. Make certain that you have satellite reception (may be a problem indoors) and look for latitude and longitude to be displayed in the bottom left corner of the 650MDS screen.
- v. Remove the calibration cup from the sonde body and install the sensor guard. It is best not to attempt this in the field since the oxygen membrane is vulnerable to being damaged if it is bumped by the guard.
- vi. Wrap the sonde in a white towel that has been soaked in tap water. The towel should cover the entire sensor guard and go around it at least twice. This will provide a humid environment for the sensors, protection from thermal extremes, and some degree of shock protection.
- vii. Transport the sonde to the field in a specially-modified white 5-gallon plastic bucket. To prepare the bucket, place approximately 8 kg of lead weight in its bottom and cover with open cell foam padding. Neoprene-covered soft weight packs containing lead shot (used by SCUBA divers) are also effective. Also put approximately 2 cm of tap water in the bottom of the bucket. Cut a hole in the middle of the lid that will accommodate the sonde body wrapped in the towel. The lid will support the sonde, help to maintain the humid environment, and keep water from spilling or sloshing out. Alternatively, an insulated cooler can be substituted for the bucket, but a breather hole should be drilled in the top to prevent pressurization of the interior.
- viii. As much as possible, keep the sonde and bucket out of direct sunlight to prevent thermal extremes.

Making Measurements

Standard Approach

- i. The sonde must be allowed to warm up for 4 to 5 minutes before taking measurements. Turn on the sonde by powering up the 650MDS and selecting <Sonde run> from the main menu. Make certain that the sonde, the GPS, and the 650MDS are all communicating properly.
- ii. Once the sonde has warmed up, and with the unit still wrapped in the wet towel inside the transportation bucket, check the dissolved oxygen percent saturation (“DOsat %local”) and record this value on the Spatial Survey Station Data Sheet.
- iii. If using a 6562 rapid pulse dissolved oxygen sensor, confirm that it reads $100\% \pm 2\%$. If the DO saturation %Local has drifted beyond these tolerances, the dissolved oxygen channel must be recalibrated on the spot. This is accomplished as follows: (These steps are not required if using the 6150 ROX optical sensor. Field DO calibrations should be avoided when using the 6150 ROX sensor.)
 - a. Press <Escape> to get to the 650 MDS Main Menu
 - b. Select <Sonde Menu>
 - c. Select <Calibrate>
 - d. Select <Dissolved Oxy>
 - e. Select <DO%>
 - f. Update the barometric pressure used for the calibration (large-font numerals) so that it exactly matches the barometric pressure as measured by the 650MDS (small-font numerals in the bottom right corner of the screen).
 - g. Press <enter> and wait for the sensor to stabilize and the sensor to calibrate. Should the sensor fail to calibrate, follow trouble-shooting guidance in the YSI 6-Series Environmental Monitoring Systems Operations Manual.
 - h. Press <Esc> to escape back to the 650MDS main menu, and then select <Sonde Run>.
 - i. Confirm that the dissolved oxygen percent saturation (“DO%”) reads $100\% \pm 2\%$ and check off the box on the Spatial Survey Station Data Sheet that the sensor was successfully recalibrated
- iv. With the sonde still wrapped in the wet towel inside the transportation bucket, select <log one sample> from the “650” side of the menu (not the “sonde” side). Select the appropriate <Filename> into which the data will be stored (see instructions above for concerning the <Logging Setup> menu) and select site number 9. This will log the “in air” data to the 650MDS as quality assurance data for this station.
- v. Lower the sonde over the side, completely submerging it in the water. The length of time required will depend upon the temperature differential between air and water. Watch for

the temperature readout to stabilize. While waiting, select <clean optics> from the “sonde” side of the menu.

- vi. Once the optics have finished wiping and the temperature has stabilized, raise the sonde so that the pressure ports are just out of the water, but the entire sensor guard is still underwater. Select <log one sample> from the “650” side of the menu and select the same <Filename> but this time site number 0 (for surface).
- vii. Lower the sonde to half of the depth of the water column and repeat the previous step, this time logging into site number 1 (mid depth). This step may be omitted for water depths less than 1.5m
- viii. Lower the sonde to within 1m of the bottom (within 20 cm for shallow systems) and repeat the process, this time logging into site number 2 (near-bottom).
- ix. Avoid running the sonde into the bottom since hydrogen sulfide will interfere with the Clarke-type electrode used for dissolved oxygen and make the output jumpy. This effect is also seen when the bottom water is anoxic and hydrogen sulfide is present. A practical method to avoid this is to attach a weight to the sonde guard on a length of line equal to the desired depth off the bottom. The operator then “feels” for the bottom with the weight.
- x. Return the sonde to the surface, wrap it in the wet towel, and replace it in the protective bucket.
- xi. If the interval of time before the next warm-up is expected to be less than 5 minutes, it is appropriate to leave the sonde running between stations. Otherwise, the sonde should be powered off to conserve battery power.

Accommodation for deep systems

Somes Sound at ACAD is over 45 m deep in some locations. The medium-depth YSI 6000-series sonde is capable of measuring to these depths, but the longest stock field cable is only 30m (100ft) long. Beyond this length, hand-lowering of the sonde becomes logistically impractical and potentially unsafe. The recommended solution for these situations is to reprogram the sonde to collect data in autonomous mode, logging continuously into sonde memory. The sonde is then lowered on a mechanically- or hydraulically-powered wire. Extra ballast will be required to assist in keeping a vertical wire angle. Since there will be no display on which to track the sonde’s depth, extra precautions must be taken not to run the sonde into the bottom. The research vessel will not be able to anchor for these stations, so vessel drift must also be taken into account in order to avoid dragging the sonde up onto shallower bottom. Program and deploy the sonde for these stations as follows.

- i. Complete steps i-iii from the standard approach to making measurements.
- ii. Select <Run> from the sonde main menu and initiate <unattended sample> mode. The next window will prompt you to enter the sampling interval (one second or 00:00:01), the start date (enter today’s date), and the start time. Enter the current time plus four minutes. This will give you time to deploy the sonde before the wipers activate and also

- allow the sonde to thermally equilibrate. Keep the sampling duration at the default (365 days). Name the file to which you will log using the spatial survey naming convention.
- iii. Select <start logging>, and start timing the four-minute delay. Disconnect the field cable from the sonde and replace it with a bulkhead connector cap.
 - iv. Deploy the sonde over the rail, suspending it from the wire so that sonde is submerged. Wait (using a timer) for the wipers to activate.
 - v. While waiting, record the current barometric pressure and position (Latitude and Longitude.) on the Spatial Survey Data Sheet. Both of these are shown at the bottom of the 650MDS display screen.
 - vi. Once the wipers have finished, raise the sonde until the pressure port is just underwater, then begin lowering the sonde through the water column. If it isn't possible use wire-out readings and wire angle to estimate depth of the sonde. This will provide only an approximation of depth, so the sonde should be lowered no closer than 3 m off the bottom.
 - vii. Raise the sonde back to the surface, reconnect the 650MDS, and connect to the sonde Main Menu. Select <run>, then <unattended sample>, then <stop logging>.
 - viii. Record the GPS position for the end of the hydrocast on the Spatial Survey Data Sheet.

Data Upload

Upload data from the 650MDS to computer at the end of each field day. Connect the 650MDS to your computer using the YSI 655174 PC interface cable, launch YSI EcoWatch software, and establish communication with the 650MDS by choosing "Comm"; from the drop down menu choose "Sonde". On the 650MDS choose <File> <Upload to PC> and upload the .dat file. The default program for this extension will be YSI EcoWatch, and the file will not be readily editable, but it will contain all the raw data collected by the sonde, regardless of whether the report parameters were properly programmed. After this upload, in EcoWatch select "File", "Open". When the file is open choose "Export", then "CDF/WMF". A pop-up window appears to identify file name, choose "comma delimited format" and uncheck the "Separate Time/Date" toggle and export this file. This comma delimited format is more generally useful for opening in spreadsheets and archiving since it does not rely on YSI EcoWatch software. Note that file names entered as part of the "Site List" are not created until data are logged into them. Only stations where data were collected will have data files. Use EcoWatch to open each data file that you just downloaded and examine them to ensure they have not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of the data files and confirm that they too are readable using EcoWatch. After these steps have been completed, reestablish communication with the 650MDS, select the <File> menu and <Delete all files>.

If survey data were logged into sonde memory rather than to the 650MDS, then follow guidance from the SOP on Continuous Water-Quality Monitoring with the YSI Sonde to upload these data. This will apply only to stations that are deeper than the available stock field cable (Acadia Somes Sound only).

Probe Care and Storage

Refer to the YSI 6-Series Multiparameter Water Quality Sondes User Manual section 2.10 on Care, Maintenance and Storage: <http://www.y.si.com/media/pdfs/069300-YSI-6-Series-Manual-RevH.pdf>. It is good practice to ensure that all of the sensors and connections are cleaned and working properly prior to extended storage (greater than one month) so that the sonde is ready for the next application. In particular, any necessary repairs/replacements must be identified before winter storage to ensure adequate time for correction in advance of the following season.

Of the probes used for this protocol, the YSI 6562 Dissolved Oxygen Probe has the most limited life expectancy. Under normal circumstances, it can be expected to perform well for at least 2-3 years, and will require resurfacing during this period. Consequently, it is advisable to keep replacement probes on hand to replace a probe that fails to calibrate properly. Note, however, that the dissolved oxygen probe also has a limited shelf life, so replacements should not be purchased too far in advance.

For short term storage of probes (one month or less), it is important to keep them in a humid environment while not immersing them in water. Simply keep the probes attached to the sonde, place approximately 1 cm of tap water in the calibration cup, and attach it tightly to the sonde. Because this protocol does not call for a pH probe, long term storage of the sonde is simplified from the standard method described in the YSI Operations Manual. Completely fill the calibration cup with tap water and attach it to the sonde body. Remove the batteries from the battery chamber and replace the battery lid following the methods detailed in the YSI Operations Manual.