

SOP 3 – Continuous 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 During deployment and collection of sonde, collect chlorophyll calibration samples from a container rather than from estuary directly	Manufacturer's improvements Manufacturer's recommendation Manufacturer's recommendation

Background and Familiarization with Instrument

This SOP describes the methods for continuous monitoring of water-quality parameters at a single station in each park during a four-week late-summer index period. Data are collected using the YSI 6600EDS multi-parameter water-quality monitor (sonde) deployed in autonomous mode. This specific instrument was selected based primarily upon the performance of the sonde in long-term deployments within estuaries with high levels of biofouling. Unlike other multi-

parameter sondes currently on the market, the YSI 6600EDS includes wipers for each of the individual sensors. Additionally, YSI was able to customize the sonde with optical sensors, mounting brackets, and wipers to allow for simultaneous measurement of underwater downwelling photosynthetically available radiation (PAR, 400-700 nm) and the PAR attenuation coefficient. It is outside the scope of this SOP to review all of the information and methods that are required to operate the YSI 6600EDS and 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 manual (including the “Configuration and Deployment Instructions for YSI Model 6600EDS Sondes” supplement) 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, deployment, and post-deployment procedures 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 with the YSI 6600EDS. YSI, Inc. offers courses on instrument calibration and maintenance. We strongly advise that all NCBN personnel involved in instrument calibration receive annual training from YSI.

YSI, Inc. offers three different depth models of the 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 will be best for most NCBN parks for continuous monitoring; however, medium-depth units will still generate acceptable depth data and may substituted if a park anticipates the need for continuous monitoring at depths greater than 9 m or if it wishes to have the capability to swap units between continuous and spatial monitoring functions and the spatial survey will include stations with depths greater than 9 m. In either case, the sonde should be non-vented for pressure, and equipped with the following sensors:

- YSI 6562 Dissolved Oxygen Probe or YSI 6150 ROX Optical Dissolved Oxygen Probe
- YSI 6136 Turbidity Probe
- YSI 6025 Chlorophyll Probe
- YSI 6560 Conductivity & Temperature Probe
- 2 LiCor LI-192SA Underwater Quantum Sensors

Note that a pH sensor is not included in the above list. The 6600EDS is routinely shipped with a pH sensor, but it should be removed from the order for a purchase credit. The LiCor sensors are a modification to the standard 6600EDS. YSI provides a bracket to hold the two cosine corrected 2- π (flat) sensors facing upward separated by a vertical distance of 50 cm. Should the specific installation conditions warrant further modifications, it is possible to make a separate bracket for the LI-192SA sensors, and run longer cables to the 6600EDS. See the SOP on Preparation of the Continuous Logging Station.

In addition to the sonde and sensors listed above, the following equipment and supplies are also required:

- 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 (including the “Configuration and Deployment Instructions for YSI Model 6600EDS Sondes” supplement), and YSI Technical Notes and Technical Documents attached herein and also at:
<<http://www.ysi.com/extranet/EPGKL.nsf/SearchDocs/1F24980CDDEE02AC85256CEF00763EC1!OpenDocument>>
- YSI 6067B Dry Calibration Cable
- Barometer (you may use the barometer from the YSI 650MDS called for in the SOP for spatial survey monitoring).
- YSI 5775 Oxygen Probe Service Kit* (membranes and O-rings for 6562 probe)
- YSI 6628 6600EDS Wiper Kit*
- YSI 6144 Wiper Pad Kit*
- Thermometer – NBS traceable with a tolerance of ± 0.2 °C
- 8 C-size alkaline batteries (do not substitute rechargeable batteries)
- Deionized water (ASTM type III, Laboratory Grade, or better)
- Conductivity standard between 10 and 50 mS/cm (YSI # 3163 and # 3169 or equivalent)
- Rhodamine WT Dye standard (Fluorescent FWT Red Dye - Lot# 257201, Kingscote Chemicals, 9676 N. Looney Road, Piqua, OH 45356. 800-394-0678)
- 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 hung so that the face of the turbidity sensor will be no less than 7.6 cm (3 in) from the inside bottom of the calibration cup sitting on the bench beneath it
- Black non-ablative marine antifouling paint such as Interlux brand ULTRA™ or Petit brand Trinidad SR™
- Laboratory glassware for preparation and dilution of standards
- LiCor LI-1400 Data Logger Instruction Manual
- LiCor LI-1400 data logger
- LiCor LI-190SA quantum sensor reserved for calibration purposes
- LiCor LI-2003S Mounting and leveling fixture for LI-190SA quantum sensor
- Two LiCor LI-2222UWB underwater cables (3 m length is adequate)
- 5-gal plastic bucket or insulated cooler box (for transporting sonde)
- Digital camera
- Continuous YSI Sonde Calibration Logs (Appendix B)

- Water sampling device capable of collecting a 1-liter sample for chlorophyll analysis from the same depth where the YSI sensors are deployed.

* These are expendable items provided in limited quantity with the original purchase of YSI 6600EDS sondes.

Instrument Preparation

Before the instrument can be deployed, it must be calibrated and several maintenance steps must be performed. Prior to assembling a sonde for the first time or prior to reassembling it at the beginning of each field season, the bulkhead, probe guard, and individual sensors must be painted with marine non-ablative antifouling paint. YSI application engineers currently recommend black Interlux brand ULTRA™ or Petit brand Trinidad SR™. Ablative paints are not recommended because they may interfere with turbidity measurements. VERY CAREFULLY follow the instructions in the “Configuration and Deployment Instructions for YSI Model 6600EDS Sondes” supplement to the YSI 6-Series Environmental Monitoring Systems manual. Failure to closely follow the painting guidance from YSI may cause individual sensors to malfunction and fail. Sloppy application of paint may also interfere with the proper function of threads on the sensors and the probe guard. Do not paint the sonde body itself. Instead, wrap it with plastic food wrap secured with electrical tape. After wrapping the sonde, make two holes in the wrapper at the position of the pressure sensor ports so that the wrapper does not interfere with depth measurements. This wrapper can then be removed and discarded at the end of each deployment. Assemble the individual sensors onto the sonde per instructions in the manual using extra care not to cross-thread the stainless steel nuts 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, be clean and free from contaminants, and be 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.

Pre-Deployment Calibration Methods

LiCor 192SA PAR sensor inter-calibration

Although LiCor recommends biennial factory calibration of its LI-192SA quantum sensors, we recommend here that all sensors used for continuous underwater deployments be factory calibrated annually. The diffuser lens of the LI-192SA is made of acrylic, which is very slightly permeable to water vapor. With long-term deployments, excessive water vapor inside the sensor has been implicated in very occasional sensor failure. Careful pre- and post-deployment

calibration checks will assure data quality. LiCor sensors that are used to make discrete light profiles for this SOP and for the spatial survey SOP may be factory calibrated every two years. Confirm the pre-deployment calibration of both LI-192SA underwater sensors for this SOP by comparing their output to that of a third, recently calibrated sensor reserved specifically for this purpose. A LI-190SA “deck cell” will work well for this purpose.

- i. Attach the two underwater quantum sensors and the third “calibration sensor” to channels I1, I2 and I3 of a LiCor LI-1400 data logger (using appropriate underwater cables for the underwater sensors).
- ii. Mount the sensors to a bracket that will keep all three of them aligned with their diffuser elements horizontal.
- iii. Program the LI-1400 to log the output from all three sensors (channels I1, I2 and I3) for a duration of one hour with the sampling period set to one second and the logging period set to one hour. Follow instructions in the LI-1400 Instruction Manual for programming the data logger. For the underwater quantum sensors, use the “in air” multipliers from their most recent LiCor calibration certificates.
- iv. Place the sensors in full sunlight within 2 hours of local apparent noon (for Eastern Daylight Time this is close to 13:00 hrs). Keep them there for the duration of the logging period.
- v. At the end of the one hour logging period, record the one-hour average value for each of the LI-192SA sensors on the Continuous YSI sonde Calibration Log. (Follow instructions in the LI-1400 Instruction Manual for viewing logged data).
- vi. Small differences in sensor output can be corrected using this intercalibration data, but if either underwater sensor differs from the air sensor by more than 20%, then it is very likely damaged and should be factory serviced. It is prudent to have back-up sensors to accommodate this scenario.

Standard solutions

Deionized Water:

Deionized water is required for calibration of many of the sensors so some guidance is provided here on the quality of water that should be used for these purposes. In general it is recommended that water be purchased or purified on site to a quality equal to or better than ASTM standards for Type III (Laboratory Grade) water. Note that a USGS sampling of supermarket distilled waters from around the country found conductivities falling well outside the standard for even ASTM Type III Laboratory Grade water (USGS Office of Water Quality Technical Memorandum 92.01). While not part of this protocol, some of the network parks already include the measurement of pH among their suite of monitoring variables. ASTM Type II (Reagent Grade) water should be used for the mixing of buffer solutions. This SOP calls for the use of pre-prepared conductivity standards for calibration of the sonde. Nevertheless, you should be further deterred from preparing or diluting your own standards by the fact that even ASTM Type II water is inadequate for these purposes. Water prepared by distillation or ion exchange should include a polishing step by passing it through a 0.45µm filter to remove some bacteria and any

ion exchange medium that escapes the columns. This is particularly relevant for calibration of the turbidity sensor (EPA Method 180.1).

Turbidity standard:

The recommended standard for this SOP is a 126 nephelometric turbidity unit (NTU) standard made of AMCO-AEPA-1® Microspheres prepared specifically for the YSI 6136 turbidity probe (YSI part number 006073). This standard can only be obtained from YSI or its dealers. Formazin based turbidity standards are less expensive and are also effective, but are not recommended for the NCBN protocol. One of the reagents used in its preparation, hydrazine sulfate, is a carcinogen and consequently requires special care in handling and disposal. Even prepared formazin suspensions can contain residual hydrazine sulfate. In addition, formazin quickly settles out of suspension and may be less stable in dilute form.

Chlorophyll standards:

This SOP calls for a two-step process to assure chlorophyll sensor performance and convert the output from the YSI 6025 chlorophyll fluorometer to actual chlorophyll *a* concentrations (in µg/l). The first calibration step, using Rhodamine WT dye, produces approximate values and allows for sensor drift to be evaluated over the deployment period. These UNCORRECTED data are logged into sonde memory. The second calibration step yields final reportable concentrations by post-calibrating the logged data against discrete water samples collected during the deployment period. Water samples are collected periodically during the deployment at the same depth as the sonde and synchronized to a logging event. The samples are filtered through glass fiber filters, and analyzed for chlorophyll *a* by extractive methods. A standard curve is generated between these extracted samples and their paired uncorrected data. The equation for this standard curve is used to generate final CORRECTED chlorophyll *a* data.

The calibration methods described here are used for the first calibration step (Rhodamine WT dye standard). Chlorophyll extraction methods for the second calibration step are described within separate SOPs (see SOP 6 – Chlorophyll *a* Sampling and Analysis and SOP 10 – Water-Quality Monitoring Data Reduction for post calibration correction methods).

Rhodamine WT Dye comes from the manufacturer as an approximate 2% stock solution (Fluorescent FWT Red Dye - Lot# 257201, Kingscote Chemicals, 9676 N. Looney Road, Piqua, OH 45356. 800-394-0678). Since the fluorescence of Rhodamine WT shows an inverse relationship with temperature, this effect must be accounted for when checking for sensor drift. YSI has prepared a temperature compensation chart for a specific dye lot (# 257201) that relates a 1:40,000 dilution of the stock dye to approximate chlorophyll *a* units (in µg/l). The reported relationship may not be accurate for other dye lots, but this calibration step is most important for confirming sensor stability over the deployment period. Absolute accuracy is less important. Each field season, prepare a fresh concentrated working standard from 2% Fluorescent FWT Red Dye. This will be a 1:200 dilution from the 2% stock solution (or approximately 100 mg/l). Use this working standard for one field season only. To prepare the 1:200 dilution, use a volumetric pipette to accurately transfer 2.5 ml of 2% Fluorescent FWT Red Dye into a 500 ml volumetric flask (or 5.0 ml into 1000 ml volumetric). Fill the flask to the volumetric mark with deionized water and mix. Transfer this concentrated standard to a glass amber bottle, and store tightly capped in a laboratory refrigerator to retard decomposition. The bottle of stock 2% Fluorescent

FWT Red Dye should also be recapped tightly, sealed with Parafilm M®, and stored in the refrigerator.

To calibrate the sonde, prepare a working standard of approximately 0.5 mg/l by mixing a 1:200 dilution of the concentrated working standard. This will be a 1:40,000 dilution of the stock dye solution. Accurately transfer 5.0 ml of the concentrated working standard into a 1000 ml volumetric flask. Fill the flask to the volumetric mark with LAB TEMPERATURE deionized water and mix. Transfer this working standard to a glass amber bottle and use immediately. If kept tightly capped and refrigerated, this dilute standard can be stored for up to 24 hours; however it must be brought back to lab temperature to perform calibrations.

6600 EDS Calibration

General notes:

Before you begin: Make sure that the sonde firmware is version 3.10 or higher. Upgrade the firmware at www.ysi.com.

Go to the sonde's Advanced Menu> Data Filter > Time Constants and change the Chl time to 30 seconds.

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.

Prior to each deployment, batteries, dissolved oxygen membranes, and wiper pads for the turbidity, chlorophyll and ROX optical dissolved oxygen probes must all be replaced with new ones. During the calibration procedure, use a standard wiper on the YSI 6136 turbidity probe. This must be replaced with an EDS turbidity wiper assembly (with brush) prior to final deployment when using a 6562 rapid pulse dissolved oxygen probe. The EDS 6628 wiper + brush assembly on the sonde and the brushes on the LiCor PAR sensors must be examined and replaced if they are excessively worn. Pay particular attention to YSI guidance on appropriate clearances for wiper assemblies since excessively tight installation could slow down or stall the wiper motor.

The 6562 rapid pulse dissolved oxygen sensor membranes are unstable for the first 3 to 6 hours after they are installed. This SOP calls for the DO membrane to be replaced and the sonde calibrated 6-12 hours before deployment. It is then put into autonomous/logging mode and allowed to collect 6-12 hours of data while still in the laboratory. After this stabilization period, just the DO sensor is recalibrated, and the sonde is ready for deployment. The advantage of this approach is that it allows the DO probe to fully stabilize while providing additional data prior to deployment. This can aid in future diagnosis of any invalid data. 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 before calibration with fresh standard solutions. It is helpful to mark the outside of the calibration cup at the appropriate levels for the amount of calibration solution required for each

sensor. 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 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. 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.

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.

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. Install a new membrane, making sure that it is tightly stretched and wrinkle free.
- 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. Then 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 membrane.
- v. After the burn-in, go to the sonde <Advanced> <Setup> menu and enable the “RS-232 auto-sleep” function. Wait 60 seconds before proceeding.
- vi. 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 review 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 and then climb upward, the sensor has a problem and must be reconditioned or replaced. Record the pass-fail status of the test on the Continuous YSI Sonde Calibration Log. Escape from the <Run> mode.
- vii. 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 last attached to the sonde body. Once these minimum times have been reached, calibrate the DO sensor using the <% Saturation> method. NOTE that if the unit is properly set up, there will be an automatic calibration after a 60-second warm-up. This is different from the calibration method used for spatial survey applications. 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 Continuous YSI Sonde Calibration Log. It should be 50 ± 25 .
- viii. When the calibration has been accepted, check the “DO gain” for the sensor and record it on the Continuous YSI Sonde 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 with air for at least one hour prior to calibration.
- ii. With the sensor guard on, place the sonde into the container of air-saturated water that 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 Continuous YSI 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 that best represents your estuary (within the range of 10 mS/cm 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; 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 of 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 appropriate 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 appropriate 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.

Chlorophyll:

Never calibrate the chlorophyll probe in direct or bright sunlight. A darkened environment is optimum.

- i. Go to the sonde’s <Advanced Menu> Data Filter > Time Constants and confirm that the Chl time is 30 seconds.
- ii. You will need deionized water and a working concentration of Rhodamine WT dye standard to calibrate the chlorophyll fluorometer. Prepare the working Rhodamine standard following the procedures in the Standard Solutions section above.
- iii. Half fill the calibration cup with deionized water and attach it to the sonde body. Swirl, tip, and gently shake the sonde in order to thoroughly wet all of the probe and bulkhead surfaces. Discard this rinse water and repeat two more times.
- iv. Half fill the calibration cup with deionized water and attach it loosely to the sonde.
- v. Select the <2 point > method from chlorophyll 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.
- vi. If the deionized water is already at laboratory temperature wait approximately 1 minute for thermal equilibration. Once a stable reading has been maintained for approximately 30 seconds, press <enter> to set the calibration. On the appropriate calibration log, record the last value displayed before pressing <enter>. Note: chlorophyll values tend to bounce more than other values, this is normal.
- vii. Pour approximately 50 ml of Rhodamine WT working standard 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.

- viii. Fill the calibration cup with at least 225 ml of fresh Rhodamine WT working standard and attach it loosely to the sonde.
- ix. Confirm that the standard is at laboratory temperature, and then use the temperature compensation chart to select the proper chlorophyll concentration to enter as the second point in the calibration from Table 2 of YSI Technical Note: Alternate Calibration Methods for the YSI 6025 Chlorophyll Sensor, Appendix C.
- x. Continue 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.
- xi. 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>. Rinse probes with DI water and pat dry with Kimwipes®.
- xii. When the calibration has been accepted, check the “Chlorophyll 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.

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.

LiCor 192SA PAR sensor installation:

Confirm that the procedures for checking the calibration of the LiCor PAR sensors have been performed and recorded in the appropriate calibration log (see section above on LiCor 192SA PAR sensor inter-calibration), then attach them to the PAR sensor mounting-bracket on the YSI. In the <Advanced> menu, under <Sensor>, confirm that the correct LiCor calibration multipliers have been entered for each of the PAR sensors, and that the sensors have not been inadvertently swapped between the upper and lower bracket arms. On the appropriate calibration log, record the LI-192SA serial numbers, their positions on the sonde, the calibration multipliers used, and the date of the last manufacturer’s calibration. Even though no pH sensor is installed on the sonde, the pH channel MUST BE ENABLED to ensure proper grounding of the PAR circuitry. Also confirm that the time constant for the PAR sensors is set to 56 seconds (<Advanced> menu settings for <time constants> “other”).

Programming and miscellaneous preparations for deployment:

If using a 6562 rapid pulse dissolved oxygen probe replace the standard wiper on the 6136 turbidity probe with the 6628 EDS wiper assembly and brush. (This is not necessary when using a 6150 ROX optical dissolved oxygen probe, which has a wiper.) Pay particular attention to YSI guidance on appropriate clearances for this wiper assembly since excessively tight installation can slow down or stall the wiper motor. Put approximately 0.5 cm of water in the bottom of the calibration cup and attach it to the sonde.

Check to confirm that there are no remaining data (.dat) files stored in the sonde's memory. These should previously have been removed from the sonde immediately after being downloaded to computer, so their presence will indicate a failure to follow the SOPs of this protocol. Proceed with caution! Download any files to a specially labeled directory and annotate the calibration log to this effect. Use EcoWatch 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, reestablish communication with the sonde, select the <File> menu and <Delete all files>.

Program the sonde with the correct date and LOCAL TIME. For all anticipated use of this SOP, this will be Eastern Daylight Savings Time. The following report parameters should be enabled in the <Report> menu: date, time, temperature, specific conductance, salinity, dissolved oxygen saturation, dissolved oxygen concentration, DO charge, depth, turbidity, chlorophyll, battery voltage, PAR1 and PAR2.

Select <Run> from the sonde's main menu and initiate <unattended sample> mode. The next window will prompt you to enter the sampling interval (15 minutes), the start date (enter today's date), and the start time (enter the current time rounded up to the next quarter hour). Keep the sampling duration at the default (365 days) and confirm that battery life and free memory are adequate to complete the intended duration of deployment.

The eight-character naming convention for the data file is:

- Station identifier for when a park has more than one logging station (one digit, using "1" as the default for parks with only one station).
- NPS park code (4 characters)
- Year (last two digits)
- Deployment sequence – sequential lettering (A, B, etc.) used to distinguish data files from sondes that are swapping out during the index period due to biofouling. First deployment shall always have "A" even if no swapping is anticipated.

Example: 1CAC004A

Site names may be more descriptive (up to 31 characters), but should start with the same first 5 characters as the data file.

Activate logging. The sonde will now record data for the water-saturated air environment within the calibration cup for the 6 to 12 hours before receiving a final dissolved oxygen calibration and being deployed. Confirm that the sonde is properly logging data by going to the <Status> menu and making sure that the sonde returns a "Logging Active" message.

Final dissolved oxygen calibration:

Just before bringing the sonde into the field for deployment, and after collecting 6 to 12 hours of pre-deployment data in the laboratory, perform a final calibration of the 6562 rapid pulse dissolved oxygen probe. It is not necessary to exit the unattended mode to do this. Simply go to

the <calibrate> menu and repeat the last 2 steps (steps vii and viii) of the instructions for calibrating dissolved oxygen. This re-calibration information will now be part of the data record and will confirm proper operation prior to the deployment. If using a 6150 ROX optical dissolved oxygen probe this step is not necessary, but a quick check that the sensor is reading 100% saturation in air-saturated water is recommended. Confirm that the sonde is properly logging data by going to the <Status> menu and making sure that the sonde returns a “Logging Active” message.

Deployment

- i. Before leaving the laboratory, remove the calibration cable from the sonde body and install the connector cap
- ii. Remove the calibration cup from the sonde body and install the sensor guard. It is best not to perform this step in the field since the oxygen membrane is vulnerable to being damaged if it is bumped by the guard.
- iii. Wrap the sonde in a white towel that has been soaked in tap water. The towel should cover the entire sonde (not just the 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.
- iv. 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. The cables and bracket arms holding the LiCor sensors may be removed from the sonde during transport.
- v. As much as possible, keep the sonde & bucket out of direct sunlight to prevent thermal extremes.
- vi. Plan the deployment so that it occurs within 2 hours either side of local apparent noon (for Eastern Daylight Time this is close to 13:00 hrs).
- vii. Prior to deploying the sonde, collect water in a large enough container to submerge the sonde (e.g. 5 gallon bucket). Submerge the sonde so that it is logging conditions in the container. At the same time as a logging event (on the quarter hour), gently mix the water in the container and collect a water sample from this container in a clean 500 ml amber polyethylene bottle for chlorophyll analysis by extractive methods. This ensures that the extractive sample for sensor calibration is collected from the same water, and at the same time, as the Chl-*a* sensor is reading. Follow the instructions in SOP 6 – Chlorophyll *a* Sampling and Analysis for sample collection and analytical methods.
- viii. Deploy the sonde at the logging station in a manner consistent with the particular mounting bracket design (see SOP on Preparation of the Logging Station). Record the

date and time of the deployment, the water depth at the time of deployment, and the distance between the substratum and the bottom of the sensor guard. This should be either 1.00 m or 0.50 m depending upon the park.

- ix. The sonde will take measurements in increments of 15 minutes on the quarter hour. All other measurements made alongside the sonde should also be taken on the quarter hour so that results can be directly compared.
- x. At one such time, make an independent measurement of PAR attenuation using the Spatial Survey Methods SOP. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde are shaded by the boat, the logging installation, or personnel.

Routine Service of the Sonde

In order to calibrate the chlorophyll sensor, discrete samples of chlorophyll must be gathered at regular intervals for analysis by extractive methods. The continuous monitoring station must also be visited regularly to make separate and independent measurement of the other monitoring metrics. To properly calibrate the sensor, discrete samples should encompass the full range of chlorophyll values encountered during the index period. The low end of the calibration curve is set with the zero chlorophyll standard, but mid and high-chlorophyll conditions are also required. The minimum frequency for this sample collection is weekly, and it shall be accomplished by incorporating a visit to the logging station into the weekly spatial survey (see Spatial Survey Methods SOP). Large phytoplankton blooms typically persist for time periods greater than a week, so this sample frequency should suffice to capture a reasonable range of values. Parks where blooms are found to persist for shorter periods may require more frequent discrete sampling at the logging station. In addition to calibration of the chlorophyll sensor, data from the spatial survey YSI sonde and LiCor PAR profiler are used to post-check all the continuous sensors and correct for any sensor drift.

Under anticipated conditions at NER coastal parks, the YSI 6600EDS can be expected to remain free from excessive biofouling and functionally operative for the duration of the index period. This will yield a total of five discrete extractions of chlorophyll against which to calibrate the YSI chlorophyll sensor. As a quality assurance and data-security measure, it is advised that data be downloaded from the sonde into memory on a YSI 650MDS, laptop computer, or other data storage device. This can be done without taking the sonde out of logging mode, but the Continuous YSI Sonde Deployment, Retrieval and Routine Maintenance Log (Appendix B) should be annotated to show when the sonde was out of the water for downloading so that non-representative data can later be clipped from the record. Upon returning from the field, the filename should be appended to identify it as a mid-deployment data dump.

Example: 1CAC004A-dump1

At some of the network parks, however, high rates of fouling will require that the sonde be replaced during the four-week deployment period (at the end of the second week). For these parks, weekly samples at the logging station would yield only three discrete samples for calibration of the chlorophyll sensor. In this case, supplemental samples must be gathered either by making additional dedicated trips to the logging station or by making multiple stops at the

logging station at different times of day during the four weekly spatial surveys. Perform the following steps for each of these routine service visits. The Continuous YSI Sonde Calibration Log forms contain fields for entering data and information related to the deployment, servicing and retrieval of the sonde (do not bring original copies into the field).

- i. The sonde will take measurements in increments of 15 minutes on the quarter hour. All other measurements made alongside the sonde should also be taken on the quarter hour so that results can be directly compared.
- ii. At one such time, make an independent measurement of PAR attenuation using the Spatial Survey Methods SOP. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde are shaded by the boat, the logging installation, or personnel.
- iii. In a clean 500 ml amber polyethylene bottle, collect a water sample for chlorophyll analysis by extractive methods. The sample should be collected at the same depth as the sonde probes (using an appropriate water sampler), and at the same time as a logging event (on the quarter hour). Sample collection and analytical methods are documented in SOP 6 – Chlorophyll *a* Sampling and Analysis.
- iv. Lower a second YSI sonde (as prepared for the spatial survey) to the same depth as the logging sonde. Actuate the wipers on the second YSI and initiate logging. Continue logging until you have captured data for the period coinciding with a logging event on the original sonde (which measures on the quarter hour). This can be accomplished by either allowing the second sonde to log for at least 15 full minutes, or by specifically targeting the quarter-hour event. If attempting the latter, be certain to log for at least 2 minutes on either side of the quarter hour. These data will be used to check for drift of the logging YSI sonde within the index period deployment.
- v. If performing a mid-deployment data-dump, do so now. Bring the sonde to the surface and attach an appropriate cable to either a laptop (dry calibration cable) or 650MDS (field cable). Connect to the sonde and from the file menu perform a “quick upload” using the PC6000 format. Redeploy the sonde.

Swapping a Sonde During the Index Period

For parks where the presence of extreme biofouling requires that the sonde be swapped out during the index period, use the following procedures to accomplish the task. This should occur during the middle of the index period (at the end of the second week). The preferred method is to prepare and calibrate a second sonde and deploy it before removing the first sonde. This will allow for a continuous data record for the entire index period and aid in the interpretation of any sensor drift. If this is not feasible, the existing sonde should be retrieved, serviced, recalibrated, and returned to the field within the shortest time possible (24 hours). In this scenario, it may not be necessary to recalibrate the chlorophyll sensor. The chlorophyll sensor is very stable and experiences little drift. If the value in deionized water is within 5% of zero after cleaning the optics and the value in the dye is within 10% of the theoretical value then record the values but do not recalibrate. The standard curve for deriving chlorophyll concentration from the logged data must be generated within a single sensor calibration. If the chlorophyll sensor is adjusted (recalibrated) then a new standard curve must be created, using a new set of discrete samples.

The Continuous YSI Sonde Calibration Log forms contain fields for entering data and information related to the deployment, servicing and retrieval of the sonde (do not bring original copies of the calibration log into the field).

- i. The day before it will be swapped into service, a second YSI 6600EDS should be calibrated following the methods of this SOP.
- ii. Plan the deployment/swap so that it occurs within 2 hours either side of local apparent noon (for Eastern Daylight Time this is close to 13:00 hrs).
- iii. When exchanging the old sonde with the new, they should be allowed to collect data at the same depth for at least one logging cycle. This can be accomplished by suspending the new sonde from the logging station at a depth equal to that of the existing sonde. Wait until just after the quarter hour to ensure that both sondes have logged a sampling event. The following two steps need to be accomplished close in time to a logging event, so two logging intervals may be needed.
- iv. At the same time as a logging event, make an independent measurement of PAR attenuation using the Spatial Survey Methods SOP. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde being retrieved are shaded by the boat, the logging installation, or personnel. (Note: This profile is used to post-calibrate PAR attenuation on the existing sonde only. At this time the sonde being newly deployed may not hang at a proper angle for making good measurements of PAR attenuation. A second discrete PAR profile will be required once the new sonde is in its final position.)
- v. Prior to deploying the new sonde, collect water in a large enough container to submerge the sonde (e.g. 5 gallon bucket). Submerge the sonde so that it is logging conditions in the container. At the same time as a logging event (on the quarter hour), gently mix the water in the container and collect a water sample from this container in a clean 500 ml amber polyethylene bottle for chlorophyll analysis by extractive methods. This ensures that the extractive sample for sensor calibration is collected from the same water, and at the same time, as the Chl-*a* sensor is reading. Follow the instructions in SOP 6 – Chlorophyll *a* Sampling and Analysis for sample collection and analytical methods.
- vi. Remove the existing YSI sonde from its mounting bracket and promptly swap in the new sonde. Take several digital pictures of the LiCor sensors and the sonde sensors. This photo documentation should be a permanent part of the record. Partially disassemble the LiCor sensor bracket to the extent necessary for transportation, and then wrap the sonde in a white towel that has been soaked in tap water. The towel should cover the entire sonde and go around the body at least twice. Transport the sonde back to the lab in the modified white 5-gallon plastic bucket keeping the sonde and bucket out of direct sunlight to prevent thermal extremes. The optical windows on the LiCor sensors should be protected from abrasion and from drying out by putting some water in the annular ring around the optical window and then capping them with the covers provided by the manufacturer.
- vii. At the same time as a logging event, make an independent measurement of PAR attenuation using the instructions in SOP 5 – Spatial Water-Quality Monitoring with LiCor PAR Instruments. Make sure that neither the LiCor PAR profiling apparatus nor the YSI sonde just deployed are shaded by the boat, the logging installation, or personnel.

Note: You are now assured proper positioning of the newly deployed YSI sonde and LiCor PAR sensors. This is the PAR profile that should be used when post-calibrating the newly-deployed YSI sonde for PAR attenuation.

Final Retrieval of the Sonde

Methods for final retrieval of the sonde are similar to the above section (Swapping a Sonde During the Index Period), except that there is no need to prepare and deploy a new YSI 6600EDS. Simply omit steps that pertain to deployment of a second sonde.

Collect water in a large enough container to submerge the sonde (e.g. 5 gallon bucket). Submerge the sonde so that it is logging conditions in the container. At the same time as a logging event (on the quarter hour), gently mix the water in the container and collect a water sample from this container in a clean 500 ml amber polyethylene bottle for chlorophyll analysis by extractive methods. This ensures that the extractive sample for sensor calibration is collected from the same water, and at the same time, as the Chl-*a* sensor is reading. Follow the instructions in SOP 6 – Chlorophyll *a* Sampling and Analysis for sample collection and analytical methods.

Keep the sonde in logging mode until back in the lab and preparing to perform the post-deployment calibration check.

Post-Deployment Calibration Check

General notes

In the laboratory, a post-deployment calibration check must be performed on all sensors both BEFORE and AFTER they are cleaned. Differences between these calibration checks can be used to interpret and correct for sensor drift. Keep the YSI 6628 EDS wiper (wiper + brush) on the turbidity probe for the first (pre-clean) calibration check. At the time the sonde is cleaned, if using a 6562 rapid pulse dissolved oxygen sensor, the YSI 6628 EDS wiper should be replaced with a standard wiper. (This is not necessary when using a 6150 ROX optical dissolved oxygen probe, which has a wiper.) Pay particular attention to YSI guidance on appropriate clearances for these wiper assemblies since excessively tight installation will scratch and damage the optical windows on these sensors.

Post-deployment calibration checks of the 6600EDS should be performed with it hanging from a ring stand or custom-mounted hook. Height is critical for calibrating 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.

Except where explicitly noted in the SOP, standards should NOT be re-used for more than one calibration (or calibration check). Used standards may, however, be saved to pre-rinse sensors and the calibration cup prior to putting them into fresh standard solutions.

Taking the above notes into account, the following calibration checks should be completed in the order presented. All requested check values should be recorded in the appropriate locations on the Continuous YSI Sonde Calibration Log - Post-Deployment (Appendix B).

Pre-Cleaning Calibration Check

- i. Before checking the calibration of the sonde, it is acceptable to pre-clean the sonde BODY by removing the plastic wrap. If, however, there is significant fouling around the pressure sensor ports, it is best to leave the wrapper in place until after the depth calibration has been checked because changes in this fouling may affect the pressure readings.
- ii. Remove the sensor guard from the sonde body and inspect all of the sensors without dislodging any fouling material. In particular, note the positions and condition of the wipers, the degree of biofouling on each of the sensors, and the condition of the membrane on the dissolved oxygen sensor. It is best not to replace the sensor guard with the calibration cup in the field since the oxygen membrane is vulnerable to being damaged if it is bumped by the guard or the calibration cup. The wet-towel method will provide better protection to the sonde and sensors.
- 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. Confirm that the sonde is still properly logging data by going to the <Status> menu and making sure that the sonde returns a “Logging Active” message.
- v. Take the sonde out of logging mode. From the sonde’s <Run> menu select <unattended sample> then select “stop logging.”
- vi. Allow adequate time for the air in the calibration cup to become saturated with water vapor and for the sonde temperature to stabilize (15 minutes to 2 hours depending upon temperature differential).

Dissolved Oxygen:

6562 Rapid Pulse Dissolved Oxygen Membrane Probe:

- i. Go to the sonde <Report> menu and enable the <DO Charge> option. Then go to the <Run> menu and switch the sonde to <Discrete Sample> mode. Set the sampling interval to 4 seconds and activate sampling.
- ii. DO 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. There must be a high to low trend. If the recorded values start

low then climb upward, the sensor has a problem. Record the pass-fail status of the test on the appropriate calibration log.

- iii. Allow the DO % Saturation value to stabilize, and then record this value in the appropriate calibration log along with the value for DO charge and the current barometric pressure.
- iv. Escape from the <Run> mode.

Dissolved Oxygen:

6150 ROX Optical Dissolved Oxygen Probe

- i. After checking all other sensors and prior to cleaning place the sonde with the sensor guard in position into the container of air-saturated water which is being continuously sparged with air. The water must be sparged for at least one hour prior to this step.
- ii. Wait for at least 10 minutes to allow the temperature and oxygen pressure to equilibrate.
- iii. Record the DO % saturation reading, the DO mg/L reading and the “ODO gain” for the sensor on the appropriate calibration log. The ODO gain is found in the ADVANCED/CAL CONSTANTS menu.

Conductivity:

- i. Before performing the calibration check, the calibration cup and all the surfaces of all the probes must be GENTLY rinsed three times with small amounts of conductivity standard. Once-used standard from previous calibrations is acceptable.
- ii. Pour approximately 150 ml of conductivity standard (within the range of 10 to 50 mS/cm) into a clean and dry calibration cup and attach it to the sonde body. Avoid aggressive shaking or swirling that can dislodge fouling material. Note that more rinse volume of standard is used here than for general calibration so that rinsing can be more gentle. Discard this rinse and repeat two more times (never reuse these rinses since they are likely contaminated with fouling material).
- iii. You will need at least 370 ml of conductivity standard for the following calibration check. Because conductivity is very vulnerable to contamination from fouling material, the “upside down” and “sideways” methods available for the initial calibration are not recommended for this pre-cleaning calibration check.
- iv. Pour 370 ml 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.
- vi. Go to the <Run> menu and activate <Discrete Sample> mode.
- 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 the reading has stabilized, record it in the appropriate calibration log.

- viii. With an NBS-traceable thermometer, verify the accuracy of the sonde temperature reading and record it on the appropriate calibration log.
- ix. Escape from the <Run> mode.

Pressure/Depth calibration:

- i. Go to the <Run> menu and activate <Discrete Sample> mode.
- ii. Once the reading has stabilized, record it in the appropriate calibration log.
- iii. Escape from the <Run> mode.

Chlorophyll and Turbidity:

- i. You will need deionized water, AMCO-AEPA-1® turbidity standard, and a working concentration of Rhodamine WT dye standard to check the calibration of the chlorophyll and turbidity probes. Prepare the working Rhodamine standard following the procedures prescribed in the section on Chlorophyll Standards (above).
- ii. Half fill the calibration cup with deionized water and attach it to the sonde body. GENTLY tip the sonde so as to thoroughly wet all of the probe and bulkhead surfaces. Discard this rinse water and repeat two more times.
- iii. Half fill the calibration cup with fresh deionized water and GENTLY 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.
- iv. Go to the <Run> menu and activate <Discrete Sample> mode with a 4-second sample interval.
- v. Actuate the wipers to remove any air bubbles from the face of the probes (press<3>). Confirm that the wiper is parked 180° away from the optical windows on the probe faces.
- vi. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde and deionized water are not all at the same laboratory temperature). Once the readings for chlorophyll and turbidity have stabilized, record them in the appropriate calibration log.
- vii. Escape from the <Run> mode.
- viii. Pour approximately 150 ml of Rhodamine WT working standard into a clean and dry calibration cup and attach it to the sonde body. GENTLY tip the sonde so-as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.
- ix. Fill the calibration cup with at least 225 ml of fresh Rhodamine WT working standard and attach it to the sonde body.
- x. Go to the <Run> menu and activate <Discrete Sample> mode with a 4-second sample interval.

- xi. Actuate the wipers to remove any air bubbles from the face of the chlorophyll probe (press<3>). Confirm that the wiper is parked 180° away from the optical window on the probe face.
- xii. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde and dye standard are not all at the same laboratory temperature). Once the chlorophyll reading has stabilized, record it in the calibration log along with the temperature of the dye standard.
- xiii. Escape from the <Run> mode.
- xiv. Use the temperature compensation chart to determine the appropriate chlorophyll equivalent of the dye standard at this temperature from Table 2 of YSI Technical Note: Alternate Calibration Methods for the YSI 6025 Chlorophyll Sensor Appendix C). Record this value in the calibration log.
- xv. Rinse probes with DI water and pat dry with Kimwipes®.
- xvi. Pour approximately 150 ml of turbidity standard (used is acceptable) into a clean and dry calibration cup and attach it to the sonde body. GENTLY tip the sonde so-as to thoroughly wet all of the probe and bulkhead surfaces. Discard this standard solution. Repeat this step two more times.
- xvii. 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 and maintain 6.7 cm clearance between the sensor face and the bottom of the calibration cup.
- xviii. Actuate the wipers to remove any air bubbles from the face of the turbidity probe (press<3>). Confirm that the wiper is parked 180° away from the optical window on the probe face.
- xix. Allow the sonde to run for at least 1 minute to ensure thermal equilibration (wait longer if the sonde and turbidity standard are not all at the same laboratory temperature). Once the turbidity reading has stabilized, record it in the appropriate calibration log.
- xx. Escape from the <Run> mode.
- xxi. Rinse probes with deionized water, put approximately 0.5 cm of tap water in the bottom of the calibration cup and firmly attach it to the sonde.

Cleaning of the YSI EDS6600 sonde and replacement of wipers

Clean the YSI sonde following guidance in the YSI Environmental Monitoring Systems Operations Manual. Do **NOT** clean the LiCor PAR sensors at this time. A soft tooth brush may be useful for removing fouling material. Use caution with abrasive cleaning agents (scouring pads) or sharp objects (dental picks), which may scratch and damage the optical windows on chlorophyll and turbidity sensors. Use a syringe to clean the pressure sensor by repeatedly rinsing clean water through the port. If using a 6562 rapid pulse dissolved oxygen sensor, replace the YSI 6628 EDS wiper + brush assembly with a standard turbidity probe wiper. Also replace the wiper pads on the chlorophyll and ROX optical sensors. Pay particular attention to

YSI guidance on appropriate clearances for these wiper assemblies since excessively tight installation will scratch and damage the optical windows on these sensors.

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. Attach the Dry Calibration Cable between the sonde and computer, launch EcoWatch software, and establish communication with the sonde.

Post-cleaning calibration check

After cleaning, the calibration of the sonde must be rechecked using the same methods as before the cleaning (see Pre-Cleaning Calibration Check). The only difference is that smaller volumes of rinse solution may be used and more aggressive rinsing is acceptable. Record all readings in the appropriate section of the Post-Deployment Calibration Checks Log (Appendix B).

LiCor 192SA PAR sensor calibration check

The LiCor PAR sensors also need to be checked for drift. Although biofouling should not be a problem with the YSI-integrated PAR sensors (with wiper brushes), the post calibration check should be done with any biofouling still in place. Repeat the steps from the initial calibration check and record results in the continuous YSI sonde calibration log. After the post-calibration, clean the PAR sensors with water and a soft sponge or brush. Be careful not to scratch the acrylic diffuser or damage the black paint within the annular ring on the face of the sensor. Do not use alcohol, organic solvents, abrasives, or strong detergents to clean the diffuser element. Mild dishwashing detergent and vinegar are acceptable cleaning agents.

Data Upload

After completing the post-deployment calibration check, immediately upload data from the sonde to computer. Connect the sonde to your computer using the dry calibration cable, launch YSI EcoWatch software, and establish communication with the sonde. From the sonde's <File> menu, <Upload> the data file to computer TWICE, once using the PC6000 format and a second time using comma delimited format. PC6000 format results in a computer file with a .dat file extension. 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. Upload a second time using comma delimited format, which is more generally useful for opening in spreadsheets and archiving since it does not rely on YSI EcoWatch software. Use EcoWatch to open the PC6000 date file and examine it to ensure it has not been corrupted and rendered unreadable in the upload process. Immediately create a CD back-up of both data files and confirm they too are readable. After these steps have been completed, reestablish communication with the sonde, select the <File> menu and <Delete all files>.

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 Rapid Pulse Dissolved Oxygen Membrane 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.