National Estuarine Research Reserve (NERRS) System-Wide Monitoring Program (SWMP)

YSI/Xylem EXO Multi-Parameter Water Quality Monitoring Standard Operating Procedure

Version 1.2

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INTRODUCTION

The National Estuarine Research Reserve System (NERRS) is a network of 28 areas representing different biogeographic regions of the United States that are protected for long-term research, water-quality monitoring, education and coastal stewardship. Established by the Coastal Zone Management Act of 1972, as amended, the NERRS is a partnership program between the National Oceanic and Atmospheric Administration (NOAA) and the coastal states.

To better meet its public trust responsibilities, in 1995 the NERRS established the System-Wide Monitoring Program (SWMP) with a primary mission to:

Develop quantitative measurements of short-term variability and long-term changes in the water quality, biotic diversity, and land-use / land -cover characteristics of estuaries and estuarine ecosystems for the purposes of informing effective coastal zone management.

SWMP is designed as a question driven monitoring program that uses the NERRS as a network of intensively studied coastal and estuarine reference sites for evaluating ecosystem function and change. Within these sites, long-term datasets with relevance to management issues of concern are collected using standard approaches with a high degree of spatial and temporal resolution.

NOAA's Next Generation Strategic Plan for 2010 recognizes the importance of "accurate and reliable data from sustained and integrated Earth Observing Systems" and that "coastal communities need observations to understand changing coastal ecosystem conditions and manage coastal resources sustainably". The plan states that "Over the long-term, NOAA must sustain and enhance observing systems (atmospheric, oceanic, inland waters, terrestrial, solar, cryospheric [Earth's surface where water is in solid form, including glaciers, sea ice and ice caps], biological, and human) – and their long-term data sets – and develop and transition new observing technologies into operations, while working in close collaboration with it's governmental, international, regional, and academic partners" (from http://www.ppi.noaa.gov/wp-content/uploads/NOAA_NGSP.pdf).

Coastal managers use these monitoring data to make informed decisions on local and regional issues, such as nutrient enrichment and dissolved oxygen depletion (hypoxia), harmful algal blooms, beach water quality, and "no-discharge" zones for boats and measuring the success of restoration projects (from <u>Buskey, et al., 2015</u>).

QA/QC is now a requirement for states and regional organizations, as well as federal agencies, involved in environmental quality measurements. (from PowerPoint presentation: <u>http://water.usgs.gov/wicp/acwi/monitoring/ppt/sanjose_0412/Hameedi.pdf</u>). With SWMP datasets linked to those collected by other organizations, an increasing number of stakeholders, improved public access to data, and applications of new and improving technologies, a higher burden of proof for data integrity is required. Using standardized protocols and equipment for data collection, SWMP data are assimilated, managed, and served by the NERRS Centralized Data Management Office (CDMO; <u>http://cdmo.baruch.sc.edu; www.nerrsdata.org</u>) for a variety of audiences from academic researchers to coastal managers to public health officials and the general public. The CDMO ensures that SWMP data are authoritative, of high quality, and easily accessible.

Buskey, E., M. Bundy, M. Ferner, D. Porter, W. Reay, E. Smith and D. Trueblood. 2015. Chapter 21 - System-wide Monitoring Program of the National Estuarine Research Reserve System: research to address coastal management needs. Chapter in *Coastal Ocean Observing Systems: Advances and Syntheses*. Y. Liu, H. Kerkering and R. Weisberg (eds.). Elsevier Press. pp. 391-415. This document outlines the standard operating procedures (SOP) for the collection of data using YSI/Xylem EXO Multi-Parameter Water Quality Sondes for the National Estuarine Research Reserve's (NERR) System-Wide Monitoring Program (SWMP). This SOP does not replace the YSI/Xylem EXO User Manual or the CDMO Data Management Manual. Users must become familiar with procedures in these manuals as well. The purpose of the SWMP SOP is to standardize instrument handling, maintenance, calibration, deployment, and post-deployment procedures for the NERR SWMP. The procedures in this SOP are the MINIMUM requirements for operating the EXO sondes; therefore all NERR sites must follow these procedures. Participating NERR sites may undertake additional procedures to ensure quality data collection, but following the procedures in this SOP will ensure consistency across the NERRS, minimize the collection of inaccurate data, and support effective data QAQC after collection. For questions or concerns about this document, please contact: cdmosupport@belle.baruch.sc.edu.

This document is separated into nine sections:

- I. EXO Communication & KORS Software
- II. Calibration
- III. EXO Programming & Deployment Preparation
- IV. EXO Deployment
- V. EXO Retrieval
- VI. EXO Data Download & Post Deployment Readings
- VII. SWMP Water Quality Monitoring Calibration Log sheet
- VIII. Probe care and storage
- IX. Appendices for troubleshooting and other detailed documents.

I. EXO COMMUNICATION & KORS SOFTWARE

A copy of KORS software, stored on a USB thumb drive, is included with purchase of each EXO sonde. This software is used for EXO communication, calibration, post-deployment readings, data file download, sonde/probe firmware updates, and any other necessary interaction with the sonde via a PC. This section briefly covers communication options using KORS. It also covers the various functions of the software and the icon-based locations of said functions.

Sonde Connectivity – EXO sondes are accessed via one of two potential options:

- 1) USB/SOA (Signal Output Adapter) Cabled Connection
 - a. Open KORS Software
 - b. Connect the USB cable to the PC and the blue SOA adapter to the sonde connector located on the top of the sonde. The connector on the right side of the sonde (when looking at the sonde from a view where the yellow plastic component faces the user) is used for wired connection.
 - c. Click the "Circular Blue/Green Arrows" icon (7th icon from the left in KORS)
 - d. Click Rescan
 - e. Click the COMM port that has the EXO USB adapter and EXO sonde ID shown
 - f. Click "Connect"
 - g. If the USB adapter or sonde do not show up at first, unplug the USB cable from the computer, plug it in again, and repeat steps 3 through 6. If this fails to show the sonde, unplug the adapter from the sonde, re-connect the adapter, and repeat steps 3 through 6.
- 2) Wireless Bluetooth Connection
 - a. Open KORS Software
 - b. Place the magnetized section of the black probe removal tool over the magnet icon located on the sonde (about halfway down the sonde within the black stickered area that says EXO2) to activate the sonde
 - c. Click the "Circular Blue/Green Arrows" icon (7th icon from the left in KORS)
 - d. Click Rescan
 - e. Click the EXO sonde ID shown
 - f. Click "Connect"
 - g. Note: make sure to connect to the appropriate EXO sonde as the Bluetooth may pick up other active EXO sondes nearby.

NOTE: Additional information about bluetooth connection and troubleshooting is available in the EXO manual

KORS Software Icons – the KORS software features eight major icons located within the software that serve various purposes. Each icon and its functions are explained below in detail

- Green Running Man (1st icon to the right of the KORS software logo) once connected to the EXO sonde, clicking this icon places the sonde into discrete mode; a mode used for making realtime measurements
- 2) Calibration Target (2nd icon to the right of the KORS software logo) clicking this icon brings up sub-menus showing each port and the port's installed probe. Each probe/parameter's rectangular icon is vertically stacked to the left of the screen. Clicking a particular parameter brings up the calibration screen for that probe. Detailed calibration steps for each parameter are discussed in the calibration section of this document.
- 3) Large Green Arrow (3rd icon to the right of the KORS software logo) clicking this icon brings up two sub menus used in preparing the sonde for unattended sampling prior to deployment;
 - a. Read Current Sonde Settings accesses the current template file present within the EXO sonde
 - b. Open Template allows the user to choose a particular template file for the sonde
- 4) Map with Blue Inverted Tear Drop (4th icon to the right of the KORS software logo) clicking this icon brings up a place where individual monitoring site names are added or removed
- 5) Data Folder (5th icon to the right of the KORS software logo) clicking this icon reveals 4 submenus icons involved in the following
 - a. Transfer used to transfer .BIN data files from the sonde to the PC
 - b. View Export used to select .BIN files and export them into .CSV or other file formats
 - c. Settings used to designate a location on the PC where files are downloaded/exported. It is recommended to leave the default pathway in place and simply copy and paste files to other locations after download and export.
 - d. View Calibration Worksheets used to access copies of calibration information sheets for each calibrated probe type. Calibration sheets can be located, accessed, sorted, and displayed within this submenu.
- 6) Gears (6th icon to the right of the KORS software logo) clicking this icon reveals seven submenu icons:
 - a. Smart QC provides a list of the sonde ports and associated probes installed within each port along with a QC score (green check mark for good)
 - b. Sonde provides an input space for the sonde ID, allows the user to update the sonde time (internal clock), select battery type, and alter the averaging mode (for SWMP this should be left at default)
 - c. User allows the user to change the language, timespan in which the sonde will drop communication (go into idle mode), select the local time zone, time format preference (should be set to 24 hr for SWMP), UTC (GMT) or time zone adjusted (should select time zone adjusted for SWMP), and a time zone check option
 - d. Units this submenu provides a stacked icon list of each parameter. Clicking on an individual parameter allows the user to change to unit of measure for said parameter by clicking to place a checkmark in next to the chosen unit of measure. For SWMP,

parameters should be set to the following units: Note: These configurations are computer settings and not changes that are sent to the sonde.

- i. Temperature (Temp): Temp C
- ii. Conductivity (Cond): SpCond mS/cm & Sal psu
- iii. Dissolved Oxygen (ODO): ODO %
- iv. pH: pH & pH mV
- v. Depth: Depth m
- vi. Barometric Pressure (Baro): Baro mmHg
- vii. Turbidity (Turb): Turbidity FNU
- viii. Sonde: Battery V
- ix. External Battery Voltage
- x. Wiper Position
- xi. Once selected, click "apply". This only needs to be done once when initially setting up the software
- e. Firmware this submenu shows each probe, the port in which it is installed, the probe serial number, address, and firmware version. Both the sonde and all probe firmware can be updated from this screen
- f. Calibration this submenu provides a stacked icon list of each parameter. Clicking a parameter provides a series of unit options. When clicked, the unit of measure screen opens allowing the user to input specific calibration points and information about the calibration standard used for calibration that probe type (type, manufacturer, and lot number). These only need to be set once unless the user is changing calibration standard lots numbers. Everything set in these menus will show up as part of the calibration screens (see Section II Calibration).
- g. Sync With Handheld allows the user to access files on an EXO handheld device, update handheld firmware, etc.
- 7) Circular Blue & Green Arrows (7th icon to the right of the KORS software logo)- clicking this icon reveals four submenu options
 - a. Rescan (see KORS software section 1)
 - b. Map shows all ports, probes installed, and probe serial numbers
 - c. Settings Bluetooth and USB adapter settings
 - d. Advanced allows comm port selection for cabled communication
- 8) Question Mark (8th icon to the right of the KORS software logo) self-explanatory help menu

II. CALIBRATION

General Calibration Considerations:

- 1) Good laboratory practices should always be followed when handling calibration standards. Please refer to MSDS sheets for any standard when necessary.
- 2) A NERRS SWMP Water Quality Calibration Log and Field Log (combined, a deployment log) must be completed for each instrument's calibration, deployment and retrieval, and post-calibration (see section VII). These deployment logs must be submitted to the CDMO via the online Deployment Log Interface. Reserves are encouraged to use hardcopy sheets to record data in the field and customized logs may be used internally in order to support additional parameters or partner program requirements, but the approved online deployment logs will be the only versions accepted by CDMO.
- 3) The sonde should be visually inspected for any abnormalities, such as a broken probe or damaged bulkhead
- 4) Remove the Wiper Brush from the sonde! The brush can trap residual standard and affect the calibration accuracy. The EXO2 sensors easily dislodge air bubbles so it is not needed during calibration. If you elect to leave it on the bristles will have to be cleaned and thoroughly rinsed between standards / buffers.
- 5) Calibrations are best performed using a guard and calibration cup that are dedicated to calibration only and never taken in the field. This insures a high level of cleanliness and non-contamination during the calibration process.
- 6) During the calibration of the probes **NEVER** accept any calibrations that display an error message. Troubleshoot the cause of the problem, correct it, and recalibrate or replace the probe before deploying the instrument.
- 7) Standards must be active (check expiration date) and fresh for all calibrations. Previously used, clean standards may be used to rinse probes but must not be used to calibrate probes. Discard and replace all expired standards.
- 8) All diagnostic parameters (pH millivolts, DO gain, and conductivity cell constant) for EXO sondes are presented after calibration of the respective parameter on the KORS generated calibration sheet and should be recorded on the NERRS data sheet once calibration of those probes is complete.
- 9) Recommended probe calibration order: Temperature (not a true calibration, but a check against NIST source), Specific Conductivity, pH, Depth, Turbidity, Dissolved Oxygen.
- 10) Prior to calibration it is a good idea to record serial number for the sonde and probe in the Calibration Log's "Datasonde & Probe Identification Numbers" section. To quickly access all serial numbers, perform the following:
 - a. Click the Gear Icon

- b. Click Firmware
- c. Record all serial numbers on the data sheet (the user can double check accuracy of the serial number during calibration of each parameter). Serial #'s may also be written during calibration, but the user loses the opportunity to quickly double check them with that method.
- 11) Batch calibrations (sometimes referred to as gang calibrations) are permissible for SWMP, but this is an advanced form of calibration that is not recommended when learning to calibrate EXO sondes. During gang calibrations, multiple versions of the same probe type are installed into an EXO sonde and calibrated one after the other. Once calibrated in this fashion, the probes are then installed into their respective EXO sondes.

Calibration Procedures - The following sections provide step-by-step SWMP approved methods for the calibration of each probe type.

- Temperature Sensor and SC Functional Check while it is not possible to calibrate the temperature probe, this does not exclude you from performing a temperature test to verify that the temperature probe is working properly. A traceable NIST thermometer will be required for this test. The Specific Conductivity check will verify that cleaned and dry electrodes read < 2 us/cm with the probe dry and in air. Note: At least once a year the temperature sensor should be checked at 3 points, ice water, room temperature, and warm water ~ 35 C.
 - a. Temperature Test
 - a. Allow the sonde to sit in a bucket of room temperature tap water for at least 5 minutes to reach temperature equilibrations,
 - b.Connect to the sonde (see I. KORS Software section 2)
 - c. Click Green Running Man icon to place sonde into discrete mode
 - d.Verify temperature readings have stabilized and record the sonde's temperature value in the Pre-Deployment section of the data sheet in the "Before Cal" field for Temp
 - e. Record the temperature reading from the NIST thermometer in the data sheet's Pre-Deployment "Standards" column.
 - f. Note: that the accuracy of the NIST device must be included in your temperature accuracy determination.
 - g. Remove the sonde from the water bucket
 - b. Conductivity Test
 - a. Rinse the SC probe with DI water after the temperature verification test
 - b.Using a compressed air source (Dust Off) blow the conductivity cells dry on the CT2 sensor, on the Wiped CT probe you can use the air or dry the open channels with a KimWipe.
 - c. The SC reading in air for both sensors must be < 2 us/cm, if greater that 2 us/cm contact Xylem/YSI for assistance

- 2) Conductivity Probe (Specific Conductivity & Salinity) 1 Point Calibration
 - a. Fill EXO calibration cup with a small amount of rinse conductivity calibration standard (rinse standard is the term for calibration standard used during the previous calibration and its use reduces cost and standard consumption)
 - b. Place the sonde with guard installed into the EXO calibration cup and shake to rinse the guard and probes
 - c. Empty out the rinse standard
 - d. Repeat the above process 1-2 more times (YSI recommends rinsing a total of three times)
 - e. Fill calibration cup to the second line with new/unused conductivity calibration standard
 - f. Screw the guard back on and place the EXO sonde into the calibration cup
 - g. Click the calibration icon
 - h. Click "Conductivity"
 - i. Click "SpCond" (Specific Conductivity)
 - j. Choose 1 point calibration and make sure the calibration standard value shows up properly (this may be entered manually each time or can be set to show up automatically by clicking the Gears icon, Calibration submenu icon, Cond icon, SpCond icon, and then entering the standard value for Cal Point 1, followed by clicking apply)
 - k. Type in the standard type, manufacturer, and lot # of the standard (standard type and manufacturer can be entered manually each time or auto-set using the procedure described in Step J.)
 - l. Click "Start Cal"
 - m. Once the temperature value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
 - n. Verify the "Pending (Post) value is correct and click "Complete"
 - o. The calibration worksheet for the probe will pop up and the user should record the following three values:
 - a. Pre Calibration Value: record this value in the data sheet's "Before Cal" column
 - b.Post Calibration Value: record this value in the data sheet's "Calibrated" column
 - c. Cell Constant: record in the data sheet's "Sensor Diagnostics/Pre-Deployment" cell constant field
 - p. Review the cell constant value to make sure it is within the proper range of 4.59 5.61 for the non-wiped CT2 sensor and 0.419 0.519 for the Wiped CT
 - q. Close the calibration worksheet
 - r. Pour the used standard into a container labelled "rinse" as this standard can be used as a rinse later
 - s. YSI Tips:
 - a. Never calibrate with a standard less than 1.0 mS/cm as they are easily contaminated by residual DI water and electrical noise

- b.Typical calibration errors are attributed to incorrect standard value input, inadequate calibration standard volume in the EXO calibration cup, or air bubbles in the conductivity cell
- pH Probe 2 Point Calibration (pH7 and pH10) (please note if you are one of the two or three Reserves that perform 3 point pH calibrations, you will simply have another round of standard (pH 4) added to the following procedure) Note: All pH sensors require periodic reconditioning and cleaning, the pH reconditioning procedure should be performed quarterly and at the beginning of a new season. Remove the Wiper Brush from the sonde prior to calibration, it can trap residual buffer and affect the calibration accuracy
 - a. Rinse the EXO calibration cup, guard, and probes with DI water to remove standard from the previous calibration
 - b. Fill EXO calibration cup with a small amount of rinse pH7 calibration standard (Rinse standard is the term for calibration standard used during the previous calibration and its use reduces cost and standard consumption)
 - c. Place the sonde with guard installed into the EXO calibration cup and shake to rinse the guard and probes
 - d. Empty out the rinse standard
 - e. Repeat the above process 1-2 more times (YSI recommends rinsing a total of three times)
 - f. Place the sonde on the work bench with the guard removed
 - g. Click the Green Running Man icon to run the sonde in discrete mode to get an air temperature reading. Once the temperature has stabilized, use that value to determine the exact values to input for your calibration standards. The standards will have pH values at various temperatures or ranges listed on the container.
 - h. Write the temperature compensated pH values in the data sheet's Pre-Deployment "Standards" column for the two or three standards to be used
 - i. Fill calibration cup to the first line with the first pH calibration standard (ph7)
 - j. Click the calibration icon
 - k. Click "pH"
 - 1. Choose 2 or 3 point calibration and enter the pH7 and pH10 standard values determined in Step G. (these values can initially be set by clicking the Gears icon, clicking the Calibration icon, clicking the pH icon, and entering the standard values for each pH cal point) It is critical to note that unlike all other parameters, the exact pH value used for calibration may change slightly based on the air temperature at which you are calibrating. If the standard values shown in the calibration page have been auto-set and do not match the values you want, simply input the correct values and proceed to the next step.
 - m. Enter the standard type, manufacturer, and lot # for each calibration point (standard type and manufacturer can be entered manually each time or auto-set using the procedure described in Step L.)
 - n. Click "Start Cal"
 - o. Once the temperature value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
 - p. Verify the "Pending (Post) value is correct and click "Proceed"

- q. Rinse the EXO calibration cup, guard, and probes with DI water to remove pH7 standard from the first part of the calibration
- r. Fill the EXO calibration cup with a small amount of rinse pH10 calibration standard
- s. Place the EXO sonde with guard installed into the EXO calibration cup and shake to rinse the guard and probes
- t. Empty out the rinse standard
- u. Repeat the above process 1-2 more times (YSI recommends rinsing a total of three times)
- v. Fill the calibration cup with the next pH calibration standard (pH10 in this case)
- w. Click "OK" when the small window pops up saying "Proceed to Standard (10.00 pH)
- x. Once the temperature value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
- y. Verify the "Pending (Post) value is correct and click "Complete"
- z. The calibration worksheet for the probe will pop up and the user should record the following values:
 - a. Pre Calibration Value for Cal Point 1: record this value in the data sheet's "Before Cal" column
 - b.Post Calibration Value for Cal Point 1: record this value in the data sheet's "Calibrated" column
 - c.Raw Value pH mV: record in the data sheet's "Sensor Diagnostics/Pre-Deployment" phX millivolts field
 - d. Record the values (Pre Calibration Value, Post Calibration Value, and Raw Value) on the data sheet for the 2^{nd} calibration point
 - e. Also record the pH probe slope in the data sheet's "Sensor Diagnostics/Pre-Deployment" next to the text "calculated pH slope"
 - f. Verify that the pH slope is within the ideal range of 160 180. pH data collected with a probe slope of less than 155 requires mandatory coding as suspect data, so it is not recommended to deploy a sonde displaying a pH slope at or below 155. A brand new pH probe will display a slope at or near 180. A probe displaying a slope of 160-165 indicates the probe tip is nearing the end of its lifespan and will require replacement in the near future.
- aa. Close the calibration worksheet
- bb. Pour the used standard into a container labelled "rinse" as this standard can be used as a rinse later
- cc. YSI Tips:

a.pH probe tips typically last 1 – 1.5 years on average

b.The entire probe does not need to be replaced when its lifespan has ended; only the probe tip (Part #599795-02).

- 4) Depth/Water Level 1 Point Calibration the directions presented here are applicable to users collecting depth with the EXO sonde. Alternative calibration procedures for water level are detailed in Appendix C
 - a. Fill EXO calibration cup with a small amount of water (the volume should not reach anywhere near the probes) to create a water-saturated air environment
 - b. Screw the guard onto the sonde and place it into the calibration cup
 - c. Allow the sonde to remain in a vertical position on the work bench
 - d. Click the calibration icon
 - e. Click "Depth"
 - f. Click "Depth m"
 - g. Determine the most current local barometric pressure value in mmHG (preferably from your SWMP weather station) and record it in the "Pre-Deployment" "Before Cal" column inside the field next to "Baro Pres. (Depth Calib). To convert other units to mm Hg, multiply inches of Hg by 25.4 or millibars by 0.75.

NOTE: Reserves located at a 'significant' elevation, MUST use the barometric pressure reading from their corresponding MET station for this calculation to ensure the most accurate depth correction possible. While this is recommended for everyone, it specifically applies to those that use an offset in their Campbell program (following NWS protocols) to adjust barometric pressure readings from their MET station to sea level. **Currently those two Reserves are OWC and LKS**.

- h. Input the mm Hg in the online Deployment Log or Depth/Offset Calculator to determine the correct barometric pressure offset to use for calibration (the charts in Appendix B may also be used to determine correct offset).
- i. Write this value on the data sheet by putting a line through 0.0 and writing the barometric pressure offset above the word offset in the "Pre-Deployment" "Standards" section. The 0.0 listed on the data sheet is an artifact of the equation present within the online Log Sheets. In the Online Log Sheets, entry of the barometric pressure automatically changes the 0.0 to the relevant barometric pressure offset.
- j. Click Advanced
- k. Click Edit
- 1. Enter the barometric pressure offset into the "Offset" field
- m. Click Apply
- n. Click OK
- o. Click Back
- p. Click "Start Cal"
- q. Once the depth value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
- r. Click "Complete"
- s. The calibration worksheet for the probe will pop up and the user should record the following values:

- a. Pre Calibration Value: record this value in the data sheet's "Before Cal" column for depth
- b.Post Calibration Value: record this value in the data sheet's "Calibrated" column for depth

NOTE: The station offset and level fields on the data sheet should be left blank when performing depth calibrations. The fields are only applicable to calibration for water level (see Appendix C for details)

- t. Close the calibration worksheet
- u. YSI Tips
 - a. Make sure the correct latitude has been entered into the EXO sonde (may be checked and changed during Step L. from the above instructions)
- 5) Turbidity probe 2-point calibrations are required at least monthly and when sensor drift is evident. As such, the following step-by-step methods detail a 2 point calibration procedure using DI water and 126 NTU standard (other DMC SWMP-approved standards include: Hach StablCal, diluted Hach 4000 NTU formazin, or standards that have been approved according to the instructions in Standard Methods, 20th ed. (Section 2130 B)).

Turbidity Probe calibration considerations:

- a. NTU (nephelometric turbidity units) and FNU (formazin nephelometric unuts) are considered synonymous for the purposes of this document
- b. The 6-Series 126 NTU standard is now relabeled and has two numbers on the bottle the value for 6-Series probe is 126 and for the EXO it is **124.** Always use the **124** entry when calibrating EXO turbidity sensors!
- c. Do not calibrate turbidity in the field as clean surfaces and solutions are essential for a good turbidity calibration.
- d. Use the EXO calibration cup for turbidity probe calibration; do not use any other calibration vessel.
- e. Bubbles over the optics will interfere with calibration therefore it is recommended to carefully pour standards into the calibration cup with the cup held at an angle to avoid aeration and to visually confirm all air bubbles are clear from the probe face before calibration.
- f. *Turbidity Zero Calibration Note*: Recent studies have concluded that in many applications it is almost impossible to clean a used sonde and its calibration cup to a level that will eliminate contamination of the zero standard. In controlled laboratory conditions with new equipment contamination levels of 0.1 ntu's were observed even when extreme precautions were used. Used equipment even when properly cleaned can contaminate the zero standard to levels between 0.2 and 0.6 ntu's.

Calibrating the sonde to a zero standard that is actually a positive number will result in negative field reading if the in-situ environment is cleaner. If you routinely see negative turbidity data (<1 ntu) from your deployments this could be the cause.

Using a small offset to account for this condition is NOT acceptable for NERRS SWMP data. SWMP data management protocols already take this small potential error into consideration by accepting small negative values and flagging/coding them automatically.

Turbidity Probe calibration:

- a. Rinse the EXO calibration cup, guard, and probes with DI water to remove standard from the previous calibration
- b. Fill EXO calibration cup with a small amount of DI water. DI water serves as a rinse in this calibration.
- c. Place the sonde with guard installed into the EXO calibration cup and shake to rinse the guard and probes
- d. Empty out the DI rinse water
- e. Repeat the above process 1-2 more times (YSI recommends rinsing a total of three times)
- f. Fill the EXO calibration cup to slightly above the first line with 0 FNU standard (DI water). Slowly pour the DI water into the cal cup to avoid introducing air bubbles
- g. Place the EXO sonde with guard installed into the calibration cup; do this slowly to avoid generating air bubbles
- h. Gently tap the EXO sonde and EXO calibration cup on the work bench at a 45 degree angle to insure air bubbles are not present on the sensor face. Visually inspect to make sure the probe face is air bubble-free before proceeding.
- i. Click the calibration icon
- j. Click "Turbidity"
- k. Click "Turbidity FNU"
- 1. Choose 2 point calibration and make sure the calibration standards shows up properly (this can be set by clicking the Gears icon, Calibration submenu icon, , Turb icon, and then entering 0.00 for Cal Point 1 and 124.00 for Cal Point 2 followed by clicking apply)
- m. Enter the standard type, manufacturer, and lot # for each calibration point (standard type and manufacturer can be entered manually each time or auto-set using the procedure described in Step L.)
- n. Click "Start Cal"
- o. Once the temperature value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
- p. Verify the "Pending (Post) value is correct and click "Proceed"
- q. Pour the DI water standard out of the calibration cup and dry the guard, cup, and probes with a KimWipe or other lint-free wipe
- r. Fill EXO calibration cup with a small amount of 126 FNU rinse standard.
- s. Place the EXO sonde with guard installed into the EXO calibration cup and shake to rinse the guard and probes
- t. Empty out the 126 rinse standard

- u. Repeat the above process 1-2 more times (YSI recommends rinsing a total of three times)
- v. Fill the EXO calibration cup to slightly above the first line with new/unused 124 FNU calibration standard by holding the calibration cup at an angle and slowly pouring the standard into the cal cup to avoid introducing air bubbles
- w. Place the EXO sonde with guard installed into the calibration cup; do this slowly to avoid generating air bubbles
- x. Gently tap the EXO sonde and calibration cup on the work bench at a 45 degree angle to insure air bubbles are not present on the sensor face. Visually inspect to make sure the probe face is air bubble-free before proceeding.
- y. Click "OK" when the small window pops up saying "Proceed to Standard (124 FNU)"
- z. Once the temperature value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
- aa. Verify the "Pending (Post) value is correct and click "Complete"
- bb. The calibration worksheet for the probe will pop up and the user should record the following two values:

a. Pre Calibration Value: record this value in the data sheet's "Before Cal" column

b.Post Calibration Value: record this value in the data sheet's "Calibrated" column

- cc. Close the calibration worksheet
- dd. Pour the used standard into a container labelled "rinse" as this standard can be used as a rinse later
- ee. YSI Tips
 - a. Holding a finger of the face of the turbidity probe (optics) should give a full response by the probe in discrete mode
 - b.YSI 126 NTU turbidity standard shelf life is 1 year

6) Dissolved Oxygen Probe - 1 point calibration

- a. Aerate a 5 gallon bucket filled ³/₄ full with tap water for at least an hour prior to calibrating a dissolved oxygen probe. This creates an air-saturated environment referred to as a 100% air-saturated water bath.
- b. Place the EXO sonde into the bucket for 15-20 minutes before calibrating the DO probe in order to achieve temperature probe stabilization. Do not allow air bubbles to flow directly towards the DO probe's face.
- c. Click the calibration icon
- d. Click "ODO"
- e. Click "ODO % sat"
- f. Review the temperature value on the screen and make sure it is stable
- g. Enter the current barometric pressure value in mmHG
- h. Choose 1 point calibration and make sure the calibration standard shows up properly as air saturated (this can be set by clicking the Gears icon, Calibration submenu icon, , ODO icon, ODO % sat icon, and then selecting "air saturated" followed by clicking apply)

- i. Click "Start Cal"
- j. Once the temperature value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
- k. Verify the "Pending (Post) value is correct and click "Complete"
- 1. The calibration worksheet for the probe will pop up and the user should record the following three values:
 - a. Pre Calibration Value: record this value in the data sheet's "Before Cal" column
 - b.Post Calibration Value: record this value in the data sheet's "Calibrated" column
 - c.DO Gain: record in the data sheet's "Sensor Diagnostics/Pre-Deployment" optical DO gain field
- m. Review the DO Gain to make sure it is within the recommended 0.87 1.25 range
- n. Close the calibration worksheet
- o. YSI Tips:
 - a. DO probe sensor caps should last 1 - 2 years (possibly longer), but do require replacement once its lifespan has ended
 - b.**Note:** For optimum performance Optical DO sensor membranes must be kept fully hydrated in water. If your DO probe has been dry stored it will need to hydrate in saturated tap water over night. If the probe was stored in saturated air the sensor should be placed in saturated water for a few hours to ensure full hydration. Always make sure that the DO membrane is clean and free of any slime or mold.
- 7) **Total Algae/chlorophyll:** Chlorophyll fluorescence is an optional SWMP parameter and calibration for this parameter is not as straight forward as other parameters. Calibration instructions are included in Appendix D.

III. PROGRAMMING & DEPLOYMENT PREPARATION

Programming – there are a number of ways the EXO sonde can be prepared for deployment. One such method involves creating a template file for each sonde in the user's inventory. This method alleviates the user from remembering to set sample and hold for telemetry stations, changing file name prefixes, and other slight tweaks as details for each sonde can be stored in the sonde's own dedicated template file. The creation of multiple template files is not required and is simply discussed due to the author's success with this method. The following instructions show how to check/update the EXO's internal clock, set up the template file for unattended mode, and fill out the programming section of the NERRS SWMP data sheet:

- 1. Click Gear icon
- 2. Click "Update Time"
- 3. Make sure your PC is set to standard time.
- 4. Click box next to "Relative to PC"
- 5. Click "Apply"
- 6. Click Large Green Arrow icon
- 7. Click "Read Current Sonde Settings" submenu icon
- 8. Click Screwdriver & Wrench icon
- 9. Enter a template file name and choose deployment Time Zone
- 10. The user will also see three clickable tabs
 - a. Basic
 - i. Logging Interval (Hour:Minute:Second): enter as 0 15 0 for SWMP
 - ii. Username: enter as user wishes
 - iii. Site Name: choose from drop-down menu list of station names entered into KORS using the Map icon (See KORS software section)
 - iv. File name prefix: characters entered here will be the first ones used in filenames.
 - b. SDI-12: used in telemetry applications
 - c. Advanced: used to enable sample and hold for sondes placed at telemetry sites along with other advanced logging options
- 11. Once the information has been set in the above three tabs, click the small green arrow icon (says Save, Deploy, Start Logging when moused over)
- 12. Choose a start time (typically using "Next Interval" and click "apply"
- 13. The "Current Deployment Summary" screen will show up containing a number of values needed for filling out the "Programming" section of the data sheet. Record the following from this summary page on the data sheet:
 - a. Start Date
 - b. Start Time (standard time)
 - c. Sonde Filename: name of the .BIN file
 - d. Battery Life (days): labelled as "Battery Life Remaining" in EXO (NOTE: This value may

be inaccurate during cabled communication as the PC is providing power to the EXO. Checking battery life via a Bluetooth connection yields a more accurate number of days)

- e. Free Memory (days): labelled as "Logging Space Duration
- f. Free Memory Status (bytes or %): labelled as Log Space Available in % in EXO
- 14. The sonde is now ready for deployment! Place the sonde back into the air-saturated water bath and allow it to take readings there until deployment time arrives. These bucket readings, or "data tails" as they are often referred to as, provide valuable data on how the sonde was functioning prior to deployment.

IV. EXO DEPLOYMENT

- 1. The NERRS Data Management Committee strongly recommends using the same sonde type at each location. If you have 2 EXO sondes, they should be rotated at the same station. If you do not have enough sondes to dedicate a specific type to each station, you may alternate but must document the sonde type used for each deployment in your metadata. Telemetered stations MUST be designated as either EXO or 6600 stations with the CDMO and should not be rotated.
- 2. When transporting the sondes, a tap-water-soaked white towel must be wrapped around each sonde. This is to be done during both deployment and upon retrieval. The wet towels reduce shock and vibration damage and ensure a saturated environment for the oxygen probe during transport. This task is <u>MANDATORY</u> to improve the oxygen data we are collecting. If there are extenuating circumstances DMC may approve another method for transport.
- 3. Sondes should be transported in a cooler of sufficient size to allow them to lie horizontal across the bottom. Suggested size is 28" x 15" x 14" for up to 4 sondes.
- 4. All sondes are to be deployed so that the probes stay submerged at low tides and are at a fixed distance off the bottom to allow for tidal and flow amplitude measurements. Suggested methods include a perforated PVC (or other plastic) tube attached to a pile of bridge abutment or a steel cage resting on the bottom (be sure probes are 0.25 to 0.5 meter off bottom). If you use a perforated tube, this tube must be periodically inspected for fouling and cleaned. YSI recommendations for tube construction are available at the following link: https://www.ysi.com/File%20Library/Documents/Guides/Long-Term-Deployment-Tube-Guide.pdf
- 5. The length of time the instrument is deployed is dependent on the rate of fouling at your site. This will range from less than a week to up to a maximum of 45 days for EXO sondes. The NERRS Data Management Committee will continue evaluating whether extended maximum deployment times for

EXO sondes (the maximum for 6-series sondes is four weeks) are feasible.

6. Independent, paired field data readings are required at all sonde retrievals/deployments. Ideally, use a hand-held meter, Winkler titration, or other properly calibrated instrument to collect this data alongside the deployed sonde for its last reading and the newly deployed sonde for its first reading. At a minimum, you must take an independent paired reading with the freshly calibrated sonde against the deployed sonde before replacing. Record the data from the independent instrument in the NERRS SWMP Water Quality Field Log with one log following each sonde through its deployment.

- 7. ABS or PVC deployment tubes:
 - a. Service the tubes annually
 - b. To check on the integrity of the installation tube, deploy a second sonde outside the tube and at the same depth. Compare the data from the two sondes. If the tube is fouled, the sonde inside will only be recording the microcosm of the pipe, not the water itself.

V. EXO RETRIEVAL

- Retrieve the sonde from the water and visually examine it and the probes for fouling and/or damage. Note any fouling type and amount in the "Fouling Presence" section of the NERRS SWMP Water Quality Field Log, however DO NOT remove fouling, so that true post-deployment readings may be obtained. NERRS Data Management Committee strongly recommends using the same sonde type at each location.
- 2. Record field data for the following mandatory parameters on the data sheets: water temperature, specific conductivity, salinity, DO percent saturation, and DO concentration. Additional optional parameters may be recorded in the "Other" field of the data sheet.
- 3. Wrap the EXO sonde in a tap-water saturated white towel and placed in a secure container, or other DMC approved transport method, in order to prevent severe vibrations to the EXO sonde during transportation.

VI. EXO DATA DOWNLOAD & POST-DEPLOYMENT READINGS

- Post Deployment Calibration Checks These checks note any changes or drift of the probe during deployment combined with effects of biofouling. This process is critical not only for data QAQC, but also for data users to know if the data were affected by biofouling, wear and tear, or other issues. Ideally these checks will take place within 24 hours of EXO sonde retrieval. If not, it is critical to make a visual inspection of the conductivity cells and note, either photographically or via notes, any visible fouling to document related drift. Bubbles and saturated water bath currents may dislodge material and significantly impact drift. Gang calibration is NOT permitted during post-deployment readings since it is important to take these readings with the sonde remaining in a condition similar to what it was while deployed in the field.
- 2. Data Download
 - a. Place the EXO sonde into a bucket of clean water that has been aerated for at least 60 minutes to create a 100% air saturated water bath. Allow the sonde ample time to reach temperature equilibration prior to beginning the download and post-deployment readings procedures.
 - b. Connect to the EXO sonde via the USB/SOA adapter or wirelessly via Bluetooth

- c. Click Large Green Arrow icon
- d. Click Stop Logging
- e. Click Data Folder icon
- f. Click "Transfer"
- g. Click to highlight the file of interest
- h. Click "Selected"
- i. Click "View/Export"
- j. If a PC folder window comes up, click the file of interest in that window and click "okay". If it does not pop up, click the small blue folder icon that says "select file" when you mouse over it (NOT the Main Data folder icon), select the file and click "okay".
- k. Click the icon next to the small blue folder icon (two white pages stacked on each other) that says "export data" when moused over
- 1. Two files should now be visible in the "Data Files" folder within the KORS folder on the PC hard drive; a .BIN file and an Excel file, the latter of which often opens automatically
- m. Do NOT remove the sonde from the aerated water bath yet
- 3. Post-Deployment Readings these readings are taken in discrete mode via the Green Running Man icon and exhibit many procedural similarities to the calibration procedure. While not specifically listed by step for brevity purposes, Reserves should follow the same rinsing procedures between readings for each probe as detailed in the Calibration Section. Values recorded during Post-Deployment checks are written in the "Post-Deployment" or "Sensor Diagnostics" sections of the NERRS WQ Data Sheet.
 - a. Reconnect to the sonde if necessary
 - b. Click the Green Running Man icon
 - c. Record the temperature reading given by the EXO sonde near the bottom of the data sheet in the "Comments- Post" section (no field is currently available in the data sheet's postdeployment section where all other readings go)
 - d. Also record the water temperature using the same NIST traceable external thermometer that was used during calibration and record that value in the same section as in line c)
 - e. Open the Excel file that was previously downloaded
 - f. Record the last two DO % saturation readings taken in the air saturated water bath in the post-deployment section of the data sheet along with the barometric pressure reading that lines up closest to the time of the second DO% reading
 - g. Remove the EXO from the saturated water bath
 - h. Remove the guard and using an Allen wrench, remove the wiper from the wiper probe. Clean all debris from the brush immediately, so the brush bristles do not dry out with gaps.
 - i. Place the freshly cleaned wiper to the side to dry as it is no longer needed for this section
 - j. Place it in a water-saturated air environment (loosely fitted calibration cup with a tiny amount of water in it or EXO wrapped in a wet towel)
 - k. Record the water depth/level value given by the EXO sonde and closest barometric pressure

reading to the measurement while the EXO sonde is taking discrete measurements

- 1. Record post-deployment conductivity reading
- m. Record post-deployment pH readings and diagnostic millivolt readings on the data sheet by running the EXO sonde in discrete mode in each of the 2 pH standards (or 3 pH standards if using 3 point calibrations)
- n. Disconnect the sonde from KORS (remove the adapter for cabled communication or put the EXO sonde to sleep with the magnet tool for blue tooth connection)
- o. Lightly clean the probe bodies removing any obvious fouling to avoid contamination of the turbidity standards. Do not clean the probe faces, the objective is to determine if fouling impacted turbidity readings in the field.
- p. Reconnect the sonde to KORS
- q. Take discrete readings in both 0 and 126 NTU standards (remember that the EXO interprets the 126 NTU YSI standard as 124 NTU) and record those values in the Post-Deployment section of the data sheet
- r. Clean the EXO sonde thoroughly. All fouling should be removed at this point in preparation for future deployments.

VII. SWMP WATER QUALITY MONITORING CALIBRATION & FIELD LOGS

NERRS SWMP Calibration and Field Logs must be filled out with every instrument deployment. They are now combined into one deployment log, which should contain all the calibration and field information for a particular sonde through its deployment in the field. Deployment logs are now completed, archived, and submitted through the Deployment Log Interface located on the CDMO's data upload page. They may be filled out live while calibrating instruments or printed out as hardcopies to be handwritten and input later. Filling the logs out thoroughly and including any additional notes on equipment or field conditions is critical. These logs will aid in data QAQC procedures, identification of anomalous data, and help to identify faulty equipment. Once completed and reviewed, SWMP Calibration and Field Logs are to be electronically submitted to the CDMO through the Deployment Log Interface by the designated annual water quality submission due date.

VIII. PROBE CARE & STORAGE

Most of the probes, except Conductivity, have a limited shelf life so do not purchase replacements too far in advance. The procedure for storage of probes is different for short-term (1 month or less) and long-term (greater than 1 month).

Short-term Storage

For short term storage, it is important to keep the probes moist but <u>not immersed in water</u>. Keep probes attached to the EXO sonde and place the sonde in approx. 0.5 in of tap water (not distilled) in the <u>sealed</u> EXO calibration cup.

Long-term Storage

- 1. Clean conductivity sensors and store them either dry or wet. If they are in contact with solution, it should not be corrosive.
- 2. The pH probe should be removed from the EXO sonde if storage will exceed 30 days and stored in the pH storage cup (the one it was shipped in) containing 1 molar KCL or pH 4 buffer.
- 3. No special precautions are necessary for the Depth sensor.
- 4. Store the turbidity probe dry in air and cover the optical surface with a cap to prevent scratching or in air saturated water (installed in the sonde with a small amount of water in the calibration cup).
- 5. Dissolved Oxygen probes should be stored in a water-saturated air environment (attached to the sonde with a small amount of water in the calibration cup to maintain humidity) to avoid the need for a 12 hour membrane rehydration at a later date. The probe can be stored dry, but if done so it must be re-hydrated in saturated water for a 12 hour period.
- 6. Remove the brush from the wiper probe and store dry (make sure it is clean and dries in original shape no gaps or forks in the bristles). The wiper itself can be stored in a humid environment or dry environment.
- 7. Remover copper tape applied directly to the sensors prior to long-term storage to prevent the glue from hardening and becoming difficult to remove. Copper tape can remain in place if a protective barrier is applied underneath like packing tape or YSI clear anti-fouling sleeves
- 8 For EXO sondes:
 - a. Remove batteries prior to storage greater than 30 days.
 - b. The battery compartment and compartment cap should be cleaned thoroughly and re-greased prior to storage.
 - c. Clean and re-grease the two sonde connectors (located at the top of the sonde) and place connector caps on both.
 - d. Plug ports of any missing sensors.

IX. APPENDICES FOR TROUBLESHOOTING & OTHER DOCUMENTS

Appendix A: Antifouling Measures

Antifouling measures have come a long way throughout the history of SWMP. YSI and NERRS personnel continue to make advancements in bio-fouling reduction. This section contains a list of recommended antifouling measures for sondes and probes along with guidance on their usage.

Sonde Antifouling Measures:

- 1. Copper Sensor Guard a copper alloy sensor guard offers biofouling properties and replaces the normal black plastic sensor guard
- 2. Duct tape may be applied to the sonde body to both reduce biofouling and aid in ease of its removal

- 3. Shrink-wrap Sleeves specially crafted plastic sleeves designed to fit the sonde body and sold by YSI. The sleeve is applied to the sonde and hit with hot air via a blow-dryer o heat-gun to "shrink" it into a form fitting protective layer
- 4. C-Spray: while it does not prevent fouling, this spray material can be applied to the sonde connector region, sensors, and internal sensor guard faces to make biofouling removal easier

Probe Antifouling Measures:

- 1. Copper Tape this adhesive-backed tape, sold by YSI and other vendors, reduces biofouling when applied to the body of each probe. Sensor faces should not be covered with copper tape and care must be taken to cut holes that correspond to the openings in the side of the temperature/conductivity probe
- 2. Temperature Conductivity Probe Screens copper alloy screen sold by YSI that prevents critters from entering the conductivity cell region when applied to the outside of the probe
- 3. C-Spray while it does not prevent fouling, this spray material can be applied to the sonde connector region, sensors, and internal sensor guard faces to make biofouling removal easier
- 4. Mesh screens 0.25" mesh screens can be applied to the outer surface of the sensor guard to prevent critters from gaining access to the probes and inner sensor guard are. The mesh should be wrapped around the guard 1.25 times and secured with a zip tie to secure it

Appendix B: Depth Offsets

Pressure	Offset	Pressure	Offset	Pressure	Offset
mb	meter	mb	meter	mb	meter
930	-0.849	980	-0.340	1030	0.170
931	-0.839	981	-0.329	1031	0.180
932	-0.829	982	-0.319	1032	0.191
933	-0.819	983	-0.309	1033	0.201
934	-0.809	984	-0.299	1034	0.211
935	-0.798	985	-0.289	1035	0.221
936	-0.788	986	-0.278	1036	0.231
937	-0.778	987	-0.268	1037	0.242
938	-0.768	988	-0.258	1038	0.252
939	-0.758	989	-0.248	1039	0.262
940	-0.747	990	-0.238	1040	0.272
941	-0.737	991	-0.227	1041	0.282
942	-0.727	992	-0.217	1042	0.293
943	-0.717	993	-0.207	1043	0.303
944	-0.707	994	-0.197	1044	0.313
945	-0.696	995	-0.187	1045	0.323
946	-0.686	996	-0.176	1046	0.333
947	-0.676	997	-0.166	1047	0.344
948	-0.666	998	-0.156	1048	0.354
949	-0.656	999	-0.146	1049	0.364
950	-0.645	1000	-0.136	1050	0.374
951	-0.635	1001	-0.125	1051	0.384
952	-0.625	1002	-0.115	1052	0.395
953	-0.615	1003	-0.105	1053	0.405
954	-0.605	1004	-0.095	1054	0.415
955	-0.594	1005	-0.085	1055	0.425
956	-0.584	1006	-0.074	1056	0.435
957	-0.574	1007	-0.064	1057	0.446
958	-0.564	1008	-0.054	1058	0.456
959	-0.554	1009	-0.044	1059	0.466
960	-0.544	1010	-0.034	1060	0.476
961	-0.533	1011	-0.023	1061	0.486
962	-0.523	1012	-0.013	1062	0.497
963	-0.513	1013	-0.003	1063	0.507
964	-0.503	1014	0.007	1064	0.517
965	-0.493	1015	0.017	1065	0.527
966	-0.482	1016	0.028	1066	0.537
967	-0.472	1017	0.038	1067	0.548
968	-0.462	1018	0.048	1068	0.558
969	-0.452	1019	0.058	1069	0.568
970	-0.442	1020	0.068	1070	0.578
971	-0.431	1021	0.079	1071	0.588
972	-0.421	1022	0.089	1072	0.599
973	-0.411	1023	0.099	1073	0.609
974	-0.401	1024	0.109	1074	0.619
975	-0.391	1025	0.119	1075	0.629
976	-0.380	1026	0.130	1076	0.639
977	-0.370	1027	0.140	1077	0.650
978	-0.360	1028	0.150	1078	0.660
979	-0.350	1029	0.160	1079	0.670

Pressure	Offset	Pressure	Offset	Pressure	Offset
in Hg	meter	in Hg	meter	in Hg	meter
27.40	-0.870	28.90	-0.352	30.40	0.166
27.43	-0.860	28.93	-0.342	30.43	0.176
27.46	-0.849	28.96	-0.332	30.46	0.186
27.49	-0.839	28.99	-0.321	30.49	0.197
27.52	-0.829	29.02	-0.311	30.52	0.207
27.55	-0.818	29.05	-0.300	30.55	0.218
27.58	-0.808	29.08	-0.290	30.58	0.228
27.61	-0.798	29.11	-0.280	30.61	0.238
27.64	-0.787	29.14	-0.269	30.64	0.249
27.67	-0.777	29.17	-0.259	30.67	0.259
27.70	-0.767	29.20	-0.249	30.70	0.269
27.73	-0.756	29.23	-0.238	30.73	0.280
27.76	-0.746	29.26	-0.228	30.76	0.290
27.79	-0.736	29.29	-0.218	30.79	0.300
27.82	-0.725	29.32	-0.207	30.82	0.311
27.85	-0.715	29.35	-0.197	30.85	0.321
27.88	-0.704	29.38	-0.186	30.88	0.332
27.91	-0.694	29.41	-0.176	30.91	0.342
27.94	-0.684	29.44	-0.166	30.94	0.352
27.97	-0.673	29.47	-0.155	30.97	0.363
28.00	-0.663	29.50	-0.145	31.00	0.373
28.03	-0.653	29.53	-0.135	31.03	0.383
28.06	-0.642	29.56	-0.124	31.06	0.394
28.09	-0.632	29.59	-0.114	31.09	0.404
28.12	-0.622	29.62	-0.104	31.12	0.414
28.15	-0.611	29.65	-0.093	31.15	0.425
28.18	-0.601	29.68	-0.083	31.18	0.435
28.21	-0.591	29.71	-0.073	31.21	0.445
28.24	-0.580	29.74	-0.062	31.24	0.456
28.27	-0.570	29.77	-0.052	31.27	0.466
28.30	-0.559	29.80	-0.041	31.30	0.477
28.33	-0.549	29.83	-0.031	31.33	0.487
28.36	-0.539	29.86	-0.021	31.36	0.497
28.39	-0.528	29.89	-0.010	31.39	0.508
28.42	-0.518	29.92	0.000	31.42	0.518
28.45	-0.508	29.95	0.010	31.45	0.528
28.48	-0.497	29.98	0.021	31.48	0.539
28.51	-0.487	30.01	0.031	31.51	0.549
28.54	-0.477	30.04	0.041	31.54	0.559
28.57	-0.466	30.07	0.052	31.57	0.570
28.60	-0.456	30.10	0.062	31.60	0.580
28.63	-0.445	30.13	0.073	31.63	0.591
28.66	-0.435	30.16	0.083	31.66	0.601
28.69	-0.425	30.19	0.093	31.69	0.611
28.72	-0.414	30.22	0.104	31.72	0.622
28.75	-0.404	30.25	0.114	31.75	0.632
28.78	-0.394	30.28	0.124	31.78	0.642
28.81	-0.383	30.31	0.135	31.81	0.653
28.84	-0.373	30.34	0.145	31.84	0.663
28.87	-0.363	30.37	0.155	31.87	0.673

Pressure	Offset	Pressure	Offset	Pressure	Offset
mm Hg	meter	mm Hg	meter	mm Hg	meter
680	-1.088	730	-0.408	780	0.272
681	-1.074	731	-0.394	781	0.285
682	-1.060	732	-0.381	782	0.299
683	-1.047	733	-0.367	783	0.313
684	-1.033	734	-0.353	784	0.326
685	-1.020	735	-0.340	785	0.340
686	-1.006	736	-0.326	786	0.353
687	-0.992	737	-0.313	787	0.367
688	-0.979	738	-0.299	788	0.381
689	-0.965	739	-0.285	789	0.394
690	-0.952	740	-0.272	790	0.408
691	-0.938	741	-0.258	791	0.421
692	-0.924	742	-0.245	792	0.435
693	-0.911	743	-0.231	793	0.449
694	-0.897	744	-0.218	794	0.462
695	-0.884	745	-0.204	795	0.476
696	-0.870	746	-0.190	796	0.489
697	-0.856	747	-0.177	797	0.503
698	-0.843	748	-0.163	798	0.517
699	-0.829	749	-0.150	799	0.530
700	-0.816	750	-0.136	800	0.544
701	-0.802	751	-0.122	801	0.557
702	-0.789	752	-0.109	802	0.571
703	-0.775	753	-0.095	803	0.585
704	-0.761	754	-0.082	804	0.598
705	-0.748	755	-0.068	805	0.612
706	-0.734	756	-0.054	806	0.625
707	-0.721	757	-0.041	807	0.639
708	-0.707	758	-0.027	808	0.653
709	-0.693	759	-0.014	809	0.666
710	-0.680	760	0.000	810	0.680
711	-0.666	761	0.014	811	0.693
712	-0.653	762	0.027	812	0.707
713	-0.639	763	0.041	813	0.721
714	-0.625	764	0.054	814	0.734
715	-0.612	765	0.068	815	0.748
716	-0.598	766	0.082	816	0.761
717	-0.585	767	0.095	817	0.775
718	-0.571	768	0.109	818	0.789
719	-0.557	769	0.122	819	0.802
720	-0.544	770	0.136	820	0.816
721	-0.530	771	0.150	821	0.829
722	-0.517	772	0.163	822	0.843
723	-0.503	773	0.177	823	0.856
724	-0.489	774	0.190	824	0.870
725	-0.476	775	0.204	825	0.884
726	-0.462	776	0.218	826	0.897
727	-0.449	777	0.231	827	0.911
728	-0.435	778	0.245	828	0.924
729	-0.421	779	0.258	829	0.938

Appendix C: Calibration Procedures for Water Level

- 1. Fill EXO calibration cup with a small amount of water (the volume should not reach anywhere near the probes) to create a water-saturated air environment
- 2. Screw the guard onto the sonde and place it into the calibration cup
- 3. Allow the sonde to remain in a vertical position on the work bench
- 4. Click the calibration icon
- 5. Click "Depth"
- 6. Click "Depth m"
- 7. Determine the most current local barometric pressure value in mmHG (preferably from your SWMP weather station) and record this value in the "Pre-Deployment" "Before Cal" column inside the field next to "Baro Pres. (Depth Calib). To convert other units to mm Hg, multiply inches of Hg by 25.4 or millibars by 0.75.

NOTE: Reserves located at a 'significant' elevation, MUST use the barometric pressure reading from their corresponding MET station for this calculation to ensure the most accurate depth correction possible. While this is recommended for everyone, it specifically applies to those that use an offset in their Campbell program (following NWS protocols) to adjust barometric pressure readings from their MET station to sea level. **Currently those two Reserves are OWC and LKS**.

- 8. Input the barometric pressure value (mm Hg) in the online Deployment Log or Depth/Offset Calculator to determine the correct barometric pressure offset to use for calibration (the charts in Appendix B may also be used to determine correct offset).
- 9. Write this value on the data sheet by putting a line through 0.0 and writing the barometric pressure offset above the word "offset" for Level in the "Pre-Deployment" "Standards" section. The 0.0 listed on the data sheet is an artifact of the equation present within the online Calibration Log. In the Online Log Sheets, entry of the barometric pressure automatically changes the 0.0 to the relevant barometric pressure offset.
- 10. Write the station offset (the station offset is the elevation of the pressure transducer at the site you are calibrating for) in the "Before Cal" field on the data sheet
- 11. Add the BP offset and Station Offset to get the value needed for calibration or simply use the Depth/Offset Calculator to generate the value
- 12. Click Advanced
- 13. Click Edit
- 14. Enter the value calculated in step K. into the "Offset" field

15. Click Apply

- 16. Click OK
- 17. Click Back
- 18. Click "Start Cal"
- 19. Once the depth value has stabilized and "unstable data" in red font changes to "stable data" in green font, click "apply".
- 20. Click "Complete"
- 21. The calibration worksheet for the probe will pop up and the user should record the following values:

a. Pre Calibration Value: record this value in the data sheet's "Before Cal" column for level

b.Post Calibration Value: record this value in the data sheet's "Calibrated" column for level

NOTE: The depth field on the data sheet should be left blank when performing water level calibration. That field is only applicable to calibration for water depth.

22. Close the calibration worksheet

Appendix D: Calibration Procedures for Total Algae (Chl and BGA) sensor

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Chlorophyll fluorescence is an optional SWMP-supported parameter that may be collected and submitted to the CDMO, but is not a required parameter.

Notes:

- 1. The EXO total algae sensor can be used to not only measure chlorophyll-a, but also phycocyanin or phycoerythrin to give a more accurate estimate of total autotrophic planktonic biomass. Depending on your interest in measuring freshwater plankton (phycocyanin) or marine plankton (phycoerythrin) pigments, you would need to order the appropriate sensor for your application. See the YSI EXO Manual for part numbers. As chlorophyll-a (reported as chlorophyll fluorescence) is the only optional SWMP-supported parameter, its calibration procedures are the only ones that will be addressed here.
- 2. For chlorophyll-a measurement, the Total Algae sensor allows calibration for two units of measure: RFU and μg/L. RFU (Relative Fluorescence Units) is used to calibrate output relative to a standard such as Rhodamine WT dye, thereby standardizing all sensors relative to each other and allowing for post-calibration at that same standard calibration value. μg/L (micrograms per liter) is used to estimate chlorophyll-a pigment concentration. A semi-quantitative estimate of chlorophyll-a μg/L can be done by calibrating the sensor using sample water of known chlorophyll concentration determined through extraction, or using a dye such as Rhodamine WT for which a correlation between its fluorescence value and chlorophyll-a concentration has been developed.
- 3. The CDMO requires chlorophyll data submitted in µg/L, so for the purpose of these Standard Operating Procedures a two-point calibration for µg/L will be the only method discussed. As chlorophyll-a extraction methods can vary, calibration in Rhodamine WT dye will be discussed here.
- 4. As with all optical probes, makes sure the optics are clean before calibration.
- 5. Since rhodamine is a strong dye, if possible, have a dedicated calibration cup for rhodamine use-only to avoid potential contamination of your zero (deionized water) standard.
- 6. To avoid aeration of the standard and interference from bubbles during calibration, it is recommended to pour standards slowly down the side of the calibration cup as it's held at an angle. Once immersed in standard, visually inspect the optics for bubbles and holding sonde at an angle, gently tap the bottom of the calibration cup against your worktop to dislodge any bubbles from the sensor optics. Alternatively you can give the sonde and cal-cup a gentle swirl to remove any bubbles that may be present on the surface of the optics.
- 7. The Rhodamine WT dye solution should be approximately 2.5% Rhodamine WT. The YSI-recommended supplier is:

Kingscote Chemicals 3334 South Tech Blvd., Miamisburg, OH 45342 1-800-394-0678; <u>http://www.brightdyes.com/</u> item# 106023 Water Tracer Dye: Fluorescent FWT Red 25 – Liquid

Instructions for making the Rhodamine WT dye solution for calibration of the Total Algae Sensor:

- 1. Accurately transfer 5 mL of the Rhodamine FWT liquid concentrate into a 1000 mL volumetric flask and bring up to 1000 mL with deionized or distilled water, to give a solution that is 125 mg/L of Rhodamine WT. Ensure the solution is mixed well. Transfer this solution to a glass bottle that you can easily pipette from.
- Accurately transfer 5 mL of the 125 mg/L stock solution into a 1000 mL volumetric flask, and bring up to 1000 mL with deionized or distilled water to give a solution that is 0.625 mg/L Rhodamine WT (200:1 dilution of the original liquid concentrate). Ensure the solution is mixed well.
- 3. The 125 mg/L stock solution from Step 1 should be stored in a refrigerator in a glass bottle in the dark to prevent decomposition, and then brought up to room temperature before using again to make the 0.625 mg/L standard solution.
- 4. The 0.625 mg/L standard solution should be used for calibration within 24 hours of preparation. Used and/or excess standard should be discarded in accordance with local regulations or can be saved for rinse only.
- 5. Suggestions:
 - a. Since this is a strong dye, it is recommended to have glassware dedicated only to the preparation of this standard.
 - b. When pipetting this solution, minimize the length of pipette tip that you immerse in the solution to avoid excess solution sticking to the outside of the tip (this is a thick solution), and when pipetting, pipette slowly so as not to break the surface tension of the solution to ensure all of the solution is drawn down out of the pipette tip.
 - c. It is handy to keep a stir bar in the glass stock bottle and to have the stock solution stirring slowly as it comes up to room temperature before making the next batch of standard solution.

Effect of temperature on fluorescence

The effect of temperature on the fluorescence of Rhodamine WT dye must be accounted for when calibrating the EXO Total-Algae sensor. The table below gives the μ g/L value that corresponds to the temperature of the calibration standard.

Temp (°C)	µg/L Chl	RFU Chl
30	56.5	14.0
28	58.7	14.6
26	61.3	15.2
24	63.5	15.8
22	66.0	16.4
20	68.4	17.0
18	70.8	17.6
16	73.5	18.3
14	76.0	18.9
12	78.6	19.5
10	81.2	20.2
8	83.8	20.8

Two point calibration for chlorophyll-a $\mu g/L$ - This calibration procedure zeroes the fluorescence sensor and uses the default sensitivity of the sensor to only semi-quantitatively calculate chlorophyll concentration in $\mu g/L$.

- 1. Using a NIST traceable thermometer, measure the temperature of your Rhodamine WT standard solution and using the included table, cross reference that temperature to the appropriate μ g/L Chl value for Rhodamine WT.
- 2. In KOR, select CALIBRATE.
- 3. Choose BGA-PC/Chlor (or -PE depending on which sensor you have).
- 4. Choose Chlorophyll μg/L. If you have both units of measure, RFU and μg/L selected for the display of chlorophyll values, you must calibrate chlorophyll twice for EACH unit, RFU and μg/L to completely calibrate this parameter. Units of measure can be turned on/off under OPTIONS -> UNITS -> CHL.
- 5. Choose a 2-point calibration and enter the values of your standards in the appropriate boxes. Standard value 1 would be 0 (deionized water) and standard value 2 would be the chl concentration from the reference table acquired in Step 1. For additional tips on this process, see below.

- 6. Fill a clean EXO calibration cup with the appropriate amount of deionized water, pouring along the side to minimize bubbles.
- 7. Rinse all of the sensors well with deionized water and install a clean (preferably calibration dedicated) black plastic EXO guard with the bottom installed.
- 8. Slowly lower the guard and sensors into the calibration cup until seated and tighten the seal on the calibration cup. Lift the sonde, and at an angle, gently tap the bottom of the calibration cup against your worktop to dislodge any bubbles from the sensor optics. Visually inspect the optics to ensure there are no bubbles attached that might affect the calibration.
- 9. Press START CAL.
- 10. Once the temperature has stabilized and the readings have stabilized, the data should change from red UNSTABLE DATA to green STABLE DATA. At this point, press APPLY. A popup window with PROCEED to next standard will appear.
- 11. Remove the sonde from the calibration cup.
- 12. Pour a small amount of Rhodamine standard into a clean (preferably dedicated) calibration cup (enough to coat sensors well), and place sonde in to the calibration cup and tighten the collar gasket.
- 13. Gently invert the sonde several times to coat all of the sensors, bulkhead and connectors with the standard. Repeat this step twice more with fresh standard rinse.
- 14. Fill the calibration cup with fresh Rhodamine WT standard to the appropriate volume and, same as in Step 8 above, gently lower the guard and sensors in to the calibration cup, then tap the sonde against your work surface to dislodge any bubbles that may be on the surface of the optics.
- 15. Press PROCEED and wait for the readings to change from UNSTABLE DATA to STABLE DATA.
- 16. Press APPLY.
- 17. Press COMPLETE.
- 18. A successful calibration will be indicated by a green check on the calibration summary screen. If a calibration error occurred, indicated by a yellow exclamation point, you will need to redo the calibration. An error could be caused by the sonde reading too high or low compared to the standard value, and could be caused by improperly made standard, contaminated standard or bubbles on the optics.
- 19. If your calibration is successful, be sure to record your pre-calibration and post-calibration values for entry into your CDMO digital calibration logs.
- 20. Save the used and any unused Rhodamine WT standard for RINSE standard for the next set of calibrations. Remember that the Rhodamine WT must be used within 24 hours of preparation, but can be saved as RINSE ONLY beyond that time.

- 21. If calibrating RFU, repeat steps 1-17 above using the RFU values from the table above. See calibration tip below for minimizing the amount of standard that you need to prepare and use.
- 22. Rinse the probes, bulkhead and connectors well with deionized water before calibrating the next probe or storing the instrument.

CALIBRATION TIP: If calibrating both μ g/L and RFU, you may conserve standard by doing a reverse calibration on your second unit. For example, you would do a 2-point calibration, deionized water (pt 1) and Rhodamine WT (pt 2) for RFU. Then, leaving the instrument in the Rhodamine WT, you can do a 2 point-calibration for μ g/L using the Rhodamine WT as your 1st point and deionized water (zero) as your 2nd point. This is especially helpful if you have multiple chlorophyll probes to calibrate and are doing a batch calibration on one instrument.