

# Calibration Tips

For YSI 6-Series Sondes & Sensors - DO Excerpt



## DISSOLVED OXYGEN CALIBRATION TIPS

ML 2/14/08

## UNATTENDED & SAMPLING DO SENSOR PREPARATION

- 1. Inspect the DO probe anodes; recondition using the 6035 reconditioning kit if they are not bright and shiny.
- 2. Install a new membrane, making sure that it is tightly stretched and wrinkle free.

<u>Warning</u>: Replace the probe o-ring if it is loose or stretched out. If you remove the DO probe from the sonde, be sure to inspect the probe port and connectors for moisture. Remove any moisture droplets from the connector and thread areas. Verify that the probe is clean and dry then apply a small amount of synthetic grease to the o-ring before it is reassembled.

Note: DO membranes will be slightly unstable during the first 3 to 6 hours after they are installed; it is strongly recommended that the final calibration of a DO sensor being used in "Unattended" studies takes place after this time period.

- 3. Go to the sonde's Report Menu and enable the "DO Charge". Now go to the Run Menu and start the sonde in the "Discrete Run" mode at a four-second rate. Allow the sonde to run (burn-in) for 15 minutes. Record the DO charge after about 5 minutes. The charge number must be between 25 and 75.
- 4. After the burn-in is complete, go to the sonde's Advanced Menu and confirm that the RS-232 Auto Sleep function is enabled. If the sonde is to be connected to an SDI-12 data logger, then the SDI-12 Auto Sleep must be enabled as well. Wait 60 seconds before proceeding to Step 5.
- 5. Start the probe in the "Discrete Run" mode at a four-second rate and record the first 10 DO % numbers on paper. The numbers must start at a high number and drop with each four second sample.

Example: 110, 105, 102, 101.5, 101.1, 101.0, 100.8, 100.4, 100.3, 100.1

It does not matter if the numbers do not reach 100%; it is only important that they have the same high to low trend. If you have a probe that starts at a low number and steadily climbs upwards, then the sensor has a problem and it must not be used.

Note: Initial power up can make the first two DO % samples read low; the first two samples can be disregarded.

6. The probe is now ready to be calibrated. Set the Auto Sleep RS-232 for the intended application: ON for UNATTENDED STUDIES and OFF for SAMPLING MEASUREMENTS. Set the sonde into the calibration cup with approximately 1/8 inch of water. Do not engage the threads, and do not allow water to contact the membrane. You may also use the wet-towel method. The sonde must now sit in this saturated environment for at least 10 minutes before the DO calibration can begin.

<u>Warning</u>: The sonde must be idle and not in the "Run" mode for 5 minutes prior to starting the DO calibration.

Calibrate the sonde in DO %; be sure to enter your local barometric pressure in mm/hg. In Unattended mode (RS-232 Auto-Sleep ON) the DO probe will be calibrated automatically once the barometric pressure is entered and the warm-up time counter counts down to zero. For Discrete or Sampling, press the Enter key when the DO readings are stable. Wait at least 3 minutes after you enter the barometric pressure before you press the Enter key again to calibrate for Discrete/Sampling DO measurements.

7. When the calibration is completed, go to the sonde's Advanced Menu and then to the "Cal Constants" and record the "DO Gain". The gain should be 1.0 with a range of -0.7 to +1.4. The probe is now calibrated and ready to prepare for the study. As with the other parameters, any warning messages displayed by the sonde during the calibration are a cause for concern and must be investigated before collecting field data with the sonde.

<u>Warning</u>: Avoid having the DO probe membrane contact the calibration cup or sensor guard during transfers. Keep the DO probe in sight when removing or installing the sensor guard and cal cup.

## ROX™ OPTICAL DISSOLVED OXYGEN CALIBRATION TIPS

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## **ROX FACTS**

- The ROX Optical Dissolved Oxygen sensor does not require any special sonde setup or burn-in.
- Calibration data is stored in the ROX so it can be calibrated in one sonde and then used in another without recalibrating the probe in the new sonde.
- Calibration data is automatically transferred to the host sonde as soon as the sonde powers up the ROX sensor.
- The ROX has excellent long term stability and will achieve  $\pm$  1% accuracy when calibrated in a (temperature-stable) saturated water or saturated air environment.
- Field DO calibrations should be avoided!
- Remove the wiper when the sensor is going to be exposed to sodium sulfite (zero DO test) or Formazin-based turbidity standards.
- The ROX sensor must remain hydrated at all times! See Appendix M in the 6-Series manual for additional information.

## CALIBRATION METHOD - ONE-POINT "SATURATED AIR"

- 1. Dry the temperature sensor and the ROX membrane to remove any water droplets.
- 2. Put 1 inch of water in the calibration cup and thread onto the endcap 1/2 thread. Do not fully thread the calibration cup onto the endcap!
- 3. Allow the sonde to sit in the calibration cup for 15 minutes to ensure that the air is 100% saturated and that the temperature is stable.
- 4. Select ODO% and enter the barometric pressure in mm hg (inches x 25.4) and press Enter. The sonde will do a 4 second count down and then say "Press Enter when the readings are stable". Wait at least 30 seconds before you press the Enter button.

Note: the sonde will only read 100% saturation if the barometric pressure is 760 mm hg. To determine the actual DO in %, divide the barometer reading by 760 and then multiply that number by 100.

Example:  $750 / 760 = 0.9868 \times 100 = 98.68\%$ 

Check the DO Gain in the Advanced Menu. An acceptable range is 0.7 to 1.4.

## CALIBRATION METHOD – ONE-POINT "SATURATED WATER"

- 1. Using a medium-size aquarium air pump and air stone, sparge a five-gallon bucket of tap water for 1 hour.
- 2. Place the assembled sonde in the bucket and allow the sonde to equilibrate to the water temperature for 5 minutes.
- 3. Select ODO% and enter the barometric pressure in mm hg (inches x 25.4) and press Enter. The sonde will do a four-second countdown and then say "Press Enter when the readings are stable". Wait at least 30 seconds before you press the Enter button.

Note: the sonde will only read 100% saturation if the barometric pressure is 760 mm hg. To determine the actual DO in %, divide the barometer reading by 760 and then multiply that number by 100.

Example:  $750 / 760 = 0.9868 \times 100 = 98.68\%$ 

Check the DO Gain in the Advanced Menu. An acceptable range is 0.7 to 1.4.

## CALIBRATION METHOD – TWO-POINT "AIR or WATER"

Note: YSI does not recommend the two-point calibration unless (A) you are certain that the sensor does not meet your accuracy requirements at low DO values and (B) you are operating under conditions where you are certain you can generate a medium that is truly free of oxygen!

- 1. REMOVE THE ROX WIPER! Sodium sulfite will be absorbed into the pad and will be applied to the ROX membrane each time the wiper is activated. This will contaminate the ROX membrane and create a problem with the second point of the calibration.
- 2. Mix 2 grams of sodium sulfite to 1000 milliliters of tap water 2 hours prior to use and keep it in a sealed bottle. Rapidly transfer this mixture into the sonde's calibration cup and fill to overflow. Install the sonde and completely seal the cup to prevent diffusion of air into the vessel.
- 3. To calibrate, select ODO % Two-Point.
- 4. The first entry for the two-point calibration will be in mg/L! Wait at least 10 to 12 minutes for the solution to re-equilibrate and then type 0 and press Enter. Remove the sonde from the sodium sulfite and thoroughly rinse all exposed surfaces, especially the ROX membrane, with tap water. Rinse at least 4 times if using a filled calibration cup.
- 5. Enter the barometric pressure in mm/hg for the second calibration point and press the Enter key when the reading is stable. Remember that you must wait 15 minutes for the air to saturate in the cal cup or 5 minutes if the sonde is in saturated water.
- 6. Verify that the DO Gain found in the Advanced Cal Constants menu is between 0.7 and 1.4.

Note: If you believe that you have calibrated a ROX probe in error (especially the zero), then you can return the probe back to its original factory calibration by using the "uncal" command. With a PC, type the word 'uncal" instead of the barometric pressure and press Enter. If using a 650, at the point where you would type in the barometer, press the Esc and Enter keys simultaneously.

## RAPID PULSE DISSOLVED OXYGEN TROUBLESHOOTING

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The Rapid Pulse DO sensor used in all 6-Series instruments can be quickly tested when a problem is suspected. The three keys to proper operation are a good charge, good gain, and a successful probe warm-up test. When a problem is suspected you must determine if it is the probe, the sonde, or both. The steps below will take you through the actions necessary to determine the cause of the problem.

## HIGH DO CHARGE (ACCEPTABLE RANGE 25 TO 75)

The first step is to isolate the problem. Remove the DO probe from the sonde, dry the port with a Kimwipe, and confirm that the charge drops to a number that is in between -0.8 to +1.2. This number can fluctuate with the typical value being 0.2. A charge number greater than 1.2 would indicate that the DO port is contaminated and there is conductivity between the pins or that the circuit board is malfunctioning. Usually connector contamination is the cause. See the cleaning instructions at the end of this document for corrective action.

If the DO charge measured on the sonde (sensor removed) is within the acceptable range, then the problem is in the probe. Remove the electrolyte from the sensor and rinse the electrolyte well with DI water and dry with a Kimwipe. Now reinstall the sensor and re-test. If the charge remains outside the -0.8 to 1.2 range, then the probe has internal leakage or contamination. You can try soaking the probe per the cleaning instructions. If this is not successful and reconditioning with the 6035 Reconditioning Kit is not successful, then the probe must be replaced.

## DO WARM-UP FAILURE

The Rapid Pulse DO sensor, like other Clark DO probes, must warm up correctly for proper operation. Failure of the probe to pass the warm-up test is grounds for probe rejection. When conducting the high to low output test remember that the RS-232 Auto-Sleep function found in the sonde's Advanced Menu must be enabled. Also remember that the first two sonde readings taken at a four-second discrete sample rate can be discounted. The first two warm-up samples can be erratic when the circuitry is first turned on after a 60 second idle period.

## Examples:

```
Good Test DO in % -44.0 104.4 99.2 97.1 95.3 94.4 93.0 92.9 92.8 Bad Test DO in % - -99.9 -99.9 30.9 49.2 54.9 57.5 59.7 61.2 62.5 65.7
```

The first step in isolating the cause of a bad DO warm-up is to test the sonde without the DO probe installed. With the probe removed the DO charge should be between -0.8 and +1.2. If the charge is greater, then clean both the probe and sonde bulkhead connector per the port cleaning instructions.

After cleaning, retest the sonde and probe after running the DO probe for 15 minutes in discrete mode. Let the sonde sit idle for at least 60 seconds before beginning the high to low warm-up test. If you are unsuccessful with the cleaning procedure and the probe still fails the DO warm-up test, then the probe must be rejected.

## HIGH OR LOW DO GAIN (RANGE -0.7 TO +1.4)

The DO gain number is a multiplier. It is usually out of tolerance when the DO charge or the High/Low warm-up fails. Corrective actions involve the same procedures used for the charge and warm-up.

## HOW TO TEST FOR MEMBRANE PUNCTURES BEFORE DEPLOYMENT

The easiest way to test for membrane punctures is to use a 1,000 mL beaker of saturated tap water. After calibrating the sonde in a wet towel or saturated air, run the sonde in discrete mode in saturated air and record the value when stable. It will read less the 100% if the sonde was calibrated for Unattended Mode use.

Now place the instrument in the beaker and watch the DO%. If it reads 98.2 % in saturated air and the readings go up by one or two percent, then the membrane is leaking. The leak could be a pinhole in the Teflon or a loose-fitting o-ring. Replace both when a leak is found.

A small aquarium pump with air-stone is required to saturate the water in the beaker. Give the pump 30 minutes to saturate your tap water.

#### PORT AND PROBE CLEANING PROCEDURE

To remove contamination -- which can include grease, saltwater electrolyte, and calibration reagents -- the probes and sonde can be submerged in hot tap water which has had a dishwashing soap (with a degreaser) added. The temperature should start at 35 to 40 degrees C. The soapy water mix must be pumped into the open sonde ports and probe connectors to be effective. Using a lab rinse bottle will help flush out the ports. Soak the probe and affected sonde connectors for one hour. Rinse thoroughly with DI water after the soak, shake or blow out a majority of the water, and allow to air-dry overnight.

In cases where severe grease contamination is an issue, a degreaser agent sold in automotive stores can be used. Wear safety goggles and follow all the manufacturer's precautions when working with any cleaning products.

## ROX™ OPTICAL DISSOLVED OXYGEN TROUBLESHOOTING

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## POST-CALIBRATION IS LOW

Verify that the ROX is in saturated environment. If yes, then remove the wiper and the DO membrane with the probe vertical while following instructions in the user manual. Inspect for moisture under the membrane inside of the o-ringed area. If water is found clean on the optics or condensed moisture is seen on the inside of the membrane surface, then rinse with DI water and dry. Inspect for damage, reassemble and retest.

Note: Moisture under the membrane is usually a sign that a problem exists that will require membrane replacement.

#### DO READS LOW IMMEDIATELY AFTER A WIPE CYCLE

This is normal! The wiper passing over the membrane will drop the reading 1.5%. The reading will return to normal in less than 30 seconds. See Appendix M for more details.

## DO READS LOW AFTER EXPOSURE TO FORMAZIN OR SODIUM SULFITE

The wiper pad will absorb these chemicals and apply it to the membrane on every wipe cycle. Remove the wipers before exposure or change the pads after exposure.

## UNATTENDED MONITORING "MAKING GOOD DO MEASUREMENTS"

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It is recommended that you take the following steps prior to deployment:

- Confirm that the RS-232 and SDI-12 Auto Sleep functions are enabled. Both can be found in the Advanced Menu under Setup.
- Confirm that the sonde Report Menu has been set up correctly.
- Confirm that the date and time are correct.
- Program the "Unattended" study and confirm that the battery voltage and system memory are adequate for the length of the deployment.
- Start the sonde logging. Confirm that the sonde is logging data by going to the Status Menu and making sure that the sonde displays a "Logging Active" message.
- Confirm that at least one sample has been logged in memory. View using "Quick View File" in the sonde's File Menu.

#### FINAL PREPARATIONS

Note: These instructions were written primarily for YSI sondes using the Rapid Pulse DO sensor. Sondes fitted with optical DO probes do not have the warm-up and burn-in requirements listed in this document.

1. Once the sonde is logging it is strongly recommended that the instrument be placed in a saturated water bath prior to deployment. The data logged in a saturated water bath can be used as your predeployment DO calibration verification. The water bath can be made with a five-gallon pail, aquarium air pump, air stone, and tap water. Run the air pump for at least one hour prior to ensure that the water bath is completely saturated.

To determine the DO saturation level of your water you can divide the barometric pressure by 7.6.

Example: 755 mm/hg / 7.6 = 99.34%

The sonde should remain in the saturated bath for at least one hour. Verify that the oxygen sensor meets the accuracy requirements. If an adjustment is needed, recalibrate in the bath between your samples. Do not stop the "Unattended" logging run to recalibrate.

2. Once the pre-deployment DO data has been checked the sonde can be prepped for transport. The best way to move instruments is assembled with the sensor guard on the sonde. Removing a calibration cup in the field exposes the probes to potential damage. Wrapping the assembled sonde in a wet white towel will provide shock and vibration protection for the instrument while maintaining a saturated environment for the DO sensor. Moving the instruments in a large (vented) white igloo cooler wrapped in wet towels will provide the best protection. The cooler keeps the instruments together, is easy to lash down in a boat or truck, and maintains the instruments in a cooler saturated environment so pre-deployment DO data can continue to be collected.

- 3. At the recovery, the process is repeated. The sonde, when recovered, would be externally wiped clean (not the sensors) and then wrapped again in the wet towel and placed back in the white cooler. The DO data now collected by the sonde will be used as part of the post calibration record. By having this information recorded on the actual data set the QA will always be available for future reference.
- 4. The other sensors will be post-calibrated when the sonde is returned to the lab. These tests will determine what effect (if any) fouling might have had on the data. When the post-calibration is completed the sonde can be returned to the saturated water bath for a final verification.

## DISCRETE SAMPLING "MAKING GOOD DO MEASUREMENTS"

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Preparing a sonde for use in a spot sampling/profiling application is the same as the preparation used in unattended monitoring. The sonde must be tested and calibrated. In addition the field display (610/650 or PC) must be charged and made ready for the field.

Sampling can be tough on the equipment -- bounced around in small boats, temperature extremes, shock, and vibration can cause Teflon-membrane DO sensors to experience a calibration shift. The field technician needs to be aware of this and must be able to document and correct for any DO drift that might occur during the day.

Other parameters on the sonde -- temperature, conductivity, depth, turbidity, pH, and chlorophyll -- are generally not affected by the above issues and do not drift during normal sampling use. The best way to determine if DO sensor drift has occurred is to check the output under a controlled condition each time you get to a site. By following the steps below you can perform a pre- and post-check at each site and greatly increase your DO data quality.

Note: These instructions were written primarily for YSI sondes using the Rapid Pulse DO sensor. Sondes with optical DO probes do not have the warm-up and burn-in requirements.

- 1. Whenever possible prepare and calibrate your equipment the day before the expected field study. Make sure that the Auto Sleep RS-232 function has been disabled for sampling applications. This is found in the sonde's Advanced Menu under Setup.
- 2. Transfer of the sonde from the calibration cup to the sensor guard puts the sonde and sensors at risk. This is when most accidents to the oxygen sensor and membrane occur. To eliminate this risk take the sonde to the field with the sensor guard installed. Instead of using the cal cup to keep sensors in saturated air, wrap the sonde in a white towel that has been saturated in tap water. The toweled sonde can be stored in a cooler or pail. The wet towel is a perfect saturation chamber. In addition it can be wrapped around the sonde to minimize shock and vibration during transit.
- 3. When you arrive at the site, the sonde/display will be turned on and started sampling; allow it to warm up/run for approximately 5 minutes. Check the DO output; it should read saturation for your local barometric pressure plus or minus the instrument's tolerance of 2%. Record the sonde's reading in your field shield under pre-deployment DO check. If you have a reading that is out of tolerance, then you would simply recalibrate the DO in the wet towel and then record that sample.
- 4. Deploy the sonde. Remember to allow the sonde a few minutes to equilibrate to the water temperature before you take your reading. Once the data has been collected, rinse the sonde with tap water, shake dry and wrap again in the wet towel. After 5 minutes perform a DO post calibration. Again, the sonde should return to saturation, plus or minus the tolerance of 2%. If the probe fails to return to saturation, then the field samples should be retaken after a recalibration.
- 5. It is strongly recommended that you store this pre- and post-calibration data in the actual site data file if you are logging the information; or record in your log sheet if manually collecting. This assures anyone who looks at the records at a later time that the sonde was calibrated and working correctly. The addition of these QA steps adds very little time to the collection process and can actually save time when unexpected data is recorded.

## **COMPARING ROX & RAPID PULSE SENSORS**

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The instructions and QA recommendations on these pages will be used to set up and calibrate YSI 6-Series sondes for use in comparison measurements of optical and steady state dissolved oxygen sensors. This document is not a substitute for the YSI manual or 6150 support documentation.

## MINIMUM REQUIREMENTS

- All YSI 6-Series sondes used in this study must be updated to firmware version 3.06. 650 displays must be updated to version 1.18.
- EcoWatch PC software must be updated to version 3.18.
- All sondes must be tested, calibrated, and be in good working order to be part of this study.
- New software /firmware available at www.ysi.com/edownloads
- Review Appendix M ROX OPTICAL DO SENSOR in your manual to become familiar with the YSI ROX luminescent DO sensor before proceeding.
- Review the 6150 Description and Instructions for Use documents for probe installation information.

## DISCRETE SAMPLING

- 1. Install the 6150 ROX optical DO sensor into the spare optical port.
- 2. Connect the sonde to a PC. At the command prompt (#) type "MDO 1". This command will allow both the Rapid Pulse and the ROX DO sensors to sample simultaneously.

Note: MDO = Multiple Dissolved Oxygen. (1/ enable, 0/disable)

- 3. Enable the ROX sensor in the sonde's Sensor Menu.
- 4. Enable the % and mg/l outputs in the sonde's Report Menu.
- 5. Disable the sonde's Auto Sleep RS-232 setting found in the Advanced Menu under "Setup".
- 6. Recondition and prepare the Rapid Pulse DO sensor with a new membrane and confirm that all diagnostics -- charge, gain, and Warm-up -- meet specifications.
- 7. Run the sonde in "Discrete Run Mode" at a four-second rate for 15 minutes to burn in the Rapid Pulse sensor. Run in saturated air.
- 8. Calibrate the Rapid Pulse DO sensor in saturated air as you normally would. DO NOT CALIBRATE THE OPTICAL DO PROBE AT THIS TIME!
- 9. Calibrate all other sensors and prepare pre-deployment and QA records.
- 10. The YSI factory-generated ROX DO calibration coefficients will automatically be transferred into the sonde at initial sensor power up.
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#### CALIBRATION PROCEDURE FOR DISCRETE SAMPLING

The YSI Rapid Pulse and ROX DO sensors can be calibrated in saturated air or saturated water; both will give the same results when done correctly. For these deployments we recommend that you calibrate in saturated water. Saturated water will allow you to check the performance of the other sensors and eliminate any chance of temperature-related calibration errors. All that is required is a clean five-gallon bucket, a good quality aquarium air pump, and two air stones.

- 1. Fill pail to at least 75% capacity with room-temperature tap water.
- 2. Connect air stone to pump and sparge water for at least one hour before calibration.
- 3. Place the sonde in the water and run in "Discrete Mode" at a one second rate.
- 4. Wait 10 minutes to ensure temperature stability.

Note: The Rapid Pulse DO sensor should read very close to the saturated air reading of your previous air calibration. If it reads more than 2% higher than the reading in saturated air, then you could have a Rapid Pulse membrane problem. Check for leaks, punctures, or a loose-fitting membrane retainer o-ring before you calibrate.

5. Calibrate both the Rapid Pulse and ROX DO sensors. Record the barometric pressure at the time of calibration.

Note: Avoid having air bubbles collect on either membrane, during the calibration.

6. Barometric pressure \_\_\_\_\_mm/Hg

Note: pressure divided by 7.6 = calculated DO

Example: 760/7.6 = 100 %

- 7. ROX Output \_\_\_\_\_\_% \_\_\_\_mg/l +/- 1%
- 8. RP Output \_\_\_\_\_\_\_ % \_\_\_\_\_mg/l +/- 2%
- 9. Temperature \_\_\_\_\_\_\_\_ppt
- 10. DO NOT recalibrate the ROX DO sensor in the field!
- 11. Do your post-calibration when you return in the same saturated water bath.

Stability and agreement must occur in this controlled environment or good field measurements will not be made. The ROX and Rapid Pulse membranes must be kept in a saturated air or water during transportation to the field and in between readings.

Notes:

#### **UNATTENDED DEPLOYMENTS**

- 1. Install the 6150 ROX optical DO sensor into a spare optical sensor port.
- 2. Connect the sonde to a PC. At the command prompt # type "MDO 1". This command will allow both the Rapid Pulse and the ROX DO sensors to sample simultaneously.

Note: MDO = Multiple Dissolved Oxygen. (1/ enable, 0/disable)

- 3. Enable the ROX sensor in the sonde's Sensor Menu.
- 4. Enable the % and mg/l outputs in the sonde's Report Menu.
- 5. Enable the sonde's "Auto Sleep RS-232" and "Auto Sleep SDI-12" settings found in the Advanced Menu under Setup.
- 6. Confirm that the sonde's Rapid Pulse DO warm-up time is set to 60 seconds. This is found in the sonde's Advanced Sensor Menu.
- 7. Recondition and prepare the Rapid Pulse DO sensor with a new membrane and confirm that all diagnostics -- charge, gain, and Warm-up -- meet specifications.
- 8. Run the sonde in "Discrete Run Mode" at a four-second rate for 15 minutes to burn in the Rapid Pulse sensor. Run in saturated air.
- 9. Calibrate the Rapid Pulse DO sensor in saturated air as you normally would. DO NOT CALIBRATE THE OPTICAL DO PROBE AT THIS TIME!
- 10. Calibrate all other sensors and record pre-deployment and QA data.
- 11. The YSI factory-generated ROX DO calibration coefficients will automatically be transferred into the sonde at initial sensor power up.
- 12. Start the sonde logging for the "Unattended Study" and place in the saturated water bath described in the next steps.
- 13. Check logged data after one hour and record the time of your observation. If the Rapid Pulse DO sensor reads more than 2% higher than its saturated air calibration point, then you could have a defective or leaking membrane. Check for air bubbles, wrinkles, punctures, or a loose-fitting membrane retainer o-ring.

#### CALIBRATION PROCEDURE FOR UNATTENDED DEPLOYMENTS

The YSI Rapid Pulse and ROX DO sensors can be calibrated in saturated air or saturated water; both will give the same results when done correctly. For these deployments we recommend that you calibrate in saturated water. Saturated water will allow you to check the performance of the other sensors and eliminate any chance of temperature-related calibration errors. All that is required is a clean five-gallon bucket, a good quality aquarium air pump, and two air stones.

- 1. Fill pail to at least 75% capacity with room-temperature tap water.
- 2. Connect air stone to pump and sparge water for at least one hour before calibration.
- 3. Place the sonde in the saturated water bath and run in "Unattended Mode". This will be the file that the sonde is deployed with.
- 4. Wait 60 minutes to ensure temperature stability.

Note: The Rapid Pulse DO sensor should be reading very close to the saturated air reading of your previous air calibration. If it reads more than 2% higher than the reading in saturated air, then you could have a Rapid Pulse membrane problem. Check for leaks, punctures, or a loose-fitting membrane retainer o-ring before you calibrate.

5. Calibrate both the Rapid Pulse and ROX DO sensors. Record the barometric pressure at the time of calibration.

Note: avoid letting air bubbles collect on the membranes, during calibration.

6.	Barometric pressure	mm/Hg
	Note: pressure divided by 7.6	= calculated DO
	Example 760/7.6 = 100 %	

7.	ROX Output	%	_mg/l +/- 1%
8.	RP Output	%	_mg/l +/- 2%
0	Tr .	1 0 0 1' '	,

- 9. Temperature \_\_\_\_\_\_deg C, Salinity \_\_\_\_\_ppt
- 10. Allow the sonde to sample for at least 60 more minutes in this controlled environment. This data will serve as a pre-deployment DO verification test and will remain part of the data set.
- 11. When the sonde is recovered, do NOT stop the "Unattended" study in the field! Return the sonde to the calibration lab and use the saturated water bath for the DO post-calibration. This data will also be part of the "Unattended" data file.
- 12. Record the barometric pressure and other parameters at recovery.

13. Barometric pressure	mm/Hg	
14. ROX Output	%	mg/l +/- 1%
15. RP Output	%	mg/l +/- 2%
16. Temperature	deg C, Salinity	ppt

Notes:



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