APPENDIX M ROX OPTICAL DO SENSOR

This appendix is in the format of "frequently asked questions", is designed to allow users to optimize the performance and the trouble-shooting of problems for your YSI 6150 ROX Optical dissolved oxygen probe by supplementing the discussion of optical dissolved oxygen measurement that is provided in the other sections of this manual (**Getting Started, Basic Operation, Principles of Operation, and Maintenance**).

How does the ROX Optical DO Sensor work?

In general, optical dissolved oxygen sensors from a variety of manufacturers are based on the well-documented principle that dissolved oxygen quenches both the intensity and the lifetime of the luminescence associated with carefully-chosen chemical dyes. The 6150 sensor operates by shining a blue light of the proper wavelength on this luminescent dye which is immobilized in a matrix and formed into a disk about 0.5 inches in diameter. This dye-containing disk will be evident on inspection of the sensor face. The blue light causes the immobilized dye to luminesce and the lifetime of this dye luminescence is measured via a photodiode in the probe. To increase the accuracy and stability of the technique, the dye is also irradiated with red light during part of the measurement cycle to act as a reference in the determination of the luminescence lifetime.

When there is no oxygen present, the lifetime of the signal is maximal; as oxygen is introduced to the membrane surface of the sensor, the lifetime becomes shorter. Thus, the lifetime of the luminescence is inversely proportional to the amount of oxygen present and the relationship between the oxygen pressure outside the sensor and the lifetime can be quantified by the Stern-Volmer equation. For most lifetime-based optical DO sensors (including the YSI 6150), this Stern-Volmer relationship (((Tzero/T) – 1)) versus O_2 pressure) is not strictly linear (particularly at higher oxygen pressures) and the data must be processed using analysis by polynomial non-linear regression rather than the simple linear regression used for most polarographic oxygen sensors. Fortunately, the non-linearity does not change significantly with time so that, as long as each sensor is characterized with regard to its response to changing oxygen pressure, the curvature in the relationship does not affect the ability of the sensor to accurately measure oxygen for an extended period of time.

Each YSI sensor module (the assembly which is attached to the face of the probe by three screws) is factory-calibrated over a range of 0-100 percent oxygen to quantify the relationship of its luminescence lifetime as a function of oxygen pressure. The Stern-Volmer parameters from this data are then fit to a third order regression equation ($ax^3 + bx^2 + cx$) and values of a, b, and c determined. These coefficients, along with the luminescence lifetime at zero oxygen pressure (Tzero), are provided to the user in coded form with each sensor membrane module or probe/sensor module combination. If you install a replacement sensor membrane assembly (YSI 6155) on your existing probe, you will be required to enter these coded constants into the sonde as described in the instructions which come with the 6155 prior to the use of the sensor. If you have purchased a probe/membrane combination, i.e. a new 6150 Optical DO sensor, the constants are already stored in your probe and will automatically be transferred to your sonde when the sensor is installed.

What are the key advantages of the ROX sensor over membrane-covered polarographic sensors?

The ROX dissolved oxygen sensor has three key advantages over the YSI Rapid Pulse sensor:

1. The set-up and maintenance of the ROX sensor is much easier since there is no membrane or electrolyte to be changed by the user.

2. Testing indicates that the ROX sensor is significantly less susceptible to field drift.

3. The ROX sensor is automatically wiped during field studies to eliminate effects of fouling. The Rapid Pulse sensor can be wiped, but only if a 6600EDS V2-2 sonde is used.

In addition, the ROX DO sensor has NO flow dependence, giving it a large advantage over systems which utilize steady-state membrane covered polarographic oxygen sensors. This advantage is minimal relative to the YSI Rapid Pulse technology which is effectively flow-independent in monitoring studies and shows only minimal flow dependence (ca. 3%) in continuous-on sampling studies.

What is the warranty period for the ROX DO Sensor?

The probe is warranted for 2 years and the sensor membrane assembly is warranted for 1 year. See **Section 9** of this manual for details of the YSI warranty policy.

What sondes can use the ROX DO Sensor?

The ROX Sensor can be used with any existing or new YSI 6-series sonde which contains an optical port. Currently available sondes which support the use of the ROX sensor are 600 OMS V2-1, 6820V2-1, 6820V2-2, 6920V2-1, 6920V2-2, 6600V2-2, 6600EDS V2-2, and 6600V2-4 which were manufactured after June 1999. If you have an older sonde with an optical port and want to determine whether it will support the use of the ROX sensor, contact YSI Technical Support. They will be able to evaluate the compatibility of your sonde with the ROX sensor by giving you a few simple software commands to issue at the "#" sign of your sonde.

If I want to use the new ROX DO Sensor with my existing sonde, what else do I have to do?

In order to use the ROX technology on an existing YSI 6-series sonde, you must do the following:

- Upgrade the firmware in your sonde from the YSI Website (ysi.com) to Version 3.00 or higher.
- Upgrade your EcoWatch for Windows PC software from the YSI Website to Version 3.18 or higher.
- Upgrade the firmware in your 650 MDS Display/Logger from the YSI Website to Version 1.18 or higher.

Note if your sonde was manufactured after June 1999, there is no need to return your existing sonde to YSI. After carrying out the software upgrades described in detail in the instruction sheet shipped with the 6150 Probe, you will be able to simply install the ROX sensor in your optical port and begin taking readings. If your sonde was manufactured prior to June 1999, then you should contact YSI Technical Support to determine if it is compatible with the ROX sensor

Can I measure dissolved oxygen with both ROX Optical and Rapid Pulse Polarographic sensors in the same sonde?

No. If you try to activate both the ROX and Rapid Pulse sensors in the sonde **Sensor** menu, only the last sensor activated will be functional.

What are the key differences between the ROX Optical and Rapid Pulse DO sensors?

The key differences between the sensors are as follows:

- The 6150 optical sensor has no flow dependence even in continuous operation during spot sampling studies while the 6562 polarographic sensor can exhibit up to 3% flow dependence.

- For the 6562 polarographic sensor, users must calibrate the sensor by different methods for sampling (Discrete Sample) and monitoring (Unattended Sample) by activating or deactivating the "Autosleep RS-232" feature found in the **Advanced|Setup** menu. The calibration of the 6150 optical sensor is the same for both sampling and monitoring applications and no changes in the "Autosleep RS-232" feature are required.

- It is possible to calibrate the 6562 polarographic sensor ONLY in oxygen-containing media at a single point. The 6150 optical sensor can be calibrated at either a single point in oxygen-containing media OR at two points, a zero-oxygen medium and an oxygen-containing medium. This allows users to maximize the accuracy of the 6150 sensor at low oxygen levels if they feel it to be necessary.

Is the ROX DO Sensor calibrated at YSI prior to shipment to the customer?

Yes. Factory calibration is required because, unlike all other sensors for YSI 6-series sondes, the response of the ROX Optical DO sensor is not linear relative to the species being measured. This non-linearity requires that the sensor be factory-calibrated at a number of oxygen values and the data fit to a third-order regression. The three constants and the sensor value at zero dissolved oxygen which define this regression analysis are automatically stored in the sensor at the time of factory-calibration. It is important to note that these constants are a function of the sensor membrane installed on the 6150 probe and NOT a function of the probe, i.e., the constants reflect the characteristics of the sensor membrane and NOT the probe. When a 6150 probe is purchased from YSI, it already has a sensor membrane installed and the constants of that membrane are transferred automatically to the sonde PCB when the sensor is run for the first time. After transfer, the constants can be viewed by accessing the **Advanced|Cal Constants** menu as described below in a separate question/answer.

Note, however, that, even with the factory calibration, the user still should perform a calibration after receipt of the sensor as described below in order to assure that the typical accuracy specification is met.

How do I calibrate my ROX DO sensor?

The ROX DO sensor is typically calibrated in the same way as the YSI Rapid Pulse sensor – a single point calibration in either air-saturated water, water saturated air, or a solution whose oxygen content has been determined by Winkler titration. Like the Rapid Pulse sensor, the ROX sensor can be calibrated in either the air-saturation mode (requiring a local barometer value input) or in concentration mode (requiring an input of oxygen content in mg/L).

It is also possible to carry out a 2-point calibration of the ROX sensor with the other point being zero oxygen content. NOTE, HOWEVER, THAT YSI DOES NOT RECOMMEND THE 2-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 TO BE ABLE TO GENERATE A MEDIUM WHICH IS TRULY FREE OF OXYGEN.

The calibration methods and recommendations for the ROX sensor are provided in detail in Section 2 of this manual and in the instruction sheet which you received with the ROX probe. The key is to remember that the single point calibration option will provide data of acceptable accuracy for the vast majority of users and it should be used in most cases.

Should I calibrate my ROX sensor in water-saturated air or air-saturated water?

Studies at YSI have shown that the sensor shows effectively the same reading in air-saturated water and water-saturated air. Thus, if the calibration is carried out properly, either medium can be used with confidence. The advantage of air-saturated water is the quick equilibration of the sonde sensors relative to thermal and humidity factors; the disadvantages are that you will require an aquarium pump, an air-stone, a large vessel to immerse the sonde in the air-saturated water, and you must sparge the water for at least 1 hour prior to calibration to assure that it is air-saturated. The advantage of using water-saturated air is that the calibration can be carried out in the cup supplied with the sonde and it can be done somewhat faster than the air-saturated water method. Note, however, that if you use water-saturated air as the calibration method, you should still wait at least 15 minutes after placing the sensors in the calibration vessel to assure thermal equilibration between the temperature and ROX DO sensors.

The bottom line is that either method will give good results as long as the considerations above are followed. The air-saturated water method may be slightly preferable if you have the equipment because of the assured equilibration, but the advantage is slight.

If I want to perform a 2-point calibration, how do I generate a zero-oxygen medium?

Two methods are generally used to provide a zero-oxygen environment:

1. Place the ROX sensor in a vessel which is filled with a flowing inert gas such as nitrogen gas

2. Place the ROX sensor in an aqueous solution of sodium sulfite at a concentration of approximately 2 g/L)

The following qualifiers apply to the zero point calibration methods:

- If you use nitrogen gas for the zero point calibration, you should make certain that the vessel you use has a SMALL exit port to prevent back diffusion of air and that you have completely purged the vessel before confirming the calibration.

- If you use sodium sulfite solution for the zero point calibration, you should make up the solution at least 2 hours prior to use and keep it sealed in a bottle which does not allow diffusion of oxygen through the sides of the container. You should also transfer the sodium sulfite solution rapidly from its container to the sonde calibration cup, fill the cup as full as possible with solution to minimize head space, and seal the calibration cup to the sonde to prevent diffusion of air into the vessel.

Whichever method you use, it is very important that you wait at least 10-12 minutes and until the readings are stable for at least 2 minutes before confirming the zero point calibration entry.

If I make a mistake in a 2-point calibration, will my sensor always be inaccurate?

No. You can either perform a new 2-point calibration with better control of the conditions or you can return to the factory default calibration by using the “uncal” command as described below.

- Select **Calibrate** from the **Main** menu, then **Optic T Dissolved Oxy**, and then run any of the ODOsat % or ODO mg/L options.

- When prompted for the input of a barometer or a concentration value, type the word “uncal” and press **Enter** if your sonde is attached to a computer. If your sonde is attached to a 650 MDS, when prompted for input, hold down the **Enter** key and press the **Esc** key.

Either procedure will reset your 6150 calibration constants to the previously input values associated with your sensor membrane.

How often should I calibrate my ROX sensor?

Our experience is that, even though the drift of the ROX sensor is minimal, it is prudent to calibrate the sensor in air-saturated water or water-saturated air prior to each deployment or field sampling study. The calibration takes very little time and should be carried out to (a) maintain the best possible accuracy for the sensor and (b) to assure that the sensor is working properly prior to proceeding to the field.

I notice that, during Discrete Sample studies, there is a lag between when I begin the sampling and when ROX DO readings actually appear on the display. What is causing this effect?

The lag between starting the study and actually seeing data is due to the fact that the readings of all optical sensors, including ROX DO, are frozen during the sensor wiping sequence. If the sonde has not been used for more than a minute, it will be in the “sleep” mode and, under these conditions, all optical sensors will automatically wipe the first time a Discrete Sample command is issued. The extent of the lag varies depending on how many optical sensors are present in the sonde and will be about 15 seconds per optical sensor. Thus, lag will be about 15 seconds for a sonde with two optical sensors and about 1 minute for a sonde with four optical sensors.

How does the ROX sensor deal with fouling in field studies?

The ROX sensor has a wiper similar to those used on all YSI optical sensors (turbidity, chlorophyll, rhodamine WT, and blue-green algae) which removes fouling from the sensor membrane. The wiper activates just prior to each measurement point in a long term study. In addition, the wiper can be activated manually from a PC keyboard or from a 650 MDS handheld logger to remove bubbles from the sensor membrane prior to spot sampling measurements.

What color wiper should be used with the ROX DO sensor?

Because the probe is digital, you can use any color wipers with the 6150 ROX sensor. In fact, if you are using the ROX sensor in the “T” port of a 6600EDS V2-2 sonde, you should use the white EDS wiper for the application. However, just to be consistent, YSI recommends that you use black wipers for the ROX sensor like those supplied with the probe for all 7non-EDS applications. The wiper is a wear item and a spare is provided with each probe along with a 0.05” hex key to loosen/tighten the wiper set screw. Black wiper packs, YSI 6625, can be ordered from YSI Technical Support or Customer Service. Alternatively, users who choose to change only the wiper pad can purchase the YSI 6144 Optical Wiper Pad Kit.

What is the response time of the ROX DO sensor?

On transfer to field water, typically the ROX sensor will reach 90% of its final value in less than 30 seconds – slightly faster than the YSI Rapid Pulse sensor. Note, however, that the response time of the ROX probes varies slightly from sensor module to sensor module.

How often should I change my ROX sensor membrane assembly?

YSI recommends that you change your membrane assembly every 12 months. The membrane assembly is simple to install and can be purchased from YSI Technical Support as the YSI 6155 Optical DO Sensor Replacement Kit.

Since the sensor is non-linear and the non-linearity is not identical for all sensors, how do I take this factor into account when I replace the sensor membrane assembly?

As described in the instruction sheet for the 6155 Optical DO Sensor Replacement Kit, you will be required to enter coded constants which are provided with the new membrane assembly into the sonde software. The process takes only a few minutes as briefly described below.

Locate the Calibration Code Label which is attached at the end of the instruction sheet provided and note the five numbers which are listed as K1 through K4 and C on the sticker. These five numbers contain the calibration code for this particular sensor membrane.

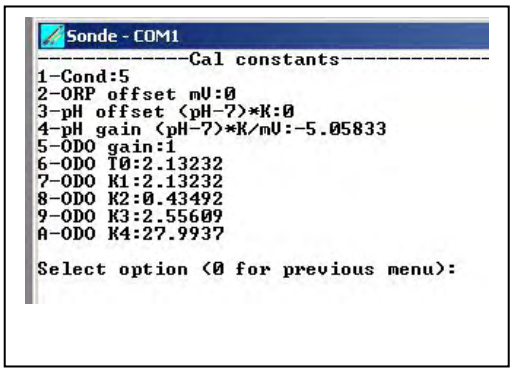
From the **Main** sonde menu, select **Calibrate|Optic T- Dissolved Oxy a**nd then select the "**3-Enter Cal Sheet**" entry. You will be prompted to enter the K1 value from the sticker. After carefully entering K1, press **Enter** to confirm the entry and then you will be prompted to enter the value of the next number. Values of K2-K4 and C should be entered in similar fashion to K1, pressing **Enter** to confirm each entry. If no error message is encountered after confirming the C value, then you have made all entries correctly and the proper constants will be transferred automatically into the sensor system for correct calculation of dissolved oxygen. If an error message is displayed after entry of C, then you have made an error (or errors) in entering the code. Following the error message you will be returned to the **Calibrate** menu from which you should again enter the K1-K4 and C numbers until the entries have been made correctly

Note that it is good idea to place the instruction sheet which contains the calibration information IN A SAFE PLACE in the unlikely event that you need to reenter it later.

How can I be sure that my calibration coefficients have been entered correctly and confirm that they are being read correctly by the sonde software?

One of the five constants (C) which you entered in coded form is a check sum value associated with the values of K1-K4 and will prevent acceptance of the constants if typographical errors are made in their entry by the user. Thus, it is not possible to make an incorrect entry of the coded constants.

After entry of the coded constants in the **Calibrate** menu, the actual constants associated with the regression fit of your particular membrane can be viewed by accessing the **Advanced|Cal Constants** menu as shown below – the regression constants are ODO K2-ODO K4 and the value at zero oxygen is K1 as shown below. Note that these constants will not appear at this location until the sonde has been run for the first time with the 6150 ROX sensor installed.



How should I store my ROX sensor when it is not in use?

When the 6150 sensor is not in field use, it MUST BE STORED IN A MOIST ENVIRONMENT, i.e., either in water or in water-saturated air with storage in water being preferable. If the sensor membrane is allowed to dry out by exposure to ambient air, it is likely to drift slightly at the beginning of your next deployment unless it is rehydrated. Thus, to make the use of the sensor as simple as possible, remember to store it WET whenever possible. The easiest storage method is to use the protective plastic cap (and enclosed sponge) which was on the probe at receipt. If you have retained this cap/sponge, then simply soak the sponge in water and replace the cap on the probe tip. Inspect the sponge every 30 days to make sure it is still moist. Alternatively, you can remove the probe from the sonde and place it directly in water (making sure that the water does not evaporate over time or leave the probe in the sonde and make certain that the calibration cup has an atmosphere which is water-saturated by placing approximately ½ inch of water in the bottom of the cup and then sealing it snugly to the sonde.

If I have inadvertently allowed my ROX sensor membrane to dry out for several days, is it ruined?

No. The sensor membrane can easily be rehydrated using the following basic procedure:

> Place approximately 400 mL of water in a 600 mL beaker or other similar glass vessel – do NOT use plastic vessels – and heat the water on a thermostatted hotplate or in an oven so that a consistent temperature of 50+/- 5 C is realized. Place the probe tip containing the sensor membrane in the warm water and leave it at the elevated temperature for approximately 24 hours. Cover the vessel if possible to minimize evaporation. After rehydration is complete, store the probe in either water or water-saturated air prior to calibration and deployment.

Once the rehydration has been performed, the sensor should be returned to its original performance specification.

Can I use alcohol or other organic solvents to clean my sensor membrane?

Absolutely NOT**.** Alcohol will dissolve the outer paint layer of the membrane assembly and other organic solvents will likely dissolve the dye itself. Under NO circumstances should you use organic solvents to clean your sensor membrane. The best method of cleaning the membrane is just to gently wipe away any fouling with a piece of lens cleaning tissue which has been moistened with water only.

I have a few pinholes in the outer paint layer of my sensor membrane so that I can see small spots of light from the probe. Will this light leakage affect the performance of my ROX sensor?

No. A few small pinholes in the outer paint layer will have no discernable effect on sensor performance. However, if there are a lot of holes or if they are relatively large (1 mm or greater in diameter), then there might be a minor compromise of sensor accuracy relative to the factory calibration. Under these latter conditions, YSI would recommend replacing the membrane assembly.

Is there any effect of ambient light on the readings from the ROX DO sensor?

Under all normal operating conditions, the ROX sensor is unaffected by ambient light, even if there are a few minor scrapes or pinholes in the protective paint layer of the sensor membrane. These conditions include exposure to room lighting during calibration and set-up and deployments in all clarities of water as long as the ROX sensor is pointed down or on its side. Our studies indicate that only if the ROX sensor is exposed directly to bright sunlight with the probe pointed upward will the readings be affected significantly. Thus, there is no reason to worry about the effect of ambient light unless your deployment sight is very unusual and requires that the sensor be pointed directly upward in shallow water with no probe

guard. It is also important to note that if the membrane is exposed to bright sunlight for extended periods, i.e., more than 60 minutes, the life of the membrane may be reduced due to photo-bleaching of the dye. Thus, make certain that you protect the membrane from bright sunlight during any storage and/or transportation to your deployment site.

Is there any effect of wiping the sensor membrane on the ROX DO readings?

Yes, there is a minor effect. Typically the optical DO reading will drop by about 1.5 % immediately after a wipe which is activated during Discrete Sample studies. After 12 seconds, the reading has typically returned to within 0.7 % of the final reading. It takes about 30 seconds for complete recovery of the ROX optical DO reading after the sensor membrane is wiped. This effect is due to the physical contact of the wiper pad with the membrane since it is not observed when the wiper assembly is removed and only the wiper shaft turns when a wipe command is issued.

Will this wiping effect cause inaccuracy in my Unattended study readings with the ROX DO sensor since I will be calibrating without wiping, but logging readings after the ROX sensor has been wiped?

The error will be minor in any case, but will vary depending on what other optical probes you have installed in your sonde. Since the effect is physical as described in the previous question, the longer it takes to log a reading after the ROX sensor has wiped, the less error there will be relative to the calibration. Under the worst case scenario, the user has ONLY a ROX sensor present with an ODO Time Constant setting of 12 seconds as set in **Advanced|Data Filter**. This set-up means that readings will be logged internally 12 seconds after the wipe has terminated and, under these conditions, there will be a typical error of 0.7 % of the reading – within the sensor specification of 1 % of the reading. This error will be further reduced if other optical probes are present since the time for them to be wiped plus their time constants will further increase the time between the wiping of the ROX sensor and the time a point is logged to internal memory. (Note that the ROX sensor is ALWAYS WIPED FIRST, no matter what optical port it is installed in as long as you have Version 3.04 or later of firmware installed in your sonde.) The extent of the error would thus be greatest with a 6920V2-1 running ROX DO only, less with a 6600-type or 6920V2-2 sonde running ROX DO and turbidity, still less with a 6600-type sonde running ROX DO and chlorophyll or BGA (since the time constant these other optical sensors is 24 seconds) and least for a 6600V2-4 sonde containing ROX DO and 3 other optical sensors. Typical errors in Unattended readings from this wiping effect are 0.7% for a sonde with only a ROX sensor and 0.3% for a sonde with a ROX and one other optical sensor. There is no discernible error for a 6600V2-4 sonde with a ROX and three other optical sensors.

Can I reduce the small error from the wiping effect?

Yes. By increasing the Time Constant value for ODO in the **Advanced|Data Filter** menu, the error will definitely be reduced. For example, with a ROX DO probe as its only optical sensor, typically your error would be reduced from 0.7% to 0.3% by increasing the ODO Time Constant from 12 to 24 seconds. Naturally, however, this will have an adverse effect on the battery life of your sonde for deployments so you should balance this factor relative to the small DO error which will be observed if you leave the Time Constant at 12 seconds.

I will be installing a ROX sensor in my 6600EDS V2-2 sonde along with another optical probe. Does it matter in which optical port I place the ROX sensor?

Yes. You should always install the ROX probe in the center (T) port of the 6600EDS V2-2 sonde and substitute the special EDS wiper for the black wiper that came with the ROX probe. The other optical probe should be installed in the outer optical port (c) with a standard wiper assembly. This installation

protocol will prevent the stiff EDS auxiliary brush which cleans the pH or pH/ORP sensor from abrading the outer paint layer of the ROX membrane. There should be no problem with the EDS wiper parking correctly even though it is white (rather than the standard black wiper supplied with the probe) since an internal Hall Effect device controls the parking in the ROX sensor.

How can I tell if my ROX sensor is not functioning is not functioning properly?

There are two factors which would indicate that your sensor is not operating up to specifications. The first is that you are observing software errors when you attempt to calibrate the ROX probe. The second is that your data (either in Discrete Sample or Unattended Sample studies becomes jumpier than normal. In addition, if your membrane surface has suffered major damage as evidenced by loss of more than 10% of the outer paint layer, then, even if the readings are stable and you do not see calibration errors, it is probably time to change your membrane assembly.

What should I do if I don't think that my ROX sensor is functioning properly?

The first step is to remove the membrane assembly from the probe face as shown in Section 2.10.2 of this manual and make certain that there is no moisture present under the membrane assembly. If moisture is present, gently remove it with lens cleaning tissue and, if possible, by a compressed air stream. Make certain that the O-ring which seals the membrane assembly to the probe face is in the groove and undamaged. Then replace the membrane assembly and evaluate the probe performance. If the performance has not improved, consult YSI Technical Support for advice on how to proceed next.