Appendix E: Calibration Procedures for Fluorescent Dissolved Organic Matter (fDOM) Sensor with KOR v2.0

Fluorescent Dissolved Organic Matter (fDOM) is recommended to be an optional SWMP-supported parameter that may be collected and submitted to the CDMO but not a required parameter.

Notes:

1. Reserves might wish to quantify colored dissolved organic matter (CDOM) in order to determine the amount of light which is absorbed by stained water and thus is not available for photosynthetic processes. In most cases, fDOM (Fluorescent Dissolved Organic Matter) can be used as a surrogate for CDOM. The EXO fDOM sensor detects the fluorescent component of DOM (Dissolved Organic Matter) when exposed to near-ultraviolet (UV) light.

2. For dissolved organic matter measurement, the fDOM sensor allows calibration for two units of measure: RFU and QSU. RFU (Relative Fluorescence Units) is used to calibrate output relative to a standard such as quinine sulfate, thereby standardizing all sensors relative to each other and allowing for post-calibration at that same standard calibration value. A surrogate for fDOM is quinine sulfate, which, in acid solution, fluoresces similarly to dissolved organic matter. The units of fDOM are quinine sulfate units (QSUs) where 1 QSU = 1 ppb quinine sulfate and thus quinine sulfate is really an indirect surrogate for the desired CDOM parameter.

3. **Fluorescently dissolved organic matter data would be submitted to the CDMO as a SWMP supported parameter must be reported in RFU and QSU**, but for the purpose of these Standard Operating Procedures a two-point calibration for RFU will be the only method discussed. As fDOM extraction methods can vary, calibration in quinine sulfate will be discussed here.

4. As with all optical probes, makes sure the optics are clean before calibration.

5. Since quinine sulfate is a sensitive reagent, if possible, have a dedicated calibration cup for quinine sulfate use-only to avoid potential contamination of your zero (deionized water) standard and contamination of the quinine sulfate calibration 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. **CAUTION: UV LIGHT Do not look directly at the end of the sensor when it is active.**

Instructions for making the quinine sulfate solution for calibration of the Fluorescent Dissolved Organic Matter sensor:

1. Purchase solid quinine sulfate dihydrate (CAS# 6119-70-6) with a high purity (>99%).

2. Purchase 0.1 N (0.05 M) sulfuric acid (CAS# 7664-93-3), to avoid the hazards of diluting concentrated sulfuric acid to make this reagent.

3. Weigh 0.100 g of solid quinine sulfate dihydrate and quantitatively transfer the solid to a 100-mL volumetric flask.

4. Dissolve the solid in about 50 mL of 0.05 M (0.1 N) sulfuric acid (H2SO4), dilute the solution to the mark of the volumetric flask with additional 0.05 M sulfuric acid, and mix well by repeated inversion. This solution is 1000 ppm in quinine sulfate (0.1%).

5. Store the concentrated standard solution created in the above step in a darkened glass bottle in a refrigerator to retard decomposition.

6. Transfer 0.3 mL of the 1000 ppm solution to a 1000 mL volumetric flask and then fill the flask to the top graduation with 0.05 M sulfuric acid. Mix well to obtain a solution of 300 µg/L (300 QSU or 100 RFU). Transfer this solution to a glass bottle that you can easily pipette from.

7. The dilute standard prepared in the previous step should be used within 5 days of preparation and should be discarded immediately after exposure to EXO's metal components. Used and/or excess standard should be discarded in accordance with local regulations or can be saved for rinse only.

Two-point calibration for Fluorescent Dissolved Organic Matter in RFU

1. In KOR, select CALIBRATION, then CALIBRATE.

2. Activate the drop-down for the fDOM.

3. **If you want to report both units of measure, RFU and QSU, you must calibrate each unit separately (RFU and QSU) to completely calibrate this parameter. So, if you are reporting fDOM in both units (RFU & QSU) you will be calibrating each fDOM probe twice.** Units of measure to be reported can be turned on/off under FILE > SETTINGS > fDOM.

4. If calibrating both units begin with RFU and press CALIBRATE.

5. Pour deionized water into a clean (preferably calibration-dedicated) calibration cup, and place sonde with a clean calibration-dedicated guard **with the bottom installed** into the calibration cup and tighten the collar. Remove the central wiper from the EXO2 sonde before proceeding to the next step.

6. Gently invert the sonde several times to rinse all of the sensors, bulkhead and connectors with the deionized water. Repeat this step twice more with deionized water rinse.

7. Fill a clean EXO calibration cup (preferably dedicated for use for 0 calibrations of chl & turbidity) with the appropriate amount of deionized water, pouring along the side to minimize bubbles.

8. Slowly lower the guard and sensors into the calibration cup until seated. Lift the sonde (holding the cal cup on, or screw the cal cup on), and at an angle, gently tap the bottom of the calibration cup against your worktop to dislodge any bubbles from the sensor optics.

9. Enter your first Standard Value of 0 (deionized water) and press enter.

10. Once the temperature has stabilized and the readings have stabilized, the Data Stability should change from red UNSTABLE to green STABLE. At this point, press APPLY.

11. Remove the sonde from the calibration cup.

12. Pour a small amount of quinine sulfate standard into a clean (preferably dedicated to use with quinine sulfate standard) calibration cup (enough to coat sensors well), and place sonde into the calibration cup and tighten the collar.

13. Gently invert the sonde several times to coat all the sensors, bulkhead and connectors with the standard. Repeat this step twice more with fresh standard rinse.

14. Fill the calibration cup with fresh quinine sulfate 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 ADD ANOTHER CAL POINT.

16. Once the temperature of the quinine sulfate standard has stabilized, use the table below to find the value (in RFU or QSU) for quinine sulfate standard at that temperature, and enter that value into the Standard Value box and press enter.

17. When the Data Stability is STABLE, press APPLY.

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. Refer to YSI's EXO User Manual for further detail.

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 quinine sulfate standard for RINSE standard for the next set of calibrations. Remember that the quinine sulfate standard must be used within 24 hours of preparation.

21. If calibrating QSU, repeat steps 1-17 above using the QSU 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 RFU and QSU, 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 quinine sulfate standard (pt 2) for RFU. Then, leaving the instrument in the quinine sulfate standard, you can do a 2 point-calibration for QSU using the quinine sulfate standard as your 1st point and deionized water (zero) as your 2nd point. This is especially helpful if you have multiple fDOM probes to calibrate and are doing a batch calibration on one instrument.

Effect of temperature on fluorescence

The intensity of the fluorescence of many dyes shows an inverse relationship with temperature. This effect must be accounted for when calibrating the EXO fDOM sensor with quinine sulfate solution. Enter the QSU or RFU value from the table below that corresponds to the temperature of the standard.

Temp (°C)	RFU	QSU	Temp (°C)	RFU	QSU
30	96.4	289.2	18	101.8	305.4
28	97.3	291.9	16	102.7	308.1
26	98.2	294.6	14	103.6	310.8
24	99.1	297.3	12	104.6	313.8
22	100	300	10	105.5	316.5
20	100.9	302.7	8	106.4	319.2