Appendix D: Calibration Procedures for Total Algae (Chl and BGA) Sensor with KOR v2.0

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 relative 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. **Chlorophyll fluorescence data submitted to the CDMO as a SWMP supported parameter must be reported in RFU**, so for the purpose of these Standard Operating Procedures a two-point calibration for RFU 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, make 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; http://www.brightdyes.com/
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.

2. 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 (preferably but not required) 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 standards 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 RFU value that corresponds to the temperature of the calibration standard of 0.625 mg/L rhodamine.

Temperature of standard solution (°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 RFU - This calibration procedure zeroes the fluorescence sensor and uses the default sensitivity of the sensor to relatively calculate chlorophyll concentration in RFU.

1. In KOR, select CALIBRATION, then CALIBRATE.

2. Activate the drop-down for the Chlorophyll and BGA-PC sensor (or -PE depending on which sensor you have).

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

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

5. Pour filtered water into a clean (preferably calibration-dedicated) calibration cup, and place sonde with clean calibration-dedicated guard with the bottom installed in to the calibration cup and tighten the collar.

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

7. Fill a clean EXO calibration cup (preferably dedicated for use for 0 calibrations of chl & turbidity) with the appropriate amount of filtered 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 (filtered water – distilled or deionized) 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 rhodamine standard into a clean (preferably dedicated to use with rhodamine standard) calibration cup (enough to coat sensors well), and place sonde in to the calibration cup and tighten the collar.

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 ADD ANOTHER CAL POINT.

16. Once the temperature of the rhodamine standard has stabilized, use the table above to find the value (in RFU or µg/L) for rhodamine 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 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 µg/L, repeat steps 1-17 above using the µg/L 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 µg/L, 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 µg/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.