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APPLICATION NOTE NO. 62 **Revised May 2011**

Calculating Calibration Coefficients for WET Labs ECO-AFL and ECO-FL Fluorometer, ECO-NTU Turbidity Meter, and ECO-FL-NTU Fluorometer/Turbidity Meter (voltage or RS-232 output sensors)

Note: Procedures in this application note are valid for Seasave V7 and SBE Data Processing version 7.21d and later.

This Application Note applies to the following WET Labs fluorometers and turbidity meters:
- ECO-AFL fluorometer - older model, not in current production
- ECO-FL fluorometer - FL, FLD, FL(RT), FL(RT)D, FLS, FLB, or FLSB
- ECO-NTU turbidity meter – NTU(RT), NTU(RT)D, or NTUS
- ECO-FL-NTU - combines a fluorometer and turbidity meter, and requires two channels in your CTD

Note:
The ECO-FL is also available as part of the WET Labs Triplet, an RS-232 output instrument that incorporates three WET Labs ECO sensors. The Triplet can include up to three ECO-FL or ECO-NTU sensors (or one of those sensors in combination with other ECO sensors). Set up each RS-232 output channel with the coefficients from the calibration sheet for the sensor on that channel.

Fluorometer Calibration Coefficients

The fluorometer has a response that is linear over the measurement range provided.

- **ECO-AFL** (no longer in production) - Measurement range is approximately 0.02 - 100 µg/l or 0.04 - 200 µg/l.

- **ECO-FL** – Measurement range for the analog output version of this single-channel sensor can be adjusted with the analog scaling value. To change the range, connect the ECO-FL directly to the computer and use WET Labs' ECOView Host software to enter a new analog scaling value.

Analog Scaling Value / Range (enter in ECOView Host)	**1**	**2**	**4 (factory default)**
Chlorophyll *a*: Nominal Range (µg/l)	0 - 30	0 - 50	0 - 125
Rhodamine: Nominal Range (ppb)	0 - 55	0 - 110	0 - 230
Phycocyanin: Nominal Range (ppb)	0 – 55	0 - 110	0 - 230
Phycoerythrin: Nominal Range (ppb)	0 - 55	0 - 110	0 - 230
CDOM: Nominal Range (ppb)	0 - 125	0 - 250	0 – 500
Uranine: Nominal Range (ppb)	0 - 100	0 - 200	0 - 400

Note: Measurement ranges specified by WET Labs have varied over the years; consult the documentation that was provided with your sensor for the appropriate ranges, or contact WET Labs.

- **ECO-FL-NTU** – Measurement range for this combination fluorometer and turbidity meter is factory-configured, and cannot be adjusted in the field. For the fluorometer channel, the range is approximately 0 to 30, 50, 75, or 125 µg/l (see *Turbidity Meter Calibration Coefficients* below for the turbidity meter channel).

- **ECO-FL as part of the Triplet** - When the ECO-FL is part of the Triplet, the measurement range is factory-configured and cannot be adjusted in the field. For the fluorometer channel, the ranges are:

Chlorophyll *a*: Nominal Range (µg/l)	0 - 50
Rhodamine: Nominal Range (ppb)	0 - 175
Phycocyanin: Nominal Range (ppb)	0 – 175
Phycoerythrin: Nominal Range (ppb)	0 - 175
CDOM: Nominal Range (ppb)	0 – 375
Uranine: Nominal Range (ppb)	0 - 300

Setting Up Configuration (.xmlcon) File for Fluorometer

1. Use the Configure Inputs menu in Seasave V7 (real-time data acquisition software), or the Configure menu in SBE Data Processing (post-processing software), to create / modify the .xmlcon file (see software Help files):
   - For the voltage (analog) output ECO FL: Select *Fluorometer – WET Labs CDOM* for the CDOM fluorometer or *Fluorometer – WET Labs ECO-AFL/FL* for all other ECO fluorometers for one of the **external voltage channels** on the CTD.
   - For the RS-232 (digital) output ECO FL (SBE 16*plus* V2, 16*plus*-IM V2, or 19*plus* V2 CTDs only): Select *Fluorometer – WET Labs CDOM* for the CDOM fluorometer or *Fluorometer – WET Labs ECO-AFL/FL* for all other ECO fluorometers for one of the **RS-232 channels** on the CTD.

2. The software prompts for Dark Output and Scale Factor and calculates concentration as:
   Concentration (µg/l or ppb, as applicable) = (Output – Dark Output) * Scale Factor (see Note)
   where:
   - Output (volts for analog output sensor; counts for digital output sensor) = *in-situ* output of the fluorometer
   - Dark Output (volts for analog output sensor; counts for digital output sensor) = measured output for a seawater blank (pure, de-ionized water) with black tape over detector (see Note)
   - Scale factor (µg/l-volts, µg/l-counts, ppb/volts, or ppb/counts as applicable) = multiplier

The fluorometer comes with a calibration sheet that lists values for Dark Output and Scale Factor (see Note). Each of these values is supplied in terms of both voltage and counts. If you changed the analog scaling value (ECO-FL series only), change the Scale Factor to correspond.

Notes:
- The configuration file can only be saved as an .xmlcon file (not a .con file) if the digital output version of the ECO-FL is one of the sensors.
- Calibration sheets from WET Labs for older fluorometers may list ***Vblank*** instead of ***Dark Output***. Use the Vblank value in place of Dark Output when setting up the configuration file.

Example Chlorophyll a Concentration Calculation in Sea-Bird Software:
Dark Output = 0.05 volts and Scale Factor = 12.35 µg/l-volts (from calibration sheet)
Measured voltage from fluorometer = Output = 4.65 volts
Calculated concentration (µg/l) = (Output – Dark Output) * Scale Factor = (4.65 - 0.05) * 12.35 = 56.8 µg/l

While the factory-supplied Scale Factor can be used to obtain approximate values, field calibration is highly recommended.
- For example, the relationship between fluorescence and chlorophyll *a* is highly variable, and is not easy to determine in the laboratory. Species distribution, ambient light level, and health of the stock are just some of the factors that affect the relationship. To accurately measure chlorophyll *a* concentration with a fluorometer, perform calibrations on seawater samples with concentrations of plankton populations that are similar to what is expected *in-situ*. Determine chlorophyll *a* concentrations independently, and use those concentrations, as well as readings from the fluorometer, to determine the correct Scale Factor. **The Scale Factor is correct as long as the condition of the plankton population does not change; the condition does change with season and geographic location.**

  Example Calculation of Scale Factor from field calibration:
  Seawater sample analysis shows chlorophyll *a* is 50 µg/l when fluorometer reads 3.2 volts; measured signal for dark output is 0.05 volts.
  concentration (µg/l) = (Output – Dark Output) * Scale Factor → 50 = (3.2 - 0.05) * Scale Factor
  Solving: Scale Factor = (50) / (3.2 - 0.05) = 15.87 µg/l → Enter new Scale Factor in configuration (.xmlcon) file.

Turbidity Meter Calibration Coefficients

The turbidity meter has a response that is linear over the measurement range provided.

- **ECO-NTU**- Measurement range for the analog output version of this single-channel sensor can be adjusted with the analog scaling value. To change the range, connect the ECO-NTU directly to the computer and use WET Labs' ECOView Host software to enter a new analog scaling value.

Analog Scaling Value / Range (enter in ECOView Host)	1	2	4 (factory default)
Sea-Bird PN 24345, 24346, and 24348: Nominal Range (NTU)	0 - 30	0 - 60	0 - 125
Sea-Bird PN 24367: Nominal Range (NTU)	0 - 250	0 - 500	0 - 1000

- **ECO-FL-NTU** – Measurement range for this combination fluorometer and turbidity meters is factory-configured, and cannot be adjusted in the field. For the turbidity meter channel, the range is 0 to 10, 25, 100, 200, or 1000 NTU (see *Fluorometer Calibration Coefficients* above for the fluorometer channel).

- **ECO-NTU as part of the Triplet** - When the ECO-NTU is part of the Triplet, the measurement range is factory-configured and cannot be adjusted in the field. For the turbidity meter channel, the range is 0 to 10, 25, 300, or 1000 NTU.

Note: If you require m^{-1} sr^{-1} output, WET Labs can provide a secondary calibration for these instruments in m^{-1} sr^{-1} at an additional cost. Alternatively, you can purchase the ECO-BB, which comes calibrated to m^{-1} sr^{-1}. See *Application Note 87* for details on how to use m^{-1} sr^{-1} output with Sea-Bird CTDs and software.

Setting Up Configuration (.xmlcon) File for Turbidity Meter

1. Use the Configure Inputs menu in Seasave V7 (real-time data acquisition software), or the Configure menu in SBE Data Processing (post-processing software), to create / modify the .xmlcon file (see software Help files):
   - For the voltage (analog) output ECO NTU: *OBS/Nephelometer/Turbidity – Turbidity, WET Labs, ECO-NTU* for one of the **external voltage channels** on the CTD.
   - For the RS-232 (digital) output ECO NTU (SBE 16*plus* V2, 16*plus*-IM V2, or 19*plus* V2 CTDs only): Select *Turbidity Meters – Turbidity Meter, WET Labs, ECO-NTU* for one of the **RS-232 channels on the CTD**.

2. The software prompts for Dark Output and Scale Factor and calculates concentration as:
   Turbidity (NTU) = (Output – Dark Output) * Scale Factor (see Note)
   where:
   - Output (volts for analog output sensor; counts for digital output sensor) = *in-situ* output of the turbidity meter
   - Dark Output (volts for analog output sensor; counts for digital output sensor) = measured output for a seawater blank (pure, de-ionized water) with black tape over detector
   - Scale factor (NTU/volts or NTU/counts, as applicable) = multiplier

The turbidity meter comes with a calibration sheet that lists values for Dark Output and Scale Factor. Each of these values is supplied in terms of both voltage and counts. If you changed the analog scaling value (single channel ECO-NTU only), change the Scale Factor to correspond.

Note: The configuration file can only be saved as an .xmlcon file (not a .con file) if the digital output version of the ECO-NTU is one of the sensors.

While the factory-supplied Scale Factor can be used to obtain approximate values, field calibration is highly recommended. The relationship between turbidity and NTU is highly variable, and is not easy to determine in the laboratory. Particle shape and size are some of the factors that affect the relationship. To accurately measure NTU with a turbidity meter, perform calibrations on seawater samples with distributions of particles that are similar to what is expected *in-situ*. Determine NTU independently, and use those values, as well as readings from the turbidity meter, to determine the correct Scale Factor. **The Scale Factor is correct as long as the distribution of particle sizes and shapes does not change; the condition does change with season and geographic location.**

Application Note Revision History

Date	Description
March 2001	Initial release
March 2003	Reflect addition of ECO-FL(RT), -FLD, -FL, -FLS, -FLB, and -FLSB to Wet Labs product line and to Seasave and SBE Data Processing .con file.
June 2003	Add information on Wet Labs ECO-FL-NTU.
December 2006	Add note that newer calibration sheets from Wet Labs may list Dark Counts instead of Vblank. You can use the Dark Count value as the entry for Vblank when setting up the .con file.
May 2007	Incorporate Seasave V7.
August 2009	• Update fluorometer and turbidity meter ranges to correspond to current listing. • Add Rhodamine fluorometer. • Update to SEASOFT V2.
February 2010	• ECO-NTU can come with secondary calibration to 0-5 m^{-1} sr^{-1}, if customer wants both units from same sensor. • Add information on .xmlcon configuration file. • Update Sea-Bird address.
April 2010	Add Phycocyanin sensor.
May 2010	Add Phycoerythrin sensor.
August 2010	SBE Data Proc and Seasave 7.20g software revision: Wet Labs ECO-NTU added to list of OBS/Nephelometers/Turbidity sensors (previously needed to select User Polynomial for this sensor).
February 2011	Add information on CDOM sensor.
May 2011	SBE Data Processing and Seasave 7.21d software revision: WET Labs RS-232 output sensors, including WET Labs Triplet, are compatible with RS-232 sensor channel on SBE 16*plus* V2, 16*plus*-IM V2, and 19*plus* V2 CTDs.