Sea-Bird Electronics, Inc.
13431 NE 20th Street
Bellevue, WA 98005
USA

Phone: +1 425-643-9866
Fax: +1 425-643-9954
E-mail: seabird@seabird.com
Web: www.seabird.com

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

Calculating Calibration Coefficients for WET Labs ECO-AFL and ECO-FL Fluorometer, ECO-NTU Turbidity Meter, and ECO-FL-NTU Fluorometer/Turbidity Meter

This Application Note applies to the following WET Labs fluorometers and turbidity meters:
- ECO-AFL fluorometer - older model, not in current production by WET Labs
- 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

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 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* Fluorometer: Nominal Range (µg/l)	0 - 30	0 - 50	0 - 125
Rhodamine Fluorometer: Nominal Range (ppb)	0 - 55	0 - 110	0 - 230
Phycocyanin Fluorometer: Nominal Range (ppb)	0 – 100	0 - 200	0 - 400
Phycoerythrin Fluorometer: Nominal Range (ppb)	0 - 55	0 - 110	0 - 230
CDOM Fluorometer: Nominal Range (ppb)	0 - 125	0 - 250	0 - 500

- **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).

In our SEASOFT V2 suite of programs, edit the CTD configuration (.con or .xmlcon) file using the Configure Inputs menu in Seasave V7 (real-time data acquisition software) or the Configure menu in SBE Data Processing (data processing software). Select one of the following as a voltage sensor when editing the .con or .xmlcon file:
- *Fluorometer – WET Labs CDOM* for the CDOM fluorometer
- *Fluorometer – WET Labs ECO-AFL/FL* for all other fluorometers

The software prompts for Vblank and Scale Factor and calculates concentration as:

concentration (µg/l or ppb, as applicable) = (Vsample - Vblank) * Scale Factor (see Note)

where:

Vsample (volts) = *in-situ* output of the fluorometer

Vblank (volts) = measured output for a seawater blank (pure, de-ionized water) with black tape over detector (see Note)

Scale factor (µg/l-volts or ppb/volts, as applicable) = multiplier

The fluorometer comes with a calibration sheet that lists values for Vblank and Scale Factor (see Note). If you changed the analog scaling value (ECO-FL series only), change the Scale Factor to correspond.

Note: Calibration sheets from WET Labs for newer fluorometers may list ***Dark Counts*** instead of ***Vblank***. Use the Dark Counts value in place of Vblank when setting up the configuration (.con or .xmlcon) file. The Dark Counts is supplied in terms of both voltage and of counts; **enter the voltage values in the Sea-Bird software**.

Example Chlorophyll a Concentration Calculation in Sea-Bird Software:

Vblank = 0.05 volts and Scale Factor = 12.35 µg/l-volts (from calibration sheet)

Measured voltage from fluorometer = Vsample = 4.65 volts

Calculated concentration (µg/l) = (Vsample - Vblank) * 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. 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 seawater blank is 0.05 volts.
concentration (µg/l) = (Vsample - Vblank) * 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 or .con) file.

Turbidity Meter Calibration Coefficients

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

- **ECO-NTU**- Measurement range 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.

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
Analog Scaling Value / Range (enter in ECOView Host)	1	2	4 (factory default)

- **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 approximately 0 to 10, 25, 100, 200, or 1000 NTU (see *Fluorometer Calibration Coefficients* above for the fluorometer channel).

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.

In our SEASOFT V2 suite of programs, edit the CTD configuration (.con or .xmlcon) file using the Configure Inputs menu in Seasave V7 (real-time data acquisition software) or the Configure menu in SBE Data Processing (data processing software).

Select the *OBS/Nephelometer/Turbidity – Turbidity, WET Labs, ECO-NTU* as a voltage sensor when editing the configuration file (the ECO-NTU turbidity meter was added to the list of voltage sensors in software version 7.20g; earlier software versions do not list it). The software prompts for Dark Voltage and Scale Factor, and calculates concentration as:

turbidity (NTU) = (V – Dark Voltage) * Scale Factor

where:

V (volts) = *in-situ* output of the turbidity meter

Dark Voltage (volts) = measured output for a seawater blank (pure, de-ionized water) with black tape over detector

Scale factor (NTU/volts) = multiplier

Note: The turbidity meter comes with a calibration sheet that lists values for Dark Counts (instead of Dark Voltage) and Scale Factor. Each of these values is supplied in terms of both voltage and of counts; **use the voltage values in the Sea-Bird software**.

Note: The configuration file can only be saved as an .xmlcon file (not a .con file) if the ECO-NTU turbidity meter is selected as 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.