



Sea-Bird Electronics, Inc.
 1808 136th Place NE
 Bellevue, WA 98005
 USA

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

APPLICATION NOTE NO. 41

revised MARCH 2003

**CALCULATING CALIBRATION COEFFICIENTS
 FOR THE WET LABS WETSTAR FLUOROMETER**

The WETStar fluorometer supplied with your Sea-Bird CTD measures Chlorophyll-*a* concentrations and provides a linear output of 0 to +5 volts. Two standard ranges are available:

- 0.03 µg/l to 75 µg/l (normal range), or
- 0.01 µg/l to 150µg/l (high range)

Consult Wet Labs for other ranges.

Select the Wetstar fluorometer when setting up the configuration (.con) file in Sea-Bird software (SBE Data Processing or SEASAVE in SEASOFT-Win32 or SEACON in SEASOFT-DOS). The software prompts for Vblank and Scale Factor and calculates chlorophyll concentration as:

$$\text{concentration } (\mu\text{g/l}) = (V_{\text{sample}} - V_{\text{blank}}) * \text{Scale Factor}$$

where:

Vsample (volts) = in situ output of the fluorometer

Vblank (volts) = measured output for a seawater blank (pure, de-ionized water)

Scale Factor (µg/l - volt) = multiplier

The fluorometer comes with a calibration sheet that lists values for Vblank and Scale Factor.

Example Concentration Calculation in Sea-Bird Software:

Vblank = 0.05 volts and Scale Factor = 14.10 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) * 14.10 = 64.9 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 indicates chlorophyll-*a* concentration is 50 µg/l when fluorometer reads 3.2 volts; measured signal for a seawater blank is 0.05 volts.

$$\text{concentration } (\mu\text{g/l}) = (V_{\text{sample}} - V_{\text{blank}}) * \text{Scale Factor}$$

$$50 = (3.2 - 0.05) * \text{Scale Factor}$$

Solving for Scale Factor: Scale Factor = (50) / (3.2 - 0.05) = 15.87 µg/l

Enter the new Scale Factor in the configuration [.con] file in SEASAVE and/or SBE Data Processing.