Green Eyes
Environmental Observing Systems

# EcoLAB and NuLAB Data Processing Guide

v1.0

**Background**: Beer's Law provides the fundamental principle for calculating sample concentration from raw light transmission values collected during colorimetric analysis. Green Eyes follows this established principle that is described below.

**The Beer-Lambert Law: $A = ecl = \log_{10} (I_0/I_1)$**

**Where:**

**A** is absorbance of monochromatic radiation or light (no units)
**e** is the molar absorbtivity of the colored solution being analyzed (L/mol cm)
**c** is the concentration of the colored solution contained in the optical cuvette (mol/L)
**l** is the optical pathlength where the light passes through the solution inside the cuvette (cm)
**$I_0$** is the intensity of the light before any of it is absorbed in the solution in the cuvette (*detector units)
**$I_1$** is the intensity of the light after passing through the solution in the cuvette of pathlength l (*detector units)

*The light detector output can have various units (e.g. volts, amps, data bits, etc.) that are not required in the calculation of absorbance because they are divided out in the ratio of $I_0/I_1$.

**Practice**: When processing laboratory or field data, the **ecl** part of the equation is seldom used and absorbance is calculated by taking the log(base-10) of the quotient of $I_0$ and $I_1$. Because it is not practical to measure the intensity of the light as it enters the cuvette, some systems use the power of a stable reference light or light emanating from the same source that is measured adjacent to the cuvette for $I_0$. With the automated EcoLAB and NuLAB, we use the power (intensity) of the light that passes through the flow cell (a small volume cuvette designed to allow liquid to pass through continuously) filled with ambient sample water to measure $I_0$. This has the significant advantage of including the optical effects of particles (turbidity) and color absorbing compounds (CDOM) in our $I_0$ reference reading. Laboratory systems often record one "turbidity blank" for an entire batch of samples, but in coastal, estuarine and riverine systems, the background optical properties of the water change rapidly so a single blank for a set of time series samples may lead to errors. The EcoLAB and NuLAB use automated syringe pumps for discrete analysis which provides considerable flexibility in sample and standard handling and can thus account for the optical properties of the water that could otherwise introduce errors into the analysis. Often these errors are small, but in colored or turbid water they can be significant.

Green Eyes instruments use light emanating diodes (LEDs) at a specific wavelength as the light source. LEDs are an excellent choice for the light source as they are readily available, low power and very stable. LED brightness is primarily a function of the current driving it (they are also effected by temperature), so Green Eyes' electronics can easily control the light intensity. Photodiodes that convert incident light to current are used to measure the light intensity after passing through the flow cell. The photodiode is fitted with a very narrow band filter set at the

maximum absorbtion wavelength of the final colored solution produced by the chemical reaction (Nitrate = 543, Phosphate 880, Ammonia 630 or 660, Silicate 810 nm).

**Absorbance Calculation:** The first step in calculating the concentration of sample data is to calculate absorbance of samples and standards. For this, the EcoLAB or NuLAB's raw received light values from the **Reference Blank ($I_0$)** that (usually) contains only pure sample water and the **Reacted Reading ($I_1$)** that contains the mixture of sample water and all the reagents required to produce the colored reaction.

Example EcoLAB data:

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 11/3/2015 16:10 | 11.4 NH4 | Smp | Bs | 2007 | 630 | 0 | 39487 | 21770 | 20342 |
| 11/3/2015 16:19 | 11.41 NH4 | Smp | Rs | 2007 | 630 | 0 | 37068 | 22168 | 20565 |

The data above contains two detector readings. The optical blank Bs which is the $I_0$ described above and reacted reading Rs which is the $I_1$ described above. The received light values (bits) are highlighted in yellow so the absorbance calculation of this sample is as follows:

Abs = Log10 (I0/I1) = Log10 (39487/37068) = 0.02745 note: light intensity units are data bits, but are divided out in the ratio

The same method is used to calculate absorbance of laboratory or on-board standards.

**Reagent Blank:** To properly calculate the concentration of nutrients in a sample, the small amount of nutrients present in the reagents (Reagent Blank) must be calculated by running high quality deionized water that should contain zero (or very close to zero) nutrients.

**Linearity:** Typically, a calibration or linear curve (Fig. 1) is made to verify that the relationship between the absorbance signal from the instrument and the concentration of standards used in the calibration follows a standard, linear relationship. Three or more analyses at each standard concentration should be run and their average should be used in the plotted data.

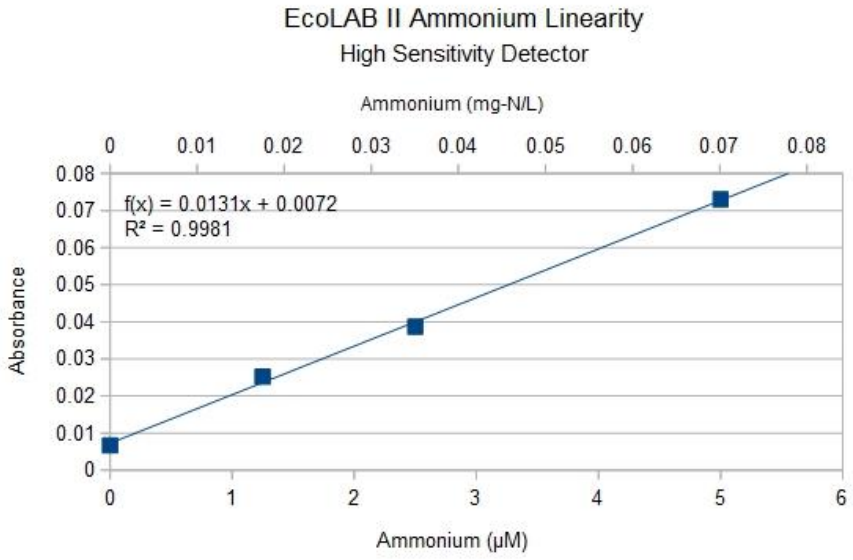


*Fig. 1: EcoLAB II Ammonium Linearity Plot*

*note: linear equation uses µM concentration units*

**Calculating concentration from the linear equation:**

$y = mx + b$

where:

y = the absorbance of the standard or f(x)
m = the slope of the linear regression line
x = the concentration of the standard
b = the y-intercept of the linear regression line

The equation is rearranged to calculate sample concentration (x) based on sample absorbance and the y-intercept.

$x = (y - b) / m$

Example: Using the linear equation from Fig. 1 and a hypothetical sample absorbance of 0.0551, we can calculate the sample concentration as follows:

Sample Concentration = (0.0551 – 0.0072) / 0.0131 = 3.66 µM (0.051 mg-N/L)

**Processing field data**: Once linearity has been proven for an instrument and a chemical method, it can be assumed that the linear relationship will hold during subsequent field deployments. However, the slope of the calibration cure or the response rate will change with variations in ambient temperature, reagent effectiveness, and other environmental conditions. To compensate for these variations, the EcoLAB uses an On-Board Standard (OBS) with a known concentration that is treated with a preservative such as chloroform or mercuric chloride to prevent changes in the concentration during the deployment.

Because linearity of absorbance with concentration has already been verified, field data is processed using the OBS concentration and absorbance in a single point calibration. Sample and OBS absorbances still must be reagent blank corrected so the field data equation becomes:

**Field Data Equation**

**Sample Concentration = ($Abs_{(Smp)}$ – $Abs_{(Rgt. Blank)}$) / ( ($Abs_{(OBS)}$ – $Abs_{(Rgt. Blank)}$) / $Conc_{(OBS)}$ )**

**where:**

**$Abs_{(Smp)}$ = the calculated absorbance of the sample**
**$Abs_{(Rgt. Blank)}$) = the calculated absorbance of pure DIW run in the lab prior to deployment**
**$Abs_{(OBS)}$ = the calculated absorbance of the OBS run in the field**
**$Conc_{(OBS)}$ = the known concentration of the OBS**

**Example:**

Using hypothetical values of $Abs_{(Smp)}$ = 0.0221, $Abs_{(Rgt. Blank)}$ = 0.0071, $Abs_{(OBS)}$ = 0.0422, and $OBS_{(conc)}$ = 2.5 µM we can calculate sample concentration as follows:

Sample Concentration = (0.0221-0.0071) / ( (0.0422-0.0071) / 2.5 ) = 0.015 / 0.014 = 1.07 µM

**Notes:** When processing field data, the OBS used in the calculation of sample concentration should be the one closest in time to the sample being calculated. Green Eyes encourages users to run the OBS frequently and many users collect OBS readings before every sample to account for chemical and environmental changes. Frequent OBS analyses also provide a useful record of the instruments performance during the deployment and are invaluable when verifying rapid changes in sample concentration.

It is also strongly recommended to collect verification samples of the OBS immediately after the treatment with preservative and after the deployment to be analyzed at an independent laboratory. Collecting hand samples adjacent to the deployment site to compare with the autonomous data is also encouraged.