Towards a protocol for the calibration of IMOS field fluorometers

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Some (simplified) terminology

In-vitro chlorophyll- a (chl-a) analysis is another term for extracted analysis. It requires filtration of a discrete water sample to concentrate phytoplankton cells onto a filter and then the extraction of the chl-a from the cells using a solvent. Chl-a concentration is then determined using a laboratory spectrophotometer, fluorometer or HPLC instrument calibrated with a primary chl-a standard.

In-vivo chlorophyll-a analysis is the measure of chl-a in living algal cells. This is measured by field fluorometers in the natural environment (in-situ).

Background

Chlorophyll-a (chl-a) is ubiquitous to phytoplankton. Field fluorometers, such as the Seabird Eco and WETStar fluorometers, are used by the IMOS national mooring facility and are deployed in-situ to collect time-series or vertical profiles of in-vivo chl-a fluorescence. These fluorometers use an excitation beam of blue light (~460nm) and detect the red light (~685nm) fluoresced by the chl-a in cells. Generally, provided the detected sample volume contains a sufficient number of cells, the amount fluoresced is directly proportional (linear) to the concentration of chl-a. When calibrated, the in-vivo fluorescence data measured by field fluorometers supplies information on the relative distribution of chlorophyll concentrations (i.e. phytoplankton biomass) and typically correlates well with extracted (in-vitro) chl-a samples.

The raw chl-a fluorescence signal detected by fluorometers is measured and reported as a voltage (analogue) or in relative fluorescent units (digital). Since the electronics and the linear response for each individual fluorometer differ, it is necessary to calibrate the instruments to a primary chl-a (and ecologically relevant) standard. This standard is the concentration of chlorophyll-a (ug/L) which is measured by in-vitro chl-a analysis using a laboratory based spectrophotometer, fluorometer or HPLC instrument. Note: Each of these laboratory instruments also requires calibration with a primary chl-a standard.

The calibration of common laboratory instruments for in-vitro measures (e.g. fluorometer) are relatively straight forward and primary chlorophyll-a standards are available from suppliers such as SIGMA-ALDRICH and TURNERDESIGNS.

For field fluorometers, calibration is slightly more complicated because field fluorometers cannot be immersed into the solvents associated with primary chl-a standards. Hence, calibration is best done with cultured phytoplankton populations using concentrations (and to a lesser extent species) that are similar to what is expected in-situ. SARDI has previously demonstrated the calibration of its fluorometer's using a serial dilution of a phytoplankton culture and in-vitro extraction methods measured on a laboratory fluorometer calibrated with a primary chl-a standard.

However, because of difficulties and expenses associated using cultures (i.e. species selection, culture maintenance, cost) there is a desire to use a less expensive, less time consuming and more stable secondary standard as an alternative to cultures. This requires two calibrations.

First, the calibration of a laboratory fluorometer (e.g. Turner 10AU) using a primary chl-a standard. A second calibration, whereby the appropriate fluorophore (fluorescent chemical) chosen for the secondary standard is calibrated against the primary standard. This is done to establish the

relationship between the primary and secondary standard (i.e. transfer function to obtain the equivalent value of the secondary standard). Earp et al. 2011 provide a review of different fluorophors and their relationship to the excitation and emission spectra of chl-a.

Once this relationship is determined, field fluorometers may be calibrated using the secondary standard; (i.e. using the linear relationship you obtained for it initially against the primary standard). An occasional (re)calibration of the secondary standard against the primary standard to recheck the stability of the secondary standard is recommended.

Ultimately, since the relationship between in-vivo fluorescence and chl-a in the field is variable, and is influenced several factors (e.g. species composition and distribution, physiology, morphology, ambient light level, temperature) chl-a concentrations measured by field fluorometers are best calibrated using in-vivo seawater samples taken in the field WHEN available.

Suggested Protocol

1. Calibrate a benchtop fluorometer using a primary chl-a standard. The calibration range should match the range expected to be measured by in-vitro methods in the field (e.g. 0-5 ug/L).
2. Determine the relationship of the secondary standard (fluorescent dye) with the primary standard. To do this, make a dilution series of the secondary standard covering multiple chl-a concentrations across the calibrated range of the benchtop fluorometer (e.g. 0.5, 1, 2, 3, 5 ug/L chl-a). Plot the concentration of the secondary standard against corresponding readings from the calibrated benchtop fluorometer. The slope of this line is the transfer function.
3. Use the concentrations from the dilution series from 2 to calibrate the field fluorometers in a dark room (no fluorescent lights). To do this, make a series of secondary standard baths (e.g. 0.5, 1, 2, 3, 5 ug/L) to put the field fluorometers in. Remember to make a zero concentration batch using filtered seawater/milliQ (or by placing black tape over the sensor). Measure the raw fluorescence signal at each concentration for 1 minute to determine the calibration curve for each fluorometer, in units of the secondary standard. Use the transfer function to convert from units of secondary standard to equivalent chl-a.
4. Repeat occasionally at a suitable interval based on the stability of the fluorescent dye or the chl-a range required for calibration.