In situ performance of the RBRtridente chlorophyll fluorometer

Introduction

Phytoplankton are photosynthetic microorganisms that represent the base of the ocean food chain and are responsible for approximately half of all global photosynthetic carbon fixation (Field et al., 1998). Despite their significance, traditional measurement techniques make resolving these fundamental organisms' spatial and temporal distributions difficult due to the logistics associated with shipboard water samples (Strickland, 1960). Modern techniques compatible with autonomous gliders and floats permit continuous, high-frequency measurements of relative phytoplankton populations in situ by observing the fluorescence of photosynthetic pigments. Pigments are large molecules which absorb photosynthetically active radiation (PAR) of specific wavelengths and transfer the energy to the reaction centres of the photosynthetic units (reviewed by Mimuro, 2005). The absorbed energy is partitioned between photosynthesis and energy dissipation as heat and fluorescence. The magnitude of each depends on the intensity of the absorbed light and the physiological status of the cell (Huot and Babin, 2010). As both chlorophylls and phycobiliproteins can re-emit absorbed energy as fluorescence but do so at different wavelengths, modern fluorometers, such as the RBR*tridente*, capture that fluorescence and use it as a proxy to estimate pigment concentrations in the ocean.

Fluorometers must be calibrated before they can be used for quantitative estimates in oceanographic field studies. Calibrations derive coefficients for a linear equation that relates the amount of light detected as a function of the concentration of fluorescing pigment. At RBR, fluorometers tuned for chlorophyll *a* measurement are calibrated using analytical grade chlorophyll *a* pigment from Sigma Aldrich (96145, purity > 95% by HPLC, CAS 479-61-8). A more detailed description of the calibration procedure is provided in Dever et al. (2024).

The isolated chlorophyll *a* pigment provides a more stable, reproducible standard for factory calibration over a live culture. However, this method produces a larger scalar on a calibrated sensor than is required to align to in situ concentrations. The misalignment of factory-calibrated fluorometers and reference quantification of in situ chlorophyll is the combined effect of two phenomena: 1. a shift in the fluorescence emission peak of chlorophyll when it is not bound to a protein (Porra et al., 1997), and 2. excitation at non-peak absorption wavelengths results in a lower fluorescence in isolated pigment than it does in situ where the organism has supplementary pigments that shuffle energy to chlorophyll and boost fluorescence. An in situ scaling factor (C2 in Equation 1) can be applied to the factory calibration equation to compensate for these combined effects.

$$[Chlorophyll\ a]\left(\frac{\mu g}{L}\right) = (C0 + C1 \times V_r) \times C2 \qquad (1)$$

where [*Chlorophyll a*] is the chlorophyll a concentration, V_r is the raw corrected optical signal from the detector, *C1* and *C0* are calibration coefficients determined in the calibration laboratory using the extracted pigment reference, and *C2* is the in situ scaling factor to align factory calibration with in situ conditions.

In situ scaling factors are derived in a monoculture and have been shown to vary from 0.5 to 6 based on the selected species (Roesler et al., 2017). The variation in scaling factor is dominated by the biological, chemical, and physical parameters which influence phytoplankton fluorescence. These sources include systemic and taxonomically constrained differences in pigment types and ratios (MacIntyre et al., 2011) as regulated responses to environmental

factors such as light, nutrient availability, and temperature (Huot and Babin, 2010) - variables that are inherent with the relative estimation of phytoplankton biomass through fluorescence.

In this paper we present the performance of RBR*tridente* chlorophyll fluorometers in a monoculture of *Thalassiosira weissflogii,* a nanoplanktonic, centric species of diatom found in both marine and brackish environments across the world. The species was chosen for its global distribution and previous use in oceanographic sensor calibration studies (Leeuw et al., 2013; Lawrenz and Richardson, 2011; Proctor and Roesler, 2010). In situ scaling factors for in vivo fluorescence measurement of this species are derived and compared at two excitation wavelengths (435 and 470 nm). An inter-sensor comparison of both excitation wavelengths is also described to explore sensor-to-sensor measurement variation in a single species.

Methods

Sensors

The RBR*tridente* is a three-channel fluorescence and backscatter instrument produced by RBR (Ottawa, ON, Canada) featuring two chlorophyll *a* channels with excitation/emission wavelength pairs of 470/695 nm and 435/695 nm. Each channel features a high-power, narrow-waveband LED to excite the target molecules and a photodiode to capture emitted fluorescence. Filters are placed in front of the LED and photodiode to isolate the channels' respective emitted and detected wavelengths (Table 1). Three instruments were tested in the culture, with the factory calibrations of each channel applied (see Table 2). Two other sensors from SeaBird Scientific (Bellevue, WA, USA) were included in the testing: a SeaOWL UV-A (FLBBFL), also known as the MCOMS on Navis profiling floats, and a WetLabs ECO Puck (FLBBFLRT2K). These two sensors were selected based on their wide use in the BGC Argo program. The factory calibration provided for each of these instruments was used to convert raw counts to µg/L chlorophyll in the data presented.

Table 1. Optics on RBRtridente chlorophyll *a* 435- and 470-nm channels. The centre wavelength (CWL) and full-width half-max (FWHM) are reported for the LED and detection filter.

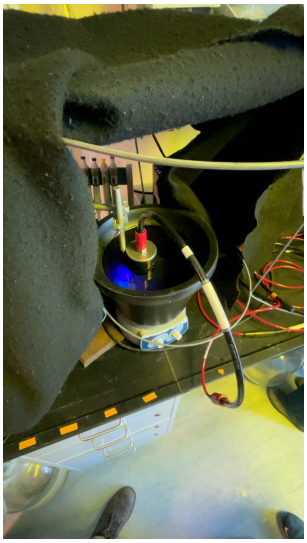
Channel	LED (CWL/FWHM)	Detection filter (CWL/FWHM)	Detection filter transmission band
RBRtridente chlorophyll *a* (435/695nm)	435nm/17nm	695nm/65 nm	663 - 728 nm
RBRtridente chlorophyll *a* (470/695nm)	470nm/20nm		

Experimental setup and protocol

Setup

Fluorometers were lowered into a non-reflective well-mixed vessel containing 4L of sample. Sensors were positioned in the vessel to ensure sufficient free space around the optical face so as not to have any wall effect. The experimental setup was covered in a black fabric shroud to limit culture light exposure and minimize sensor signal pollution from reflected light (Figure 1). To further minimize light absorbed by the dark-acclimated culture prior to testing the windows in the test area were covered and the space illuminated by fluorescent bulbs covered with green acetate.

A.



B.

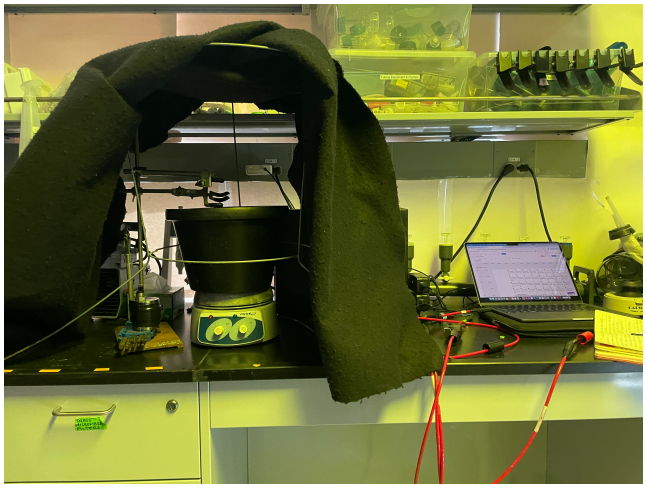


Figure 1. The experimental set up for fluorometer in situ monoculture measurement at Dalhousie University (Halifax, NS). The black shroud has been pulled up for visibility of top-down (A) and side angle (B) views of the set up. The greenish hue in the test area is due to green acetate covered fluorescent lighting (to minimize light absorption by dark-acclimated cultures during transfer to the test container).

Culture growth and acclimatization

A monospecific isolate of the diatom *Thalassiosira weissflogii* (Clone CCMP1050; NCMA, ME, USA) was maintained in balanced growth in a semi-continuous culture (MacIntyre and Cullen, 2005) by Cat London in the MacIntyre lab in the Department of Oceanography at Dalhousie University, NS, Canada. The culture was grown at 18 ± 0.5 °C in a constant-temperature incubator at an irradiance of c. 80 µmol photons m^{-2} s^{-1}, with continuous illumination from cool-white fluorescent bulbs. The growth medium was based on tangential flow-filtered coastal seawater (salinity 30), enriched with f/2 macronutrients, vitamins, and trace minerals (Guillard 1975) plus 106 µM Na_2SiO_3 and 10 nM H_2SeO_3.

The culture was acclimated to the growth conditions in 40-mL cultures and was monitored daily with variable chlorophyll fluorescence (Brand et al., 1981; MacIntyre and Cullen, 2005). A two litre Pyrex bottle of medium was inoculated with the 40 mL cultures after full acclimation and stirred continuously with a magnetic stirrer. Testing was performed with the culture in exponential phase growth.

Preparation of dilution series

A nine-sample dilution series was prepared with nominal concentrations ranging from 0.1 - 20.0 µg/L chlorophyll *a*. Concentrations were estimated using a direct sample of the final two litres into a 3:2 (V:V) mixture of dimethylsulfoxide (DMSO) and 90% ACS-grade acetone (Shoaf and Lium, 1976). Prior to measurement, all nine diluted aliquots were held in the growth incubator (constant temperature) in darkness to ensure the photosystems were fully relaxed prior to fluorometer measurement.

Workflow and procedure

Ten solutions, including a seawater blank, were measured with each of the five sensors listed in Table 1. All sensors, sampling for one minute each (two minutes for SBE sensors), were cycled through one solution before moving on to the next, starting with the blank and increasing in concentration. For culture samples the solution was allowed to stir for one minute before the first fluorometer measurement was taken.

Once fluorometer measurements were completed over a single dilution series, triplicate subsamples were taken for quantification. Culture subsamples were filtered onto Whatman GF/F glass fibre filters using a vacuum pump at low pressure (< 17 kPa). Pigments were extracted in 6 mL of 3:2 (V:V) dimethylsulfoxide (DMSO):90% ACS-grade acetone for a minimum of 15 minutes in the dark at room temperature. The filtered volumes were adjusted in response to the approximate concentration of the diluted culture to ensure that the pigment retained on the filter (16.7-21.0 µg/L in solution) was in the mid-range of the calibration curve.

Reference quantification

The Welschmeyer (1994) fluorometric method was used to quantify chlorophyll *a* concentration. A Turner 10-005R fluorometer with digital output was configured for the non-acidification method and calibrated on the day of analysis using a c. 5 mg/L primary solution of purified chlorophyll *a* (Sigma Aldrich C6144) in 90% ACS-grade acetone.

Turner fluorometer calibration

The specific absorption coefficient (Jeffrey et al., 1997) was used to determine the concentration of the purified chlorophyll *a* standard for calibration of the Turner 10-005R fluorometer. Absorption by standard was measured against a blank of 90% ACS-grade acetone with a Cary 4000 spectrophotometer. Both samples were in 1-cm Hellma quartz cuvettes. After correcting for absorption by the blank and residual scattering at 750 nm, the concentration was calculated from the specific absorption coefficient, 87.67 L g^{-1} cm^{-1} at 664 nm.

The primary standard was diluted to produce triplicate aliquots at five concentrations between 4.75 and 23.8 µg L^{-1} and fluorescence was measured across the sensitivity settings of the Turner fluorometer. Regression of the blank- and range-corrected values of fluorescence on the chlorophyll concentration in the tertiary standard solutions produced the calibration factor (R^2 = 0.9999). The lower limit of detection (Anderson, 1989; Miller and Miller, 2005) was 0.22 µg L^{-1} in solution, approximately an order of magnitude lower than the extracts of the culture.

A quality control step on the primary standard was performed to ensure that no degradation had occurred during storage. After scanning (see above), a sample of the standard was acidified with 100 µL of 10% HCl (aqueous) to degrade the chlorophyll *a* to phaeophytin *a.* The degraded sample was scanned and the concentration of phaeophytin *a* calculated from the specific absorption coefficient, 51.2 L g^{-1} cm^{-1} at 667 nm (Jeffrey et al., 1997). If the sample had been partially degraded in storage the estimated chlorophyll *a* concentration would be less than the estimated phaeophytin *a*. There was a quantitative conversion (initial chlorophyll *a* > final phaeophytin *a*), indicating no detectable prior degradation.

Data processing

Each of the five instruments underwent ten rounds of sampling - the seawater blank and nine dilute culture aliquots (nominal concentrations of 0.1, 0.5, 0.9, 1.2, 1.5, 3.0, 5.0, 10.0, 20.0 µg L^{-1}). For each test solution, the 100-sample window with the smallest standard deviation was selected and the chlorophyll *a* concentration was averaged to produce a single value. The standard deviation within that 100-sample window is reported as the sensor measurement error. On RBR instruments an additional 5% initial calibration error (2.5 µg L^{-1} chlorophyll *a*) is added to the sensor measurement error (or y-axis error contribution) as stated in the factory calibration certificate on any standard RBR*tridente*. During this experiment, both RBR*tridente's* 435- and 470-nm chlorophyll *a* channels are used with their factory calibration C0 and C1 coefficients to produce chlorophyll *a* concentration from raw signal counts. C2 is set to 1.0 for all RBR data.

Analysis of spectrophotometric and fluorometric data for culture chlorophyll *a* concentration was performed by Cat London and Hugh MacIntyre at Dalhousie University. The average concentration from the triplicate samples of each diluted culture aliquot was provided with the standard deviation across the triplicate measurements. The standard deviation is reported as the error in chlorophyll *a* quantification.

A linear regression was performed between the in situ fluorometer data and the fluorometrically quantified chlorophyll *a* reference concentrations. Following the recommendation of Proctor and Roesler (2010), the intercept of the regression was not forced to zero nor was the data corrected for the blank. The slope for each sensors' individual chlorophyll *a* channels response in the culture aliquots was used to generate an in situ correction scalar –or C2 in Equation 1– equal to the inverse of the slope. For each wavelength (i.e., 435 and 470 nm), C2 values were compared with a one-way ANOVA to check for statistical differences. Finally, an average C2 was produced for each wavelength and applied to all respective data.

The processing code used in the analysis is available upon request.

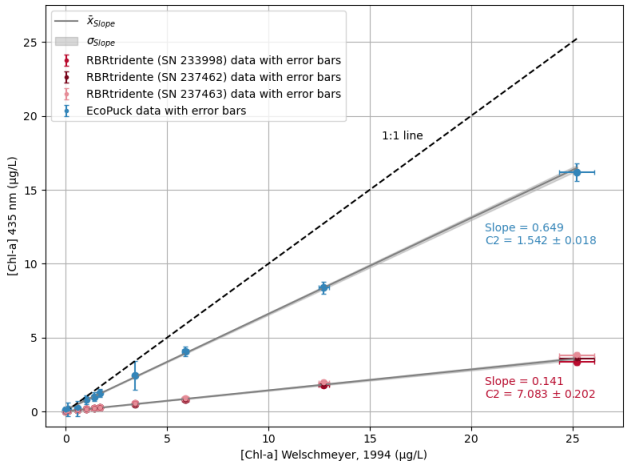
Results

Deriving in situ correction scalars

A one-way analysis of variance (ANOVA) demonstrated that the computed slopes of 435- and 470-nm RBR chlorophyll *a* sensors were not statistically different across sensors within their associated uncertainty (p-value > 0.05). This shows the repeatability of RBR's factory calibration and a small sensor-to-sensor variation. The average in situ correction scalar (C2) for the RBR*tridente* fluorometers was 7.083 ± 0.202 at 435 nm, and 0.258 ± 0.007 at 470 nm (Figure 2).

The same process was repeated for 470-nm chlorophyll *a* channels on SeaBird instruments. A comparison of their in situ values by one-way ANOVA also found no statistical differences (p-value > 0.05). The average C2 between the two SBE instruments (SeaOWL and WetLabs ECO) tested is 3.002 ± 0.105. The 435-nm chlorophyll *a* sensor on the WetLabs ECO was the only SeaBird sensor examined in this test, and yielded a C2 of 1.542 ± 0.018.

A.



B.

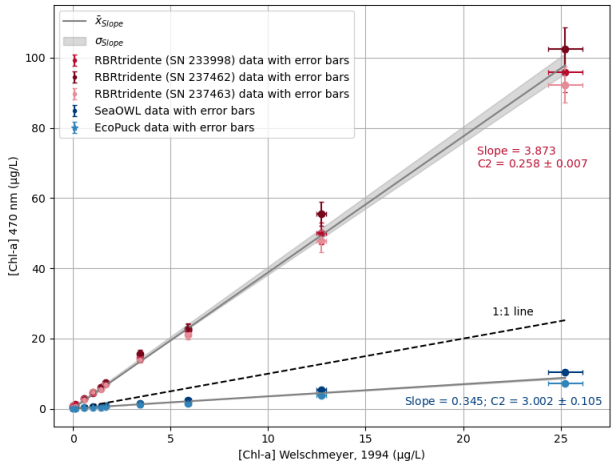
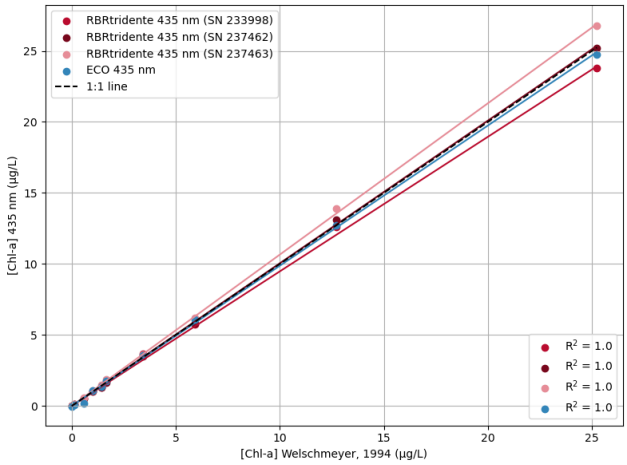


Figure 2. RBRtridente 435- and 470-nm chlorophyll *a* sensor measurement (A & B respectively) in aliquots of dilute *T. weissflogii* culture, 0.1 - 20.0 µg L^{-1} chlorophyll *a*. X- and Y-axis error bars represent the uncertainty associated with each method of measurement. Secondary, in-situ correction scalars (or C2 values) for both chlorophyll channels/excitation wavelengths are listed next to the solid regression line with the uncertainty shaded.

Applying in-situ correction scalars

The wavelength-specific in situ correction scalars described above were applied to their respective manufacturers (Figure 3). With the applied C2, each RBR*tridente* 435- and 470-nm chlorophyll *a* dataset has a strong linear correlation ($R^2 > 0.999$) and a typical root mean squared error (RMSE) smaller than 0.06 µg/L with respect to the 1:1 line. Similarly, the SeaBird instruments also demonstrate a strong linear relationship across 0 - 20.0 µg L^{-1} ($R^2 > 0.999$). However, application of a unique, averaged, C2 value for SeaBird 470-nm chlorophyl *a* sensors results in an average 0.23 µg/L RMSE.

A.



B.

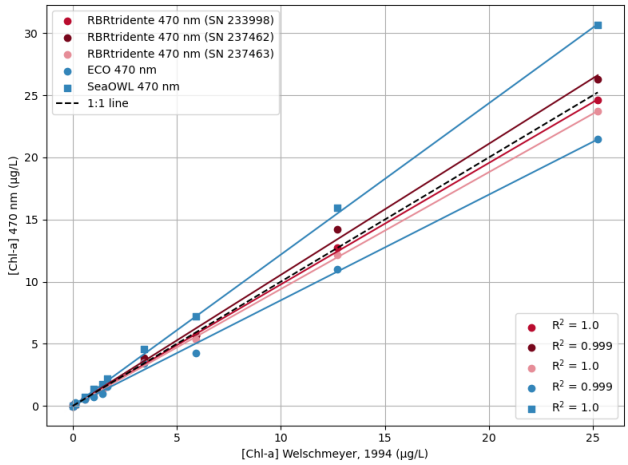
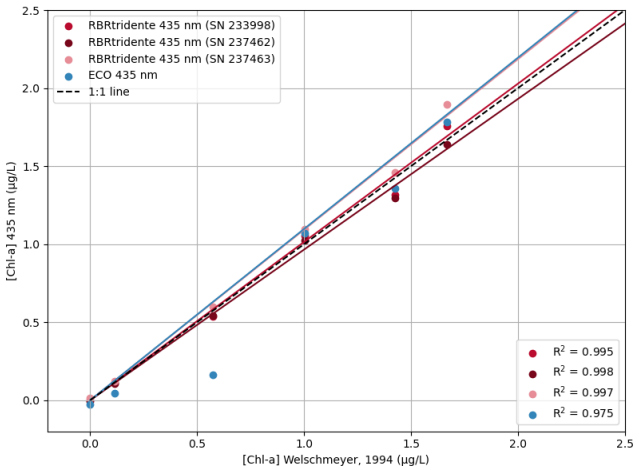


Figure 3. RBR*tridente* and SeaBird 435- and 470-nm (A & B, respectively) chlorophyll *a* sensor data corrected with the computed, wavelength-specific, in situ correction scalars (C2).

Examining linearity at low concentrations

Characterizing the linearity and sensitivity of the fluorometer in the low concentration range (<2.0 µg) is important, as the majority of the open ocean falls within this range. This ensures reliable chlorophyll measurements in oligotrophic and mesotrophic waters. When focusing on low concentrations only (0 - 2.0 µg L^{-1}), the calculated R-squared values for each sensor with respect to the 1:1 line show a strong linear behavior (R^2 > 0.99; Figure 4). The linearity of the ECO Puck (435 and 470 nm) at low concentrations is slightly lower than when considering the entire range (R^2 < 0.975).

A.



B.

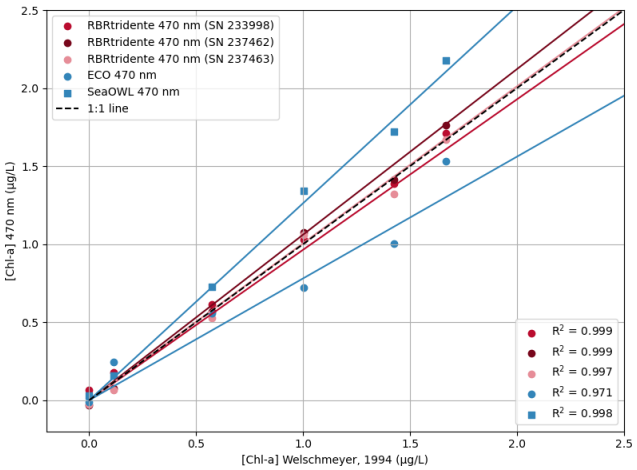


Figure 4. RBR*tridente* and SeaBird 435- and 470-nm chlorophyll *a* sensor data from 0 - 2.0 µg L^{-1} corrected with manufacturer and wavelength-specific in situ correction scalars.

Inter-comparison of sensor consistency

To ensure the consistency of chlorophyll-fluorescence measurements with other commonly used fluorometers, a coefficient is currently used at RBR to scale factory calibrated RBR*tridente* measurements to match SeaBird 470-nm chlorophyll measurement. The scalar was derived by comparing the 470-nm RBR*tridente* chlorophyll *a* sensor to a 470-nm chlorophyll SeaOWL channel in a fluorescein dye solution (C2 = 0.103). This coefficient has no meaning other than to align measurements to ensure consistency across datasets if needed. It is recommended to collect, for each deployment, water samples that could be used to derive an appropriate C2 for the specific community being sampled by the fluorometer (Bittig et. al 2019).

In Figure 5, the RBR*tridente* 470-nm chlorophyll *a* data has had this default scalar applied. The 470-nm RBR sensor data, within its associated uncertainties, overlaps with the SeaOWL data in the *T. weisflogii* culture with an average root mean square error of 0.22 µg L^{-1}. This demonstrates the alignment of the two manufacturer instruments through this default scalar. The data from the WetLabs ECOpuck does not align with the RBR or SeaOWL data, suggesting that the default C2 value might not be general to all SBE sensors. Instead, RBR strongly recommends deriving scaling scalars in situ, based on the regional community composition.

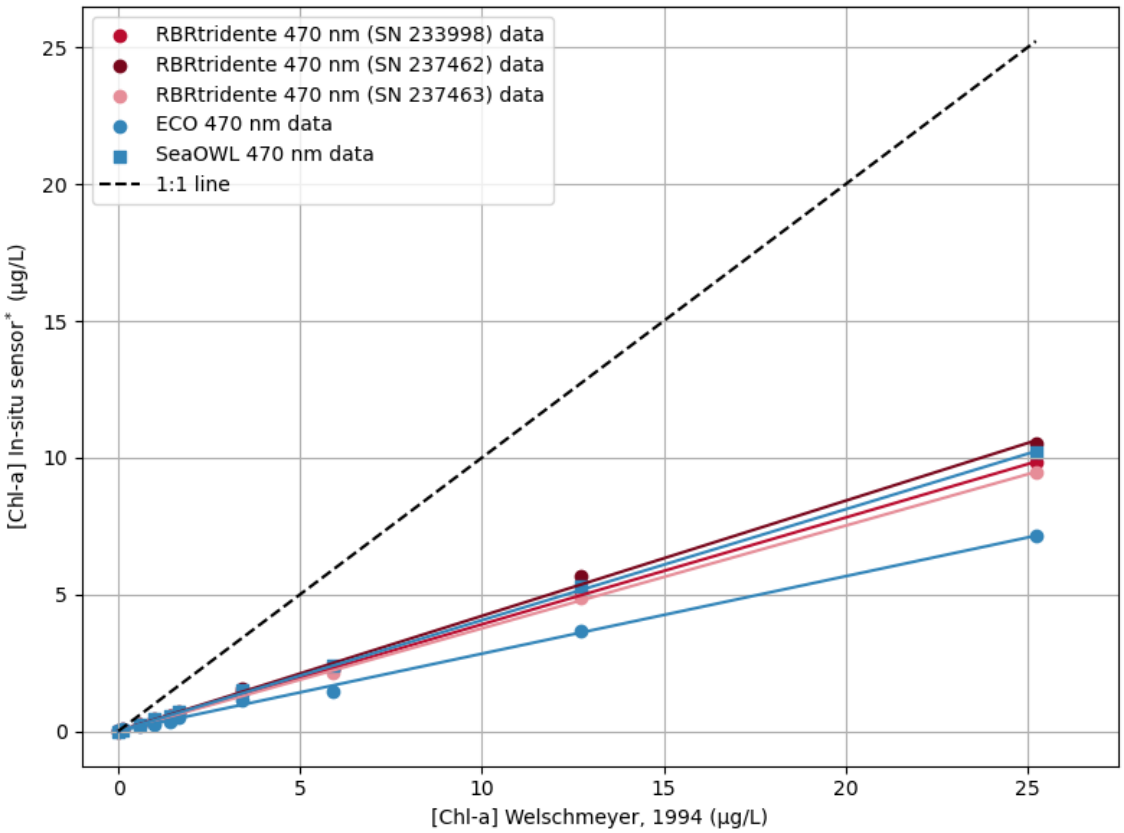


Figure 5. RBR*tridente* and SeaBird 435- and 470-nm chlorophyll *a* sensor data. RBR data are corrected with default sensor alignment scalar of C2 = 0.103.

Presentation of scalars

While RBR maintains the recommendation that in situ scalars be derived for regional community composition, the scalars that have been derived by RBR thus far are presented in Table 3. This is intended to serve as a collection of values to which future derived scalars can be compared. Diatom cultures like the one used to derive the in situ scalars in this experiment have previously generated in situ scalars close to the median scalar in a range of test cultures (Procter and Roesler, 2010). However, it is important to note that growth conditions or treatment before measurement can have a significant impact on the derived scalar.

Table 3. Secondary scalars derived to align 470-nm RBR chlorophyll *a* sensors with the listed sources. Note that the SeaOWL is also referred to as the MCOMMS on Navis floats.

Source of alignment	Scalar (C2)
SeaBird SeaOWL in fluorescein	0.103
SeaBird SeaOWL in *T. weissflogii**	0.108 +/- 0.002
SeaBird WetLabs ECO in *T. weissflogii**	0.074 +/- 0.008
T. weissflogii (Welschmeyer, 1994)*	0.258 +/- 0.007

*Growth conditions and treatment of the *Thalassiosira weissflogii* culture are detailed in the Methods section. Scalar derivation in a *T. weissflogii* culture with different growth conditions may result in a different scalar.

Key conclusions and recommendations

1. RBR*tridente* chlorophyll *a* sensors (435- and 470-nm excitation) demonstrate high linearity (R^2> 0.999) up to 20.0 µg L^{-1}.
    - Strong linearity is also maintained at low concentrations (<2.0 µg L^{-1}) (R^2 = 0.995 – 0.999).
2. RBR*tridente* chlorophyll *a* sensors demonstrate high instrument repeatability.
    - A unique C2 scalar can be successfully derived for both 435- and 470-nm wavelengths to align the RBR*tridente* with a culture of *Thalassiosira wiessflogii* with well-defined and reproducible growth characteristics.
    - RMSE from the average C2 for RBR at 470 nm is 0.05 µg L^{-1}
    - RMSE from the average C2 for SBE (SeaOWL and ECOpuck) at 470 nm is 0.25 µg L^{-1}
3. To align the RBR*tridente* with the *Thalassiosira wiessflogii* culture from this experiment:
    - RBR 470-nm chlorophyll *a* to the culture, C2 = 0.258 ± 0.007
    - RBR 435-nm chlorophyll *a* to the culture, C2 = 7.083 ± 0.202
4. To align the RBR*tridente* to the SBE
    a. at 470 nm, $C2_{SBE}(470nm)$ = 0.108 ± 0.002
    b. at 435 nm, $C2_{SBE}(435nm)$= 4.460 ± 0.834
5. RBR strongly recommends that unique C2 scalars are derived in situ based on the environment and community composition.

Acknowledgements

The team at RBR would like to express our sincere gratitude to Hugh MacIntyre for dedicating the time, space, and resources in his lab at the University of Dalhousie, Department of Oceanography to this study. Cat London was also an instrumental part of the in-lab work, growing and monitoring the cultures to ensure they were in balanced growth and analyzing samples on the day of testing. Additionally, our team would like to recognize their combined efforts in the writing of the Methods section of this paper.

References

Anderson, D. J. “Determination of the Lower Limit of Detection.” *Clinical Chemistry* 35, no. 10 (October 1989): 2152–53.

Bittig, Henry C., Tanya L. Maurer, Joshua N. Plant, Catherine Schmechtig, Annie P. S. Wong, Hervé Claustre, Thomas W. Trull, et al. “A BGC-Argo Guide: Planning, Deployment, Data Handling and Usage.” *Frontiers in Marine Science* 6 (August 22, 2019). https://doi.org/10.3389/fmars.2019.00502 .

Brand, Larry E., Robert R.L. Guillard, and Lynda S. Murphy. “A Method for the Rapid and Precise Determination of Acclimated Phytoplankton Reproduction Rates.” *Journal of Plankton Research* 3, no. 2 (January 1, 1981): 193–201. https://doi.org/10.1093/plankt/3.2.193 .

Dever, M., J. Taylor, and G. Johnson (2024). Calibration Procedure of a Combined Fluorescence-Backscattering Sensor: The RBRtridente" *IEEExplore*, doi: 10.1109/OCEANS55160.2024.10754154.

Field, Christopher B., Michael J. Behrenfeld, James T. Randerson, and Paul Falkowski. “Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components.” *Science* 281, no. 5374 (July 10, 1998): 237–40. https://doi.org/10.1126/science.281.5374.237 .

Guillard, Robert R. L. “Culture of Phytoplankton for Feeding Marine Invertebrates.” In *Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport*, edited by Walter L. Smith and Matoira H. Chanley, 29–60. Boston, MA: Springer US, 1975. https://doi.org/10.1007/978-1-4615-8714-9_3 .

Huot, Yannick, and Marcel Babin. “Overview of Fluorescence Protocols: Theory, Basic Concepts, and Practice.” In *Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications*, edited by David J. Suggett, Ondrej Prášil, and Michael A. Borowitzka, 31–74. Dordrecht: Springer Netherlands, 2010. https://doi.org/10.1007/978-90-481-9268-7_3.

Jeffrey, S.W., R.F.C. Mantoura, and T. Bjørnland. “Data for the Identification of 47 Key Phytoplankton Pigments.” In: Jeffrey SW, Mantoura RFC, Wright SW (Eds) *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*, pp 449–559. Paris: UNESCO Publishing, 1997.

Lawrenz, Evelyn, and Tammi L. Richardson. “How Does the Species Used for Calibration Affect Chlorophyll a Measurements by In Situ Fluorometry?” *Estuaries and Coasts* 34, no. 4 (2011): 872–83.

Leeuw, Thomas, Emmanuel Boss, and Dana Wright. “In Situ Measurements of Phytoplankton Fluorescence Using Low Cost Electronics.” *Sensors* 13, no. 6 (June 19, 2013): 7872–83. https://doi.org/10.3390/s130607872.

MacIntyre, Hugh L., and John J. Cullen. “Using Cultures to Investigate the Physiological Ecology of Microalgae.” In: Andersen, Robert A. (Ed) *Algal Culture Techniques.*, pp 287–326. Elsevier, 2005. https://doi.org/10.1016/B978-012088426-1/50020-2.

MacIntyre, Hugh L., Adrienne L. Stutes, William L. Smith, Carol P. Dorsey, Ann Abraham, and Robert W. Dickey. “Environmental Correlates of Community Composition and Toxicity during a Bloom of Pseudo-Nitzschia Spp. in the Northern Gulf of Mexico.” *Journal of Plankton Research* 33, no. 2 (February 1, 2011): 273–95. https://doi.org/10.1093/plankt/fbq146 .

Miller, J. N., and Jane C. Miller. *Statistics and Chemometrics for Analytical Chemistry*. 5th ed. Harlow, England, New York: Pearson Prentice Hall, 2005.

Mimuro, Mamoru. “Visualization of Excitation Energy Transfer Processes in Plants and Algae.” In *Discoveries in Photosynthesis*, edited by Govindjee, J. Thomas Beatty, Howard Gest, and John F. Allen, 171–76. Dordrecht: Springer Netherlands, 2005. https://doi.org/10.1007/1-4020-3324-9_16 .

Porra, R. J., E. E. Pfundel, and N. Engel. “Metabolism and Function of Photosynthetic Pigments.” In *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*, Jeffrey, S.W., Mantoura, R.F.C,. and Wright S.W. (Eds) 87. 10. United Nations Educational, Scientific and Culture Organization, Paris, (1997)

Proctor, Christopher W., and Collin S. Roesler. “New Insights on Obtaining Phytoplankton Concentration and Composition from in Situ Multispectral Chlorophyll Fluorescence.” *Limnology and Oceanography: Methods* 8, no. 12 (2010): 695–708. https://doi.org/10.4319/lom.2010.8.0695.

Roesler, Collin, Julia Uitz, Hervé Claustre, Emmanuel Boss, Xiaogang Xing, Emanuele Organelli, Nathan Briggs, et al. “Recommendations for Obtaining Unbiased Chlorophyll Estimates from in Situ Chlorophyll Fluorometers: A Global Analysis of WET Labs ECO Sensors.” *Limnology and Oceanography: Methods* 15, no. 6 (2017): 572–85. https://doi.org/10.1002/lom3.10185 .

Shoaf, W. Thomas, and Bruce W. Lium. “Improved Extraction of Chlorophyll a and b from Algae Using Dimethyl Sulfoxide.” *Limnology and Oceanography* 21, no. 6 (1976): 926–28. https://doi.org/10.4319/lo.1976.21.6.0926 .

Strickland, J D H. “Measuring the Production of Marine Phytoplankton,” Bulletin 112, Ricker, W.E., and Carter, N.M., (Eds), Fisheries Research Board of Canada, Pacific Oceanographic Group, Nanaimo, BC, (1960), pp 172

Welschmeyer, Nicholas A. “Fluorometric Analysis of Chlorophyll a in the Presence of Chlorophyll b and Pheopigments.” *Limnology and Oceanography* 39, no. 8 (1994): 1985–92. https://doi.org/10.4319/lo.1994.39.8.1985 .