

Calibration procedure for Slocum glider deployed optical instruments

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Abstract: Recent developments in the field of the autonomous underwater vehicles allow the wide usage of these platforms as part of scientific experiments, monitoring campaigns and more. The vehicles are often equipped with sensors measuring temperature, conductivity, chlorophyll a fluorescence (Chl a), colored dissolved organic matter (CDOM) fluorescence, phycoerithrin (PE) fluorescence and spectral volume scattering function at 117 degrees, providing users with high resolution, real time data. However, calibration of these instruments can be problematic. Most *in situ* calibrations are performed by deploying complementary instrument packages or water samplers in the proximity of the glider. Laboratory calibrations of the mounted sensors are difficult due to the placement of the instruments within the body of the vehicle. For the laboratory calibrations of the Slocum glider instruments we developed a small calibration chamber where we can perform precise calibrations of the optical instruments aboard our glider, as well as sensors from other deployment platforms. These procedures enable us to obtain pre- and post-deployment calibrations for optical fluorescence instruments, which may differ due to the biofouling and other physical damage that can occur during long-term glider deployments. We found that biofouling caused significant changes in the calibration scaling factors of fluorescent sensors, suggesting the need for consistent and repetitive calibrations for gliders as proposed in this paper.

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References and links

1. J. J. Cullen, F. M. H. Reid, and E. Stewart, "Phytoplankton in the Surface and Chlorophyll Maximum off Southern-California in August of 1978," *J. Plankton Res.* **4**(3), 665–694 (1982).
2. C. Belzile, C. S. Roesler, J. P. Christensen, N. Shakhova, and I. Semiletov, "Fluorescence measured using the WETStar DOM fluorometer as a proxy for dissolved matter absorption," *Estuar. Coast. Shelf Sci.* **67**(3), 441–449 (2006).
3. R. M. W. Amon, G. Budeus, and B. Meon, "Dissolved organic carbon distribution and origin in the Nordic Seas: Exchanges with the Arctic Ocean and the North Atlantic," *J. Geophysical Research-Oceans* **108**, (2003).
4. D. Stramski, R. A. Reynolds, M. Babin, S. Kaczmarek, M. R. Lewis, R. Rottgers, A. Sciandra, M. Stramska, M. S. Twardowski, B. A. Franz, and H. Claustre, "Relationships between the surface concentration of particulate organic carbon and optical properties in the eastern South Pacific and eastern Atlantic Oceans," *Biogeosciences* **5**, 171–201 (2008).
5. T. D. Dickey, "Emerging ocean observations for interdisciplinary data assimilation systems," *J. Mar. Syst.* **40**, 5–48 (2003).
6. M. S. Twardowski, H. Claustre, S. A. Freeman, D. Stramski, and Y. Huot, "Optical backscattering properties of the 'clearest' natural waters," *Biogeosciences* **4**, 1041–1058 (2007).
7. C. S. Roesler, and E. Boss, "In situ measurement of the inherent optical properties (IOPs) and potential for harmful algal bloom detection and coastal ecosystem observations," in *Real-Time Coastal Observing Systems for*

- Ecosystem Dynamics and Harmful Algal Bloom*, M. Babin, C. S. Roesler, and J. J. Cullen, eds. (UNESCO Publishing: Paris, France, 2008), pp. 153–206.
8. M. Lehaitre, L. Delauney, and C. Compère, "Biofouling and Underwater measurements," in *Real-Time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Bloom*, M. Babin, C. S. Roesler, and J. J. Cullen, eds. (UNESCO Publishing: Paris, France, 2008), pp. 463–494.
 9. K. Niewiadomska, H. Claustre, L. Prieur, and F. d'Ortenzio, "Submesoscale physical-biogeochemical coupling across the Ligurian current (northwestern Mediterranean) using a bio-optical glider," *Limnol. Oceanogr.* **53**, 2210–2225 (2008).
 10. M. J. Perry, B. S. Sackmann, C. C. Eriksen, and C. M. Lee, "Seaglider observations of blooms and subsurface chlorophyll maxima off the Washington coast," *Limnol. Oceanogr.* **53**, 2169–2179 (2008).
 11. E. Boss, D. Swift, L. Taylor, P. Brickley, R. Zaneveld, S. Riser, M. J. Perry, and P. G. Strutton, "Observations of pigment and particle distributions in the western North Atlantic from an autonomous float and ocean color satellite," *Limnol. Oceanogr.* **53**, 2112–2122 (2008).
 12. B. H. Jones, G. Toro-Farmer, I. Cetinic, N. Nezhlin, and M. Ragan, "Validation of MODIS algorithms for remotely sensed coastal ocean color using autonomous vehicles," presented at the Ocean Optics Conference XIX, Braga, Italy, 2008.
 13. E. Boss, and S. Maritorena, "Chapter 3, Uncertainties in the Products of Ocean-Colour Remote Sensing," in *Remote Sensing of Inherent Optical Properties: Fundamentals, Tests of Algorithms, and Applications*, Z.-P. Lee, ed. (IOCCG: Dartmouth, Canada, 2006).
 14. T. R. Parsons, Y. Maita, and C. M. Lalli, "Manual of Chemical and Biological Methods for Seawater Analysis," (Oxford, UK: Pergamon Press, 1984).
 15. J. J. Cullen, and R. F. Davis, "The blank can make a big difference in oceanographic measurements," *Limnol. Oceanogr. Bull.* **12**, 29–35 (2003).
 16. D. Ian, Walsh, Wet Labs, 620 Applegate St., Philomath, Oregon, 97370 (personal communication, 2008)
 17. K. Wróbel, and K. Wróbel, "Determination of aspartame and phenylalanine in diet soft drinks by high-performance liquid chromatography with direct spectrofluorimetric detection," *J. Chromatogr. A* **773**(1-2), 163–168 (1997).
 18. K. Hayakawa, T. Schilpp, K. Imai, T. Higuchi, and O. S. Wong, "Determination of aspartic acid, phenylalanine, and aspartylphenylalanine in aspartame-containing samples using a precolumn derivatization HPLC method," *J. Agric. Food Chem.* **38**(5), 1256–1260 (1990).
 19. C. S. Roesler, "Phytoplankton dynamics in the Gulf of Maine: Secrets revealed by seven years of hourly optical observations on a moored array," presented at the Ocean Optics Conference XIX, Braga, Italy, 2008.
 20. A. W. Decho, T. Kawaguchi, M. A. Allison, E. M. Louchard, R. P. Reid, F. C. Stephens, K. J. Voss, R. A. Wheatcroft, and B. B. Taylor, "Sediment properties influencing upwelling spectral reflectance signatures: The "biofilm gel effect,"" *Limnol. Oceanogr.* **48**, 431–443 (2003).
 21. R. E. Davis, M. D. Ohman, D. L. Rudnick, J. T. Sherman, and B. Hodges, "Glider surveillance of physics and biology in the southern California Current System," *Limnol. Oceanogr.* **53**, 2151–2168 (2008).
 22. R. L. Townsin, "The ship hull fouling penalty," *Biofouling* **19**(1 Suppl), 9–15 (2003).

1. Introduction

The development of the new class of autonomous underwater vehicles (AUVs) allows observations of the ocean with higher spatial and temporal coverage than most traditional profiling systems used in oceanographic studies, providing better resolution data necessary for understanding more complex biological and physical processes such as eddies, mesoscale ocean fronts, phytoplankton blooms, river and stormwater plumes. Slocum gliders are relatively small (about 2 m in length), buoyancy-driven underwater vehicles that complete dives to depths up to a few hundred meters in the span of a few hours while covering approximately 3 km horizontally. Gliders can be deployed for several months carrying a variety of oceanographic instruments tailored to a particular mission. Optical measurements from gliders can provide proxies for phytoplankton biomass [1], dissolved organic material absorption [2], dissolved organic carbon [3], total particulate mass and particulate organic carbon [4], evaluation of light availability for photosynthesis, optical tracking of the water masses and much more. Gliders can acquire large optical data sets during a single deployment, for example a three-week glider deployment in a coastal area can complete ~4,000 profiles up to 60 m depth, with most of the instruments sampling at ~2 Hz, resulting in more than 240,000 data points per instrument per deployment. Data received can be processed in almost real time and assimilated into physical and biogeochemical models [5].

Many issues arise due to the nature of the instrument and long time deployments. Factory calibrations usually differ from calibrations performed by the user after receiving the instrument for many reasons: calibration standards used by the factory, factory calibration performed prior the insertion of the optical instrument into the glider, or offsets that can occur

on the instruments during shipping. Instruments signals can fluctuate and drift due to power and temperature oscillations [6,7]. Biofouling and physical damage to the sensors can occur during long-term deployments, especially in coastal areas. Optical instruments deployed in the ocean are strongly affected by continuous biofilm growth on the optical windows [8]. Only 10% coverage of the optical path can have a strong effect on the quality of the data, and based on previous experiments [8], that amount of biofouling can be acquired on an instrument after one to three weeks deployment, which is within the timescales of a Slocum glider deployment.

In situ calibrations of instruments deployed onboard gliders are done by deploying calibrated complementary instrument packages in the proximity of the glider [9] or subtracting the deep water values in case of chlorophyll fluorescence [10]. Vicarious calibrations using remote sensing data from satellites can be performed [11,12], but retrievals of the inherent optical properties in the coastal areas are not so reliable.

In order to use these data sets for future biogeochemical models, or to constrain uncertainties related to the ocean color remote sensing measurements [13] we must be able to distinguish natural from instrumental variability, and ensure that we are not trading quantity for quality.

Laboratory calibrations are complicated due to the placement of the instruments within the body of the vehicle. Removal of these sensors is generally not practical, since sensors become an integrated with the glider. Therefore, in order to calibrate these instruments with some standard, the whole vehicle, or a section of it has to be shipped to the manufacturer for an extended period of time. Additionally, instruments should be calibrated in the mounted position, so the reference calibration pertains to the actual deployment calibration as much as possible.

Here we present a calibration procedure using a small chamber that enables easier laboratory calibrations of Slocum glider optical sensors. Additionally, we evaluate several different calibration standards and describe the calibration chamber that allows easy pre- and post-deployment calibrations, providing correction of the long deployments data sets. We also look at the impact of biofouling on the optical fluorescence instruments onboard gliders during long term deployments.

2. Materials and methods

2.1 Instrumentation testing

Two Slocum gliders were tested during the laboratory and field experiments, each one equipped with a SeaBird non-pumped, low-drag conductivity, temperature, depth (CTD 41CP) recorder optimized for lower power operation, an Airmar altimeter, and two WETLabs Inc Environmental Characterization Optics (ECO) Pucks. These ECO Pucks house a triplet of fluorometers to measure chlorophyll *a*, colored dissolved organic matter (CDOM) and phycoerythrin/rhodamine. The excitation/emission wavelengths were 470/695 nm, 370/460 nm and 540/570 nm, respectively. The second ECO Pucks is a backscattering sensor that will not be used in this experiment. The ECO Pucks are mounted in the bottom part of the science bay, facing downwards (Fig. 1(a)).

2.2 Calibration

Raw voltages from the instrument are converted to concentration of the targeted constituent using the following linear relationship:

$$[constituent] = scale\ factor * (output - dark\ counts) \quad (1)$$

where *dark counts* (DC) is the signal measured in the absence of the source light and *scale factor* is the linear fit between the instrument *output* and the real constituent concentration. Initial calibration of the tested fluorescence sensors was performed by the manufacturer, using a suspension of chlorophyll standard for chlorophyll fluorescence, and a quinine sulfate standard for CDOM fluorescence.

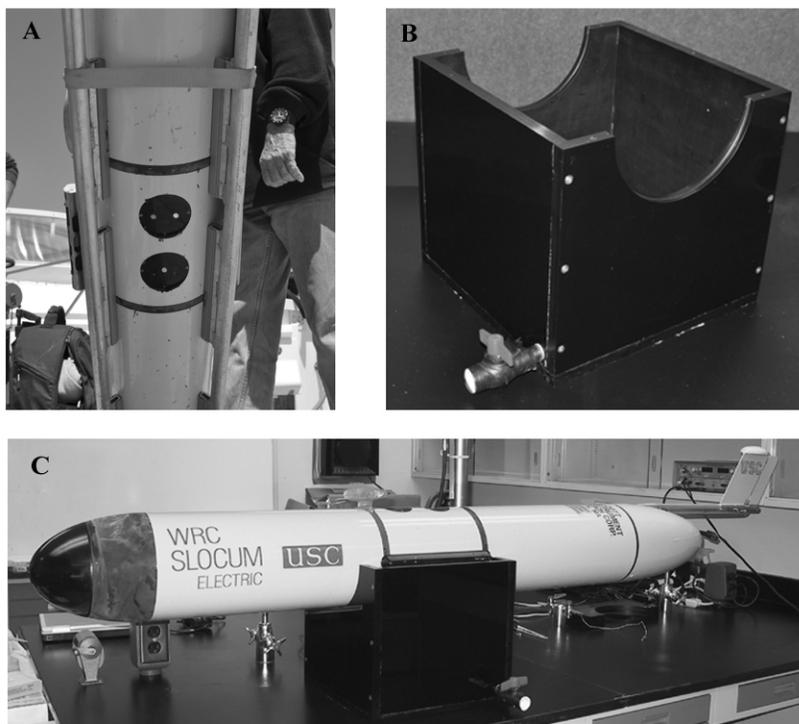


Fig. 1. (a) Slocum glider with science bay where optical instruments are located, (b) calibration chamber, (c) calibration chamber with glider mounted on it, ready for calibration

2.3 Dark counts measurements

The most accurate way of measuring the dark counts in these instruments is to cover all the detector windows with black electrical tape, but leaving the emitter windows uncovered [6]. While measuring dark counts, we repeated the measurements several times, and readjusted the dark tape, so we were sure that the measurements were consistent and with minimal or no light penetration possible. In order to test the temperature dependency of the dark counts, both laboratory room temperature and Milli-Q water within the chamber were set at 3 different temperatures (16, 20, and 25 °C). Additionally, the effect of environmental and platform dependent (power) parameters on the dark count values was tested in the field, with gliders deployed with covered pre cleaned sensors (as explained above) on several dives prior to a normal glider deployment.

2.4 Chlorophyll standard

For the chlorophyll calibration procedure we used a mixture of local phytoplankton isolates from Southern California Bight. Cultures of *Lingulodinium polyedra*, *Prorocentrum gracile*, *Thalassionema sp.*, *Pseudo-nitzschia spp.* and unidentified nano protists were grown in F/2 media, on 20° C and 12:12 light/dark regime. The primary standard was made by mixing specific percentage of different cultures with locally collected, filtered (0.2 µm pore size) sea water bringing the total volume to ~20 L (chamber volume). Lower concentrations were made by diluting the primary standard with the filtered sea water, and the 0 mg chl L⁻¹ (blank) by using Milli-Q water. This non-standard serial dilution approach ensured that the standards are comparable, and there are minimal species composition differences between the standards. Number of the calibration points throughout our experiment differed, due to the chlorophyll standard (healthy culture) or time availability (glider turnover). Calibration was always

performed in the period of 6 – 10 hours from the beginning of the light cycle, and cultures used were in their stationary growth phase.

For each of the calibration dilutions, 50 mL of the standard was filtered onto GF/F filters, and after 24 hours extraction in 90% acetone, chlorophyll concentration of the sample was determined in vitro using a Turner bench top fluorometer [14].

Due to the previously reported differences between the Milli-Q water and filtered sea water (FSW), and the importance of using FSW as a blank for the correct calibration [15], we ran a series of tests on both of the gliders and measured differences between FSW (0.2 μm pore size filtered calibration standard) and Milli-Q water. For our sensors, we found that values for FSW were 0.05 ± 0.02 , which is well within the confidence range of our instruments, and therefore used Milli-Q water as our “blank” calibration standard.

2.5 Colored Dissolved Organic Matter (CDOM) standard

The standard calibration procedure for WETLabs CDOM fluorometers is performed using a solution of quinine sulfate in 0.5 M H_2SO_4 [2]. Because the solution’s low pH could potentially damage the instrument and the glider body itself, quinine sulfate standard was not used for calibration of the glider deployed instruments. Instead, we used several dilutions of Sprite Zero[®] (The Coca-Cola Company) and MilliQ water to make a calibration curve for the glider CDOM fluorometer [16]. Sprite Zero[®] was chosen because its optical characteristics are similar to dissolved organic matter, possibly due to the presence of the aspartame [17,18], easy availability and because it is made to a consistent standard [16]. Walsh [16] found Sprite Zero/quinine sulfate scale factors ratio to be 0.73, when tested on a series of fluorometric measurements. To remove the carbonation of the Sprite Zero, ~20 L of the solution was de-bubbled overnight using a slow turning magnetic stirrer.

2.6 Calibration chamber

The calibration chamber is a box shaped container built out of 1.9 cm thick black acrylic, and shaped so the length and the width (35.5 cm) of the box is 7 cm longer and wider than the Slocum glider science bay (Fig. 1(b)). The side plates of the chamber are 32.5 cm high, while two opposite side plates of the calibration chamber are shaped such that the optical sensors mounted in the glider science bay are immersed in the calibration standard (Fig. 1(c)), and the optical windows of the instruments are 20 cm above the bottom of the chamber but still submerged into the standard. Leaks are prevented from the chamber using molded rubber seals where the glider sits in. The inside of the chamber is black and matted to minimize the possibility of light contamination. The chamber and glider are draped with an opaque black blanket to prevent external light from entering the chamber.

2.7 Glider deployments

During the fall and winter of 2008 and the spring of 2009 two Slocum gliders (named HeHaPe (H) and Rusalka (R)) were deployed a total of 3 times in a coastal area of the Southern California Bight. Deployments lengths varied, from 7 to 10 days for glider R (fall) and a 21 day deployment for glider H (early spring). Prior to the deployments, gliders were calibrated in the lab, using the above mentioned standard and calibration procedure (*pre* calibration). Upon recovery, the science bay was wrapped in wet cloth to prevent the drying of the possible biofouling growth, and post-calibration was done in the lab within 24 hours of recovery. First post calibration (*post A*) was done without cleaning the sensor windows. Therefore those measurements were expected to be affected by biofouling and instrumental drift. After the first calibration, all sensor windows were cleaned using isopropyl alcohol, and another calibration was performed to identify non-fouling instrument drift (*post B*). Time dependent linear interpolation between *pre* and *post A* calibration was used to calculate new scale factors that were used to process the data into concentration units [7].

3. Results and discussion

3.1 Dark counts (DC)

Measured dark counts for the glider (R) mounted chlorophyll fluorometer (immersed in the calibration chamber filled with Milli-Q water) ranged from 50 to 53, with a mean value of 51 counts compared to the factory values of 50 counts. Dark counts measured for the glider mounted CDOM fluorometer varied between values of 35-37, with a mean value of 36 (compared to dark counts of 34 from factory calibrations). Average DC for the chlorophyll fluorometer deployed on glider H was 56 (range 53-58), compared to the 60 from the factory calibration, while mean DC for the CDOM fluorometer was 50 (range 47-52), compared to the value of 62 from the factory calibration. For all our DC measurements, uncertainties were defined as the DC range and propagated in further calculations. Repetitive measurements of the dark counts for both of the gliders (performed during the pre and post deployment calibrations) for 80% of the points varied in the predetermined uncertainties range (Fig. 2 dashed lines). During the field measurements of the dark counts performed on the instruments deployed on glider H, we again found discrepancies from the factory observed values (10-20% lower), but most of the data points (~70%) are still within the values observed pre and post deployment in the lab (Fig. 2, dashed lines). Observed offsets from the factory calibration dark scale numbers have been reported [6,11], and may result from the different procedure in dark count measurements, as well as the fact that the instruments were not mounted within the glider bodies when the factory calibrations were performed.

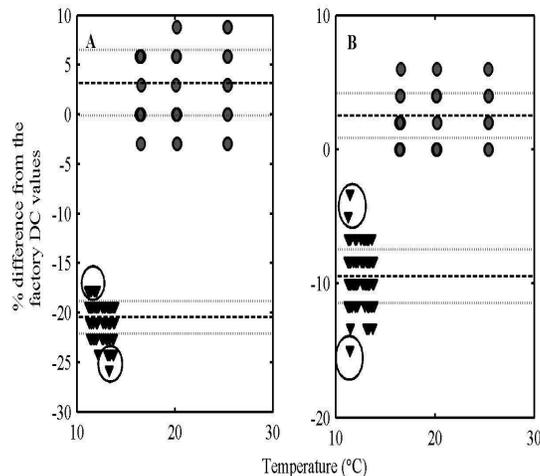


Fig. 2. Variability of Dark Counts as a function of temperature, observed for (a) CDOM fluorescence sensor and (b) chlorophyll fluorescence sensor deployed on the gliders, triangles - field deployment (instruments on H glider), and circles lab measurements (instruments on R glider). Circled triangles are measurements collected on the top or bottom of the dive. Dashed line represents in laboratory predetermined mean values at 20°C, while dotted lines represent standard deviation range for the same DC values.

It has been previously noted that fluctuations of the dark counts are dependent on temperature [15,19], other environmental parameters [6], and power oscillations induced by other instruments and motors on the platform (glider). Temperature dependency of dark counts was tested in the lab using three different temperatures (16, 20, and 25 °C). Around 75% of measured dark counts were found to fall within the previously established standard deviation range, and no trend associated with temperature was observed in the data set (Fig. 2). Dark counts measured in the field to a depth of 40 m showed no significant temperature or pressure dependency (temperature having inverse relationship to pressure; 11°C ~40 m depth). As can be seen in Fig. 2, highest outliers for both CDOM and chlorophyll DC are found at the top or the bottom of the dive (circled triangles). These outliers could result from a

power oscillation caused by the ballast pump and the battery positioning motor turning on at the top and bottom of the diver profiles. Additionally, these outliers fall outside of 2 standard deviation range, and should not be considered for the final calculation of the DC that will be used in the data calculation and analysis.

Both laboratory and field measurements of the DC confirm the importance of repetitive laboratory measurements of DC, since differences in DC are significant enough (~20% difference) to affect the final measurements. Additionally, these differences are not comparable between the 2 instruments, and do not follow changing trends in the environment. At the top and bottom of the dive, results must be used with caution because platform dependent variability cannot be compensated by measurable environmental effects.

3.2 Scale factors

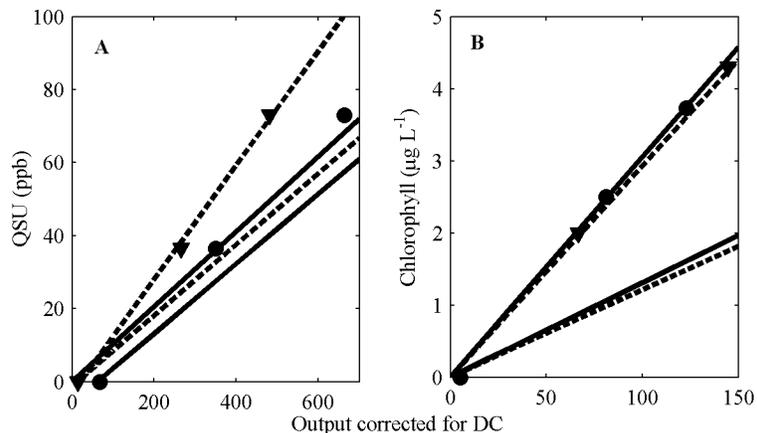


Fig. 3. Comparison of the factory and in house calibrations for glider deployed fluorometers. X axis presents the instrument output minus dark counts values, and y-axis presents concentration of the standard. Dashed line with triangles represents in-house calibration points for the H glider, while solid line with circles calibrations for R glider. Dashed line (H) and solid line (R) with no markers are factory calibration values. A) CDOM fluorescence sensor and B) chlorophyll fluorescence sensor deployed on the glider.

Factory calibration scale factors differed from the slope obtained from our experiments (Fig. 3). For both of the glider deployed chlorophyll fluorimeters our scale factor ($R = 0.0305 \mu\text{g chl L}^{-1} \text{ count}^{-1}$, $H = 0.0293 \mu\text{g chl L}^{-1} \text{ count}^{-1}$) was higher than the factory provided scale factors ($R = 0.0131 \mu\text{g chl L}^{-1} \text{ count}^{-1}$, $H = 0.0121 \mu\text{g chl L}^{-1} \text{ count}^{-1}$). Uncertainties (range) for our measurements were $R = \pm 0.09 \mu\text{g chl L}^{-1}$ and $H = \pm 0.14 \mu\text{g chl L}^{-1}$. As suggested by the manufacturer, and other authors [11], our calibration was performed using a local species assemblage. Experimental scale factors were found to be up to 3 times higher than the factory ones, possibly because of the differences between the pigment composition within the local community and the chlorophyll standards used by the factory. As noticed before, the excitation light used by this chlorophyll fluorometer (470 nm) is not directly absorbed by chlorophyll, but rather by the photosynthetic accessory pigments [10], which makes the fluorescence response dependent on the phytoplankton community composition.

In situ calibrations from a parallel CTD casts or *in situ* water samples could be affected by the variations in quantum yield of fluorescence and/or by the community composition, especially in cases where the environment is highly stratified. Cultures grown under constant conditions (light, nutrients, and temperature) provide repeatable calibrations that facilitate accumulation of long term intercomparable data sets.

CDOM fluorometer calibrations showed similar offsets where the scale factor ($H = 0.1561 \text{ ppb/count}$, $R = 0.1027 \text{ ppb/count}$) differed up to 50% from the initial factory calibrations ($H = 0.0939 \text{ ppb/count}$, $R = 0.0908 \text{ ppb/count}$), as it can be observed on Fig. 3(b). Uncertainties for

our measurements were $R = \pm 0.21$ ppb and $H = \pm 0.62$ ppb. Large differences in scale factors could be attributed to the calibration standard, but when the same standard is applied to the stand-alone ECO triplet (data not shown) difference on scale factors (factory vs. in laboratory) is only 5%. This suggests that large differences between scale factors are due to the placement of the instrument in the body of the glider, i.e. i.e., the factory calibration is not performed while the instrument is mounted on the vehicle or platform.

3.3 Assessment - Glider deployment

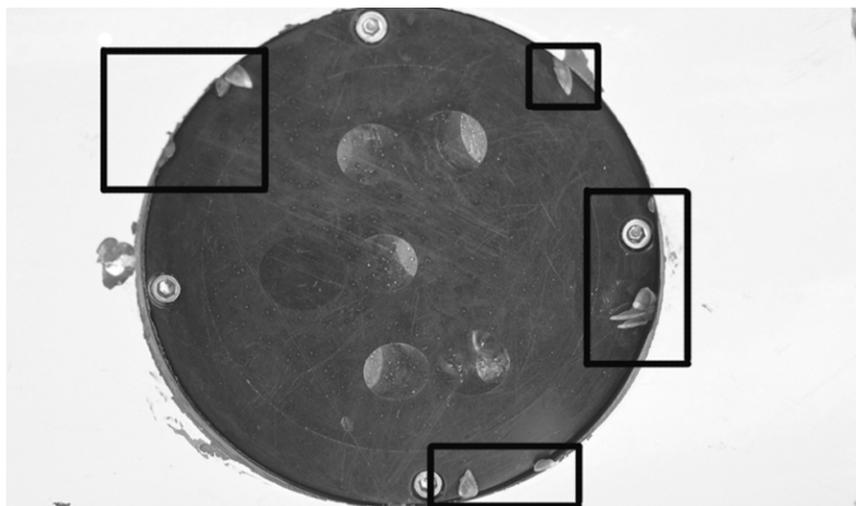


Fig. 4. Biological growth found on body of HeHaPe glider after 3 weeks of deployment in Southern California Bight. Black squares highlight goose-neck barnacles growing on the sides one of the ECO pucks, near the optical window.

Our gliders were deployed within a productive coastal area, and spent ~50% of their time in the euphotic zone, with most dives going to 60 m, and deepest dives to 80 m (along San Pedro shelf). Extensive biological growth was found on the glider bodies after each deployment (Fig. 4), even the short 7-day deployment. Following the pre and post calibration procedure for the several glider deployments, we observed differences (up to 120%) between *pre*, *post A* and *post B* calibration values (Fig. 5(a) and (b)).

Instrumental drift, defined as the difference between the *pre* and *post B* calibration, could be due to the changes at both the detector, as seen in the pre and post dark counts differences, and the emitter. Instrumental drift did not seem to have a large effect on our calibrations, 5% for the CDOM fluorometer and <1% for the chlorophyll fluorometer, which falls within the error previously determined for the DC (Fig. 2).

Biofouling caused drift, defined as the difference between *post A* and *post B* calibrations, and was large for both the CDOM and chlorophyll scale factors (Fig. 5). Biofouling caused large drift, but no trend based on the deployments length was obvious.

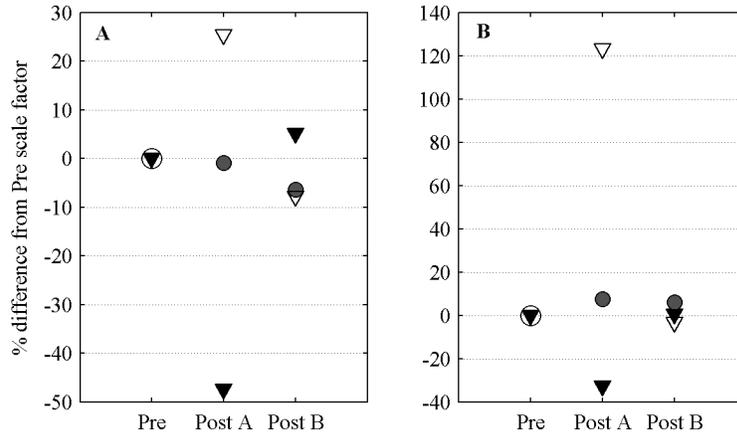


Fig. 5. Comparison of *pre* (all values equal 0), *post A* (without optical window cleaning) and *post B* (with cleaning of the optical windows) scale factors values for R 10 days deployment (filled circles) and H 10 day deployment (black triangles), and H 3 week deployment (open triangles) expressed as percentage difference from the initial *pre* calibration. Panel A – CDOM fluorometer, Panel B Chlorophyll fluorometer.

The formation of biofilms, although highly dependent on environmental factors, is thought to have 5 successional stages [8] starting with adhesion of inorganic and organic molecules, progressing through bacterial and algal communities, and maturing into a macroorganismal community. It is possible that differences between the biofouling related drifts are due to the different stages in the succession of the biofilm, as well as the ambient community and other environmental conditions present during the deployments. The glider deployments used in our analysis are from different periods of the year (7 days – summer/fall, 10 and 21 days – winter/early spring) perhaps resulting in seasonal effects on the community composition of the biofilm layer. Organic molecules (predominantly exopolysaccharides), chlorophyll, and other pigments assumed to be part of the biofilm deposited on the optical windows. The pigments absorb light in the portion of the spectra where the fluorometer excitation for CDOM and chlorophyll are located (370 and 470 nm respectively) [20]. It is possible that growth on the optical windows blocks or absorbs some of the light coming from the emitter, therefore lowering the expected (calibrated) amount of light reaching the instrument detector. If the biofilms fluoresce at the detector wavelengths, a cumulative effect that includes both concentration in the measurement volume and the biofilm on the instrument windows will result in higher than expected measurements.

In order to correct our data set, we assumed that the scale-factor drifts linearly with time between the *pre* and *post A* calibrations. Biofilm growth is exponential for up to 3 weeks of deployment [8 and references therein], but biofilm dispersal processes and shedding due to the vehicle movement could change the spatial coverage and/or thickness of biofilm and make the growth curve somewhat non-linear. Such oscillations from the assumed linear biofilm growth trend could affect our data, but in absence of a continuous calibration reference (such as internal dark counts), we find this approach is the most appropriate one.

When Eq. (1) with temporally corrected scale factors is applied to our data set (Fig. 6), both chlorophyll and CDOM fluorescence values fall outside of the measurement uncertainties established in pre-deployment calibration after only one week of deployment. For longer deployments, where we observed higher differences in scale factors, the uncertainty in the corrected values is even greater. If the biofilm on the optical window is sufficiently developed that it totally masks the ambient concentrations, even the best calibration procedures will not be able to reconstruct the data.

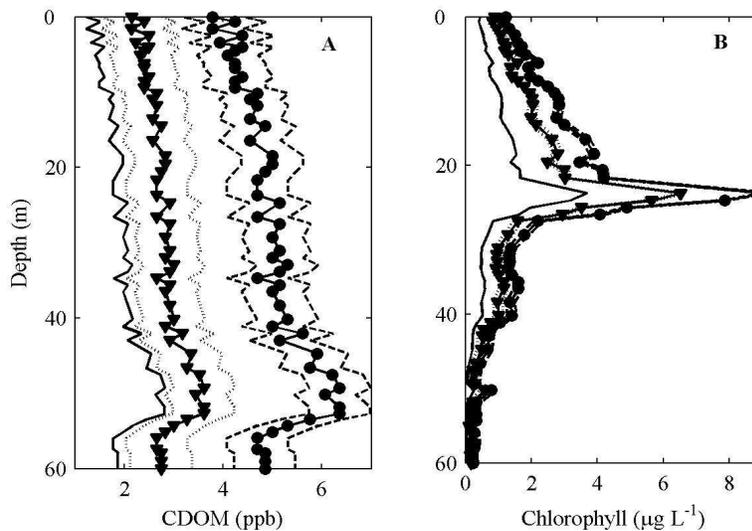


Fig. 6. Selected profile from the 7th day of the 10-day H glider deployment. Panel A shows CDOM data and panel B shows the chlorophyll data for the same profile. Black solid lines indicate values calculated using factory calibration; black solid lines with circles indicate the pre-deployment calibration; dashed lines – confidence values; black solid line with triangles using a temporal derived calibration, dotted lines – confidence values.

The observed effect that biofouling had on our data has not previously been reported for gliders deployed in this area. Davis et al. [21] noticed minimal biofouling during the 97 days of the Spray glider deployment in the Southern California area. The reason for it could be that the Spray gliders were diving all the way to 500 m during that mission, which could result with the die-off of all organisms attached to the surface of the glider [10]. Moored instruments in the coastal ocean, on the other hand, are usually equipped with biofouling preventing technology such as *bio-wiper*, copper tubing or/and copper tape which allows for longer deployments with higher quality data [19].

4. Summary

In this study we reaffirm the importance of proper calibrations when dealing with data sets acquired from long term deployments of optical sensors. The differences between factory established calibrations and pre- and post-deployment calibrations, emphasize the importance of the consistent and repetitive sensor calibration procedures.

The calibration chamber allowed easier, lab-controlled, repetitive calibrations that can be used for both fluorescence sensors and backscattering sensors on the gliders, due to the low reflective nature of the chamber. It can be also used for intercalibration of multiple instruments deployed within an observational network, to ensure consistent, interchangeable data sets. Repetitive calibrations and dark count measurements enable us to track instrumental drift and constrain uncertainties within our measurements. These large data sets then become valuable inputs to biogeochemical models and for calibration and validation of remote sensing observations.

Biofouling proved to be a significant concern for retrieving calibrated data. Connection between the length of the deployment and effect of the biofouling was not straightforward, due to the variability of the scale factors from deployment to deployment. These results point to the need for effective antifouling strategies optical instruments deployed autonomous platforms. Additionally, macrobiofouling observed on gliders raises the question about its effect on platform drag, velocity and power consumption of the glider, a well known problem for all aspects of the marine industry [22].

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