

EXPORTS 2018 Calibration Report  
**Chlorophyll Fluorescence Calibration to HPLC Chlorophyll a**

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**Overview**

Below is a document that fulfills a primary objective of calibration the chlorophyll fluorescence sensor on the CTD-rossette (hereafter referred to as CTD ChlF) through match-ups with high performance liquid chromatography (HPLC) samples.

Calibration of the CTD data should follow this form using values from Table 1:

$$\text{CTD ChlF Corrected (ug/L)} = [\text{Raw CTD ChlF (V)} - \text{Dark Value (V)}] / [\text{Slope of Calibration Curve (V/(ug/L))}]$$

**Table 1.** Summary of calibration coefficients for ChlF sensors.

Coefficient	Sally Ride	Revelle	Sikuliaq	Wirewalker	Seaglider	Lagrangian Float
Slope	0.64 ± 0.01 V/(ug/L)	0.69 ± 0.01 V/(ug/L)	4.0 ± 0.2 (ug/L / ug/L)	243.5 ± 0.3 Counts/ (ug/L)	269 Counts/ (ug/L)	0.00181 Counts/(mg/ m <sup>3</sup> )
Dark	0.087 ± 0.0009 V	0.051 ± 0.002 V	0.03 ± 0.01 ug/L	53 counts	42 Counts	53 Counts

The key steps to the calibration are:

- Subtract the average deep value from the ChlF sensor data as a dark value
- Use the bottle file spreadsheet matched to HPLC data restricting matches to PAR values below 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  to linearly fit the data
- Correct the CTD ChlF sensor using the computed calibration curve
- Calibrate your sensor to the corrected CTD sensor by matching within casts along isopycnals using the same PAR threshold

**Table of Contents**

1. Calibration of the CTD ChlF Sensors using HPLC from the Bottle Files for the EXPORTS 2018 Cruise in the North Pacific (Aug-Sep 2018) - detailed information on how the calibration was determined as well as choices for dark values
2. Calibration of the CTD ChlF Sensor using HPLC from Bottle Files for the Recovery Cruise (Nov-Dec 2018)
3. Comparison between the 1m binned (down-cast) FL and the bottle-sampled HPLC (upcast)
4. Calibration of Autonomous Assets
  - A. Wirewalker
  - B. Seaglider
  - C. Lagrangian Float

## 1. Calibration of the CTD ChlF Sensors using HPLC from the Bottle Files

HPLC data were used from the bottle file spreadsheet version 2- <https://drive.google.com/drive/u/o/folders/1shhZmFYHcfGEzkt2igvAD5FpFjiCsMhy> . A summary table of uncertainties associated with the pigment analysis are summarized in table 2. In section A, we provide a recommendation for a linear calibration -- converting the raw voltages of the CTD ChlF sensors on the Revelle (RR) and Sally Ride (SR) to units of ug/L (hereafter referred to as CTD Chl). In section B, we apply the calibrated CTD Chl data to the Wirewalker data as a demonstration of the steps that should be followed for calibration of the other ChlF sensors used during EXPORTS 2018.

**Table 2.** Summary of uncertainties reported in the HPLC total Chla analysis.

Uncertainty	Sally Ride	Revelle
HPLC TChla Analysis Precision CV (%)	0.43	0.35
HPLC TChla Replicate Filter Precision CV (%)	1.97	5.31 (reported also as 4.37 without one pair)

At-A-Glance Calibrations Steps for HPLC-->CTD ChlF:

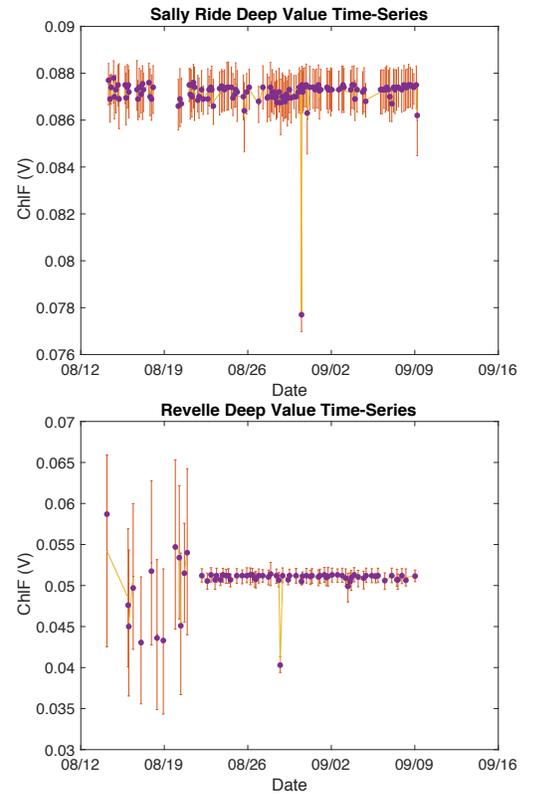
- 1) Subtract sensor dark counts. We used the average deep value. There were also dark counts from a taped cast and from a pre-cruise test on this spreadsheet, which we compared against (FL\_compare\_EXPORTS01 file - <https://docs.google.com/spreadsheets/d/1pIq3ee5EKUv6UKOBZA2zPuuKozN6umKTzRcyVPJ2CHg/edit#gid=1798388318>)
- 2) Match HPLC stations to CTD cast station numbers
- 3) Match the HPLC depths to the bottle-file CTD ChlF (preferred method 1, section 1), and extracted directly from the 1m binned CTD ChlF profiles (method 2, section 3).
- 4) Evaluate using only night-time values (sunset+2h to sunrise) (matlab function suncycle.m) or using a PAR threshold to avoid quenching impacts
- 5) Linearly fit the data using least squares fitting (Matlab curve fitting toolbox). Calculate the uncertainty on the slope and the R<sup>2</sup> value.

### *Dark Value Evaluation*

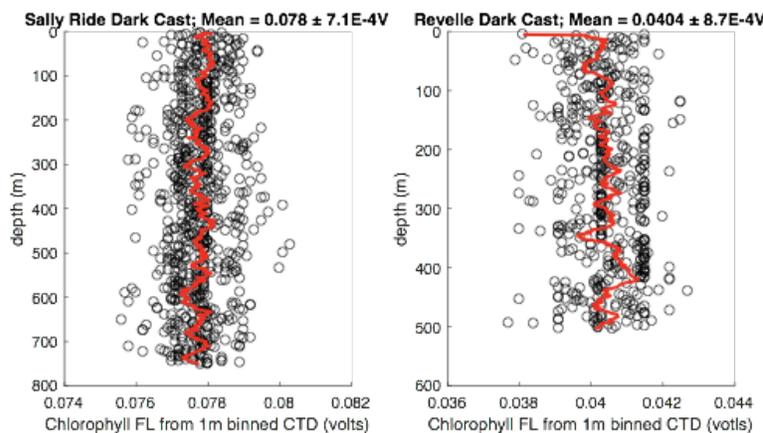
On the Revelle (RR), the FLNTU3003 sensor was deployed on the CTD package. No prior calibration files were available for this sensor. A dark count measurement of  $47 \pm 2$  was collected at the beginning of the EXPORTS experiment. Since the CTD logs in voltage, the conversion between these units (given the instrument dynamic range of 5V/4096 counts) yields a dark value of  $0.0574 \pm 0.0024$  volts. We also evaluated the dark counts (in voltage) from deep values, determined as the mean of all fluorescence values from the CTD binned data greater than 475 meters for all casts (with deep enough values). This depth was chosen to be able to compare with deep values from the Wirewalker, which travelled to 500 m. Figure 1 shows the time-series of the deep values. The Sally Ride was consistent, whereas the Revelle had large

variations in the beginning of the time-series where the sensor appeared to have much more noise in the measurements. The low values on each plot correspond to the taped casts. This gave a value of  $0.051 \pm 0.002$  volts. Cast number 048 was conducted with tape as a dark cast. The median of this entire cast was  $0.0403$  V and this value was consistent with the deep value of this dark cast, as the median of values below  $475$  m was also  $0.0403$  V. The mean of the cast was  $0.0404 \pm 8.7E-4V$  (figure 2). The difference between the taped cast values and the estimated in situ dark counts is probably due to non-chlorophyll fluorescing matter (such as CDOM) and thus, we are using the deep value estimated in situ dark counts for the calibration. **The deep value of  $0.051 \pm 0.002$  V was used as the dark count for the RR sensor. This value was subtracted from the raw CTD ChlF data for final calibration.**

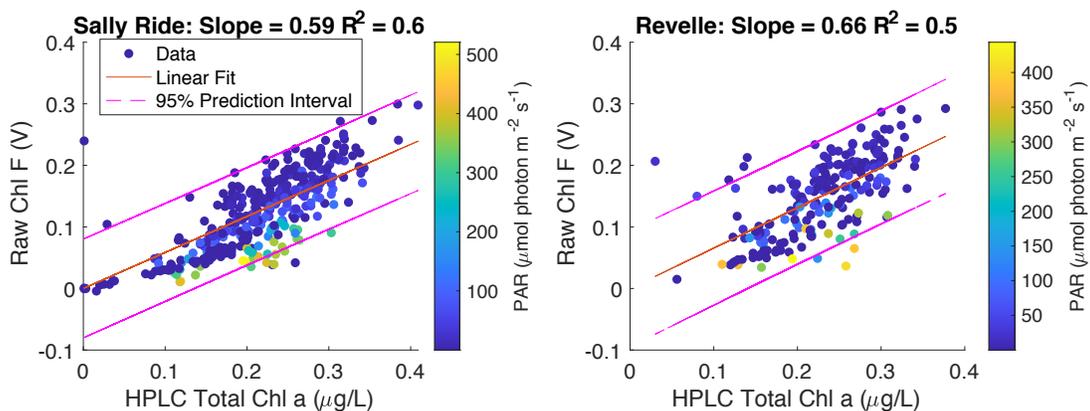
For the Sally Ride (SR), the FLBBRTD3522 was deployed on the CTD package. No prior calibration files were available for this sensor. A dark count measurement of  $46 \pm 1$  counts, or  $0.0492$  V was collected at the beginning of the EXPORTS experiment. We also evaluated the dark counts (in voltage) from deep values, determined as the mean and median of all fluorescence values from the CTD binned data greater than  $475$  meters for all casts (with deep enough values) to be  $0.087 \pm 0.0009$  volts. The mean of the taped cast was  $0.078 \pm 7.1E-4V$ . **The deep value of  $0.087 \pm 0.0009$  volts was used as the dark count for the Ride sensor. This value was subtracted from the raw CTD ChlF data for final calibration.**



**Figure 1.** The average fluorescence greater than  $475$  m for each cast.

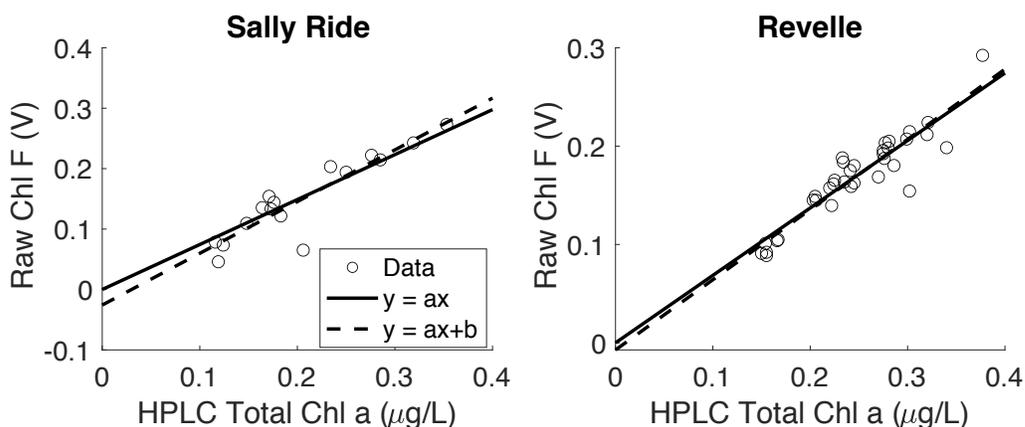


**Figure 2.** Dark values obtained from the cast by taping over the sensor: cast 048 for the Revelle and 092 for the Sally Ride. Mean values of the cast were  $0.0404 \pm 8.7E-4V$  and  $0.078 \pm 7.1E-4V$ , respectively. Black points show actual values and red line shows  $20$  m running average computed using `movmean.m`



**Figure 3.** This shows all of the bottle file data matches for the Sally Ride (left) and Revelle (right) HPLC Chla to the raw ChlF. Data are colored by the PAR values to show that the matches at higher values of PAR are decreased by quenching, which are lowering the slope and should be excluded for correcting the sensor values.

Once dark values were subtracted, for the calibration between HPLC and ChlF, only the night time values were used to avoid influences of non-photochemical quenching (NPQ) (figure 4). This was done by using a function in Matlab called `suncycle.m` adapted by R. Pawlowicz from `Air_Sea Toolbox` version 2.0. This code determines the time of sunrise and sunset using latitude, longitude, and date. All match ups from at least two hours after sunset until sunrise were kept as values not affected by NPQ (Roesler et al., 2017). Figure 4 below shows the match ups of HPLC to CTD fluorescence using bottle-file matchups. The data were linearly fit using the Matlab Curve Fitting Toolbox (also can use `polyfitZero.m` for fitting and `rsquare.m` for computing the coefficient of determination ( $R^2$ )). The same fitting functions were also used for future fits computed in this document. It is also important to note that the fits from figure 4 and table 2 were not chosen as the final method for calibration, but are provided here as a reference.

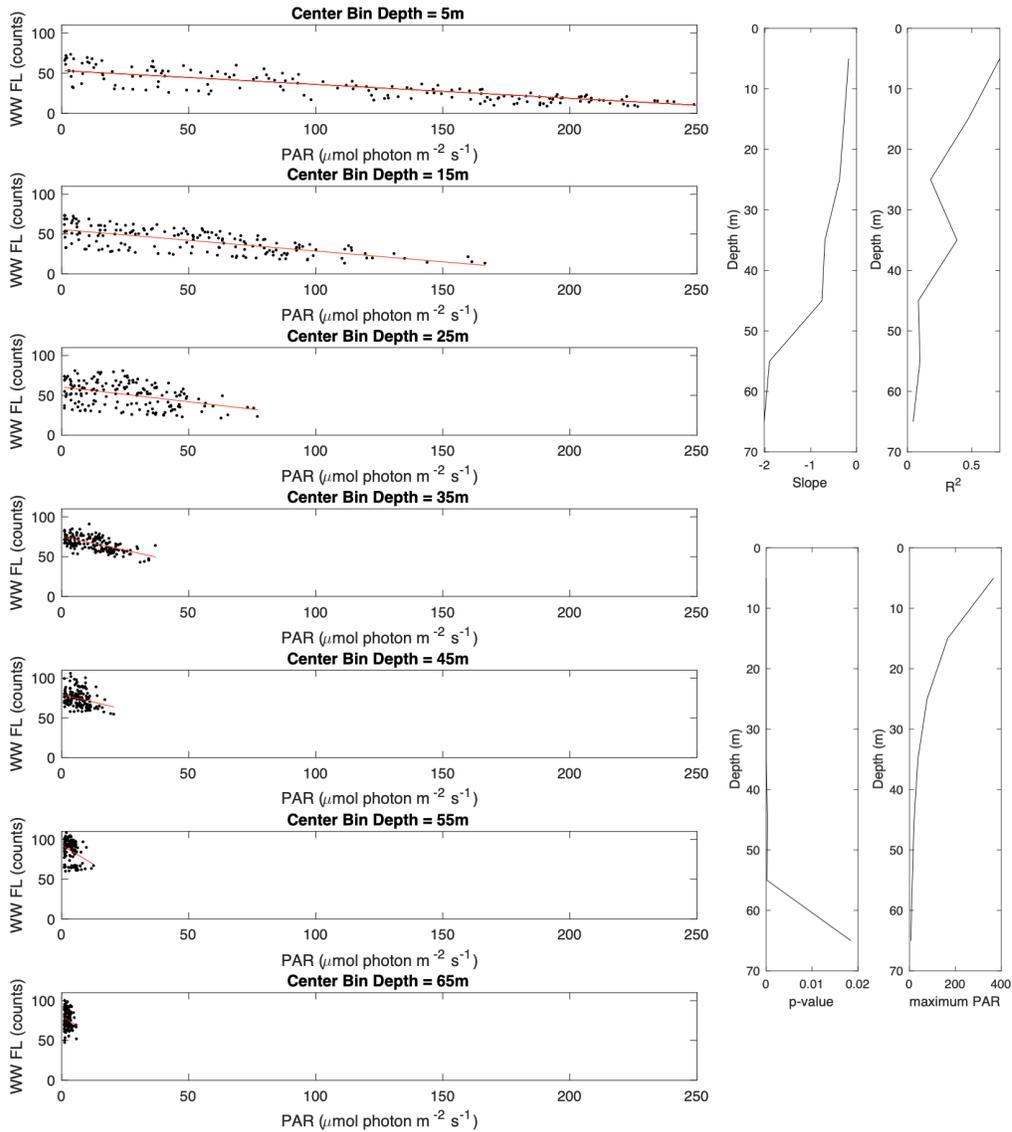


**Figure 4.** The two plots show the Sally Ride and Revelle binned CTD fluorescence from bottle files matched to the HPLC Chl a with both a linear fit through zero. These are the points from two hours after dark until dawn only, so no quenching is impacting the data. See table 2 below for fit equations.

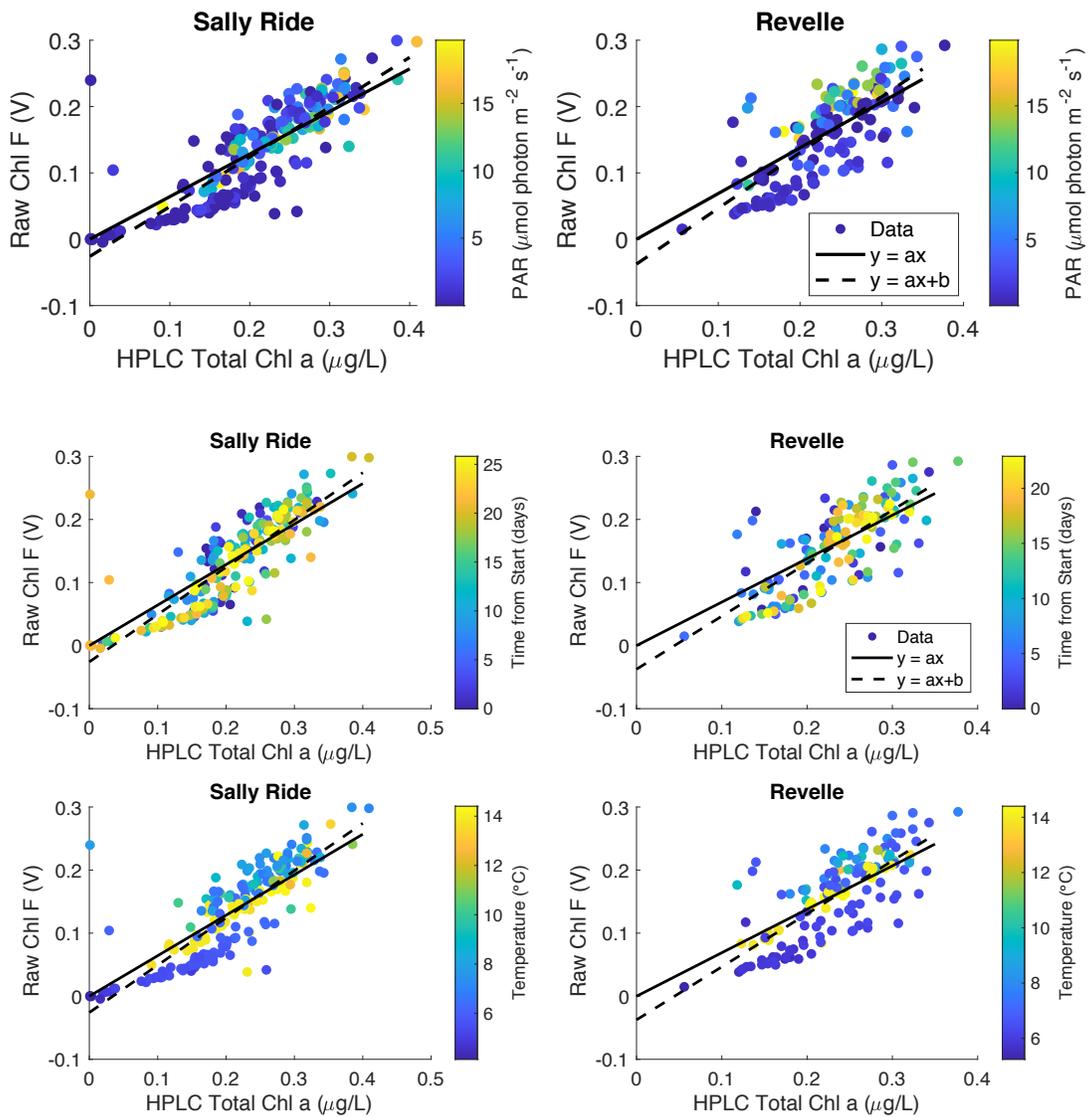
**Table 2.** Equations of fits for the night-time only bottle HPLC to CTD ChlF matches. (see fig 4).

<b>Cruise</b>	<b>Method</b>	<b>Dark Value (V)</b>	<b>Equation</b>	<b>R-Squared</b>
Sally Ride	Night-time bottle data	0.087	$(0.74 \pm 0.03) * X$	0.81
			$(0.86 \pm 0.1 * X) + -0.03 \pm 0.99$	0.83
Revelle	Night-time bottle data	0.051	$(0.69 \pm 0.01) * X$	0.86
			$(0.71 \pm 0.05 * X) + -0.007 \pm 0.8$	0.86
Sally Ride	Night-time bottle data	0.078	$(0.78 \pm 0.03) * X$	0.83
			$(0.86 \pm 0.1 * X) + -0.02 \pm 0.98$	0.83
Revelle	Night-time bottle data	0.0404	$(0.73 \pm 0.01) * X$	0.86
			$(0.71 \pm 0.05 * X) + -0.003 \pm 0.76$	0.86

We also assessed the possibility of using a **PAR threshold instead of night-time only casts** to increase the number of data points included in the calibration to include deep values that would also be unimpacted by NPQ. To determine a reasonable PAR threshold, we binned the Wirewalker fluorescence observations by 10 m bins and plotted against PAR (figure 5). Then we used the determined 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  threshold to compute slopes (figure 6). This value is similar to the 15  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  from Xing (2018).



**Figure 5.** Wirewalker fluorescence observations binned by 10 m depth intervals plotted against PAR shows that quenching occurs in the upper ~40 m. In the 50 m bin, the R-squared decreases and in the 60 m bin the p-value increases. The maximum PAR value in the 40 - 50 m bin was 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . From this, we determined that using the maximum PAR value of 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the 40-50 m bin was a reasonable cutoff.

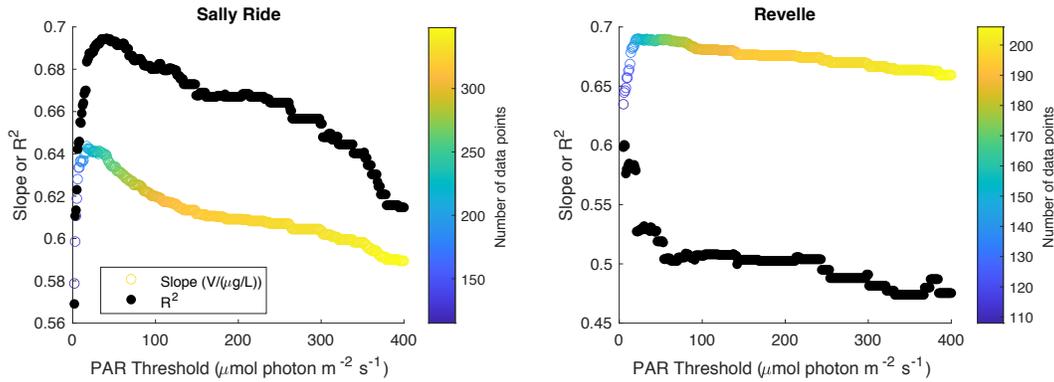


**Figure 6.** This assesses the use of a PAR threshold of  $20 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  instead of using only night-time casts to increase the number of data points see table 3 for equations. Upper plots are colored by PAR, middle by the time since the start of the casts, and lower by the CTD temperature.

**Table 3.** Equations of fits for the PAR threshold of 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for bottle file HPLC to CTD ChlF matches. (see fig 6). Fits in bold indicate they were chosen for calibration.

<b>Cruise</b>	<b>Method</b>	<b>Dark Value (V)</b>	<b>Equation</b>	<b>R-Squared</b>
Sally Ride	PAR Threshold bottle data	<b>0.087</b>	<b><math>0.64 \pm 0.01 *X</math></b>	<b>0.69</b>
			$(0.75 \pm 0.03 *X) + -0.03 \pm 0.8$	0.70
Revelle		<b>0.051</b>	<b><math>0.69 \pm 0.01 *X</math></b>	<b>0.58</b>
			$(0.84 \pm 0.06 *X) + -0.04 \pm 0.9$	0.60
Sally Ride		0.078	$0.697 \pm 0.01 *X$	0.697
			$(0.75 \pm 0.03 *X) + -0.0168 \pm 0.8$	0.704
Revelle		0.0404	$0.73 \pm 0.01 *X$	0.589
			$(0.84 \pm 0.057 *X) + -0.027 \pm 0.92$	0.599

We also assessed the robustness of the PAR thresholds by plotting the slope and fit of the data by computing these values over a range of PAR values, with a minimum of 100 CTD-HPLC matches (figure 7). This analysis determined thresholds to be 17  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the Sally Ride and 24  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the Revelle, which are similar to the 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  cutoff, supporting the previously determined PAR threshold.



**Figure 7.** The upper two plots show the range of slopes and  $R^2$  computed at varying PAR thresholds for both ships' sensors. When using the PAR threshold that resulted in the maximum slope, 17  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the Sally Ride and 24  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for the Revelle, the same slopes were obtained from using the previously determined PAR threshold of 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ .

**Table 4.** Equations of fits for the PAR threshold of 17 and 24  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for Sally Ride and Revelle, respectively, bottle file HPLC to CTD ChlF matches. (see fig 7).

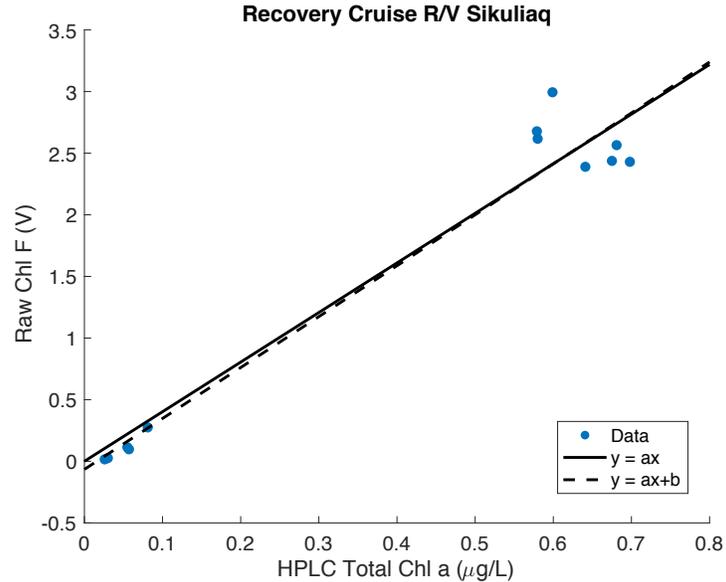
Cruise	Method	Dark Value (V)	Equation	R-Squared
Sally Ride	PAR Threshold bottle data	0.087	$(0.64 \pm 0.01)*X$	0.68
	17 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$		$(0.75 \pm 0.03 * X) + -0.03 \pm 0.8$	0.7
Revelle	PAR Threshold bottle data	0.051	$(0.69 \pm 0.02)*X$	0.53
	24 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$		$(0.76 \pm 0.06 * X) + -0.02 \pm 0.83$	0.53
Sally Ride	PAR Threshold bottle data	0.078	$(0.68 \pm 0.01)*X$	0.7
	17 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$		$(0.75 \pm 0.03 * X) + -0.02 \pm 0.8$	0.7
Revelle	PAR Threshold bottle data	0.0404	$(0.73 \pm 0.015) * X$	0.53
	24 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$		$(0.76 \pm 0.06 * X) + -0.007 \pm 0.83$	0.53

## 2. Recovery Cruise R/V Sikuliaq Nov-Dec. 2018:

This section is only relevant for calibration of autonomous vehicles recovered post-EXPORTS. The recovery cruise sensor on the R/V Sikuliaq was the FLBBRTD-3522. Bottle file headers indicated that calibration was conducted on the sensor on May 9, 2018 (Scale factor = 9; Vblank=0.086). Since the raw voltages were not carried through to the bottle files, the calibration to HPLC data was conducted on the dataset with a dark value was the Vblank of 0.086. The units were unreported in the bottle files, but should be ug/L.

Looking at the in situ data from cast 2 which profiled to 1000 m shows that below 475 m the deep dark value is  $0.03 \pm 0.01$  ug/L. This was subtracted from the CTD calibrated data to account for any non-chlorophyll fluorescence contamination.

**Figure 8.** This plot shows the night-time recovery cruise CTD fluorescence from bottle files matched to the HPLC Chl a with a linear fit through zero chosen for calibration (see table 5).



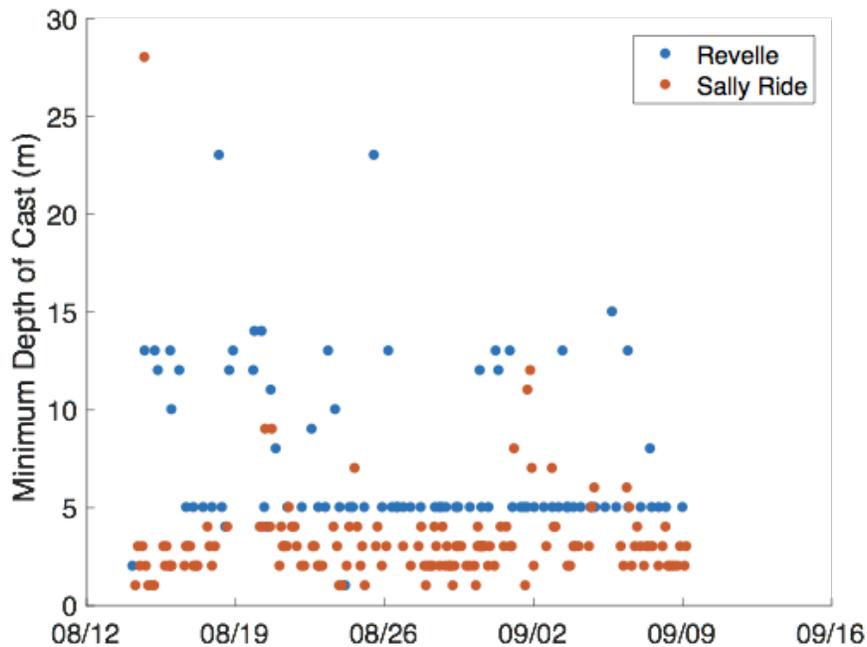
Since there were very few data points, including all of the matches was assessed as a possible option, given that the maximum value of PAR was  $11.8 \text{ umol photons m}^{-2} \text{ s}^{-1}$ . Including the additional data points added 6 matches did not change the slope.

**Table 5.** Recovery cruise fitting CTD bottle files to the HPLC measurements. Bold text indicates the fit was chosen for calibration.

Cruise	Method	Equation	R-Squared
RV Sikuliaq	Use CTD calibrated data from bottle files to compare to HPLC	<b><math>(4.0 \pm 0.2) * X</math></b>	<b>0.95</b>
		$(4.1 \pm 0.3 * X) + -0.07 \pm 4.5$	0.95

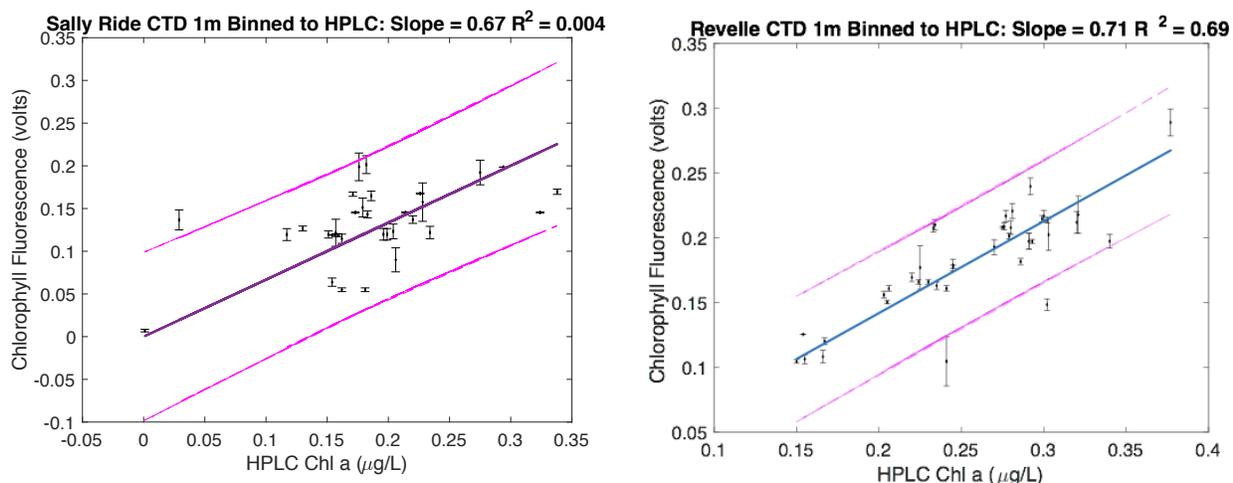
### 3. Comparison between the 1m binned (down-cast) FL and the bottle-sampled HPLC (upcast)

Previously (section 1) we looked at the correlation between the bottle-file FL (up-cast) FL and the bottle-sampled HPLC (up-cast). Here we also evaluate whether the 1 meter binned down cast CTD raw ChlF values would align with the HPLC Chl a. One issue that arose in directly extracting the 1m binned profile data, was that not all of the profiles start at the surface. In 56 of 84 profiles on the RR, and 133 of 144 profiles on the SR, the profile starts below 5m depth (Fig 9).



**Figure 9.** The shallowest depth value for each cast from the binned dataset for all casts longer than 10 data points, blue dots are the Revelle casts and orange are the Sally Ride.

In order to align the 1 m binned CTD data with the HPLC measurements, data were first matched by reported station number. If the binned CTD data had a length of the profile that spanned less than 10 meters than it was excluded (only one cast removed). Once the casts were matched, then the closest in depth measurement from the binned data were compared to the sampled depth from the HPLC file. Any match greater than 1 m offset between binned CTD data and HPLC were excluded. To determine the uncertainty, we averaged  $\pm 1$  meter from the binned CTD ChlF match where data were available. This is represented by the error bars on figure 10.



**Figure 10.** Binned 1 m down cast CTD ChlF data matched to the HPLC Chl<sub>a</sub>. Error bars are +/- 1 m standard deviations of the mean for each data point, which is the CTD-HPLC match at a given depth. Given the cutoff of surface processed binned CTD data and the poor fits this was not chosen as a method for calibration. However, the slopes were similar to other methods.

**Table 6.** Binned 1 m CTD to HPLC comparison of linear fits.

Cruise	Method	Dark Value (V)	Equation	R-Squared
Sally Ride	Binned CTD down-cast to HPLC matches during night-time only	0.087	$0.67 \pm 0.04 * X$	0.004
Revelle		0.051	$0.67 \pm 0.01 * X$	0.69
Sally Ride		0.078	$0.67 \pm 0.04 * X$	0.004
Revelle		0.0404	$0.71 \pm 0.01 * X$	0.69

### Further Discussion

Overall, we find that the correlation coefficient ( $R^2$ ) between the HPLC and bottle-file CTD ChlF (collected concurrently, Fig 4) is higher than the  $R^2$  between the HPLC and 1m binned data for both the RR and SR (collected during the up and down cast respectively, Fig 10). This is possibly due advection of horizontal gradients in the time elapsed between the down and up cast. These results highlight that even over the fairly short time/space differences between the up- and down-cast, the ChlF values can vary enough to cause a substantial decrease in the correlation.

For doing instrument match-ups to the CTD data, our overall recommendation is to apply a PAR threshold (less than  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). However we note that the  $R^2$  obtained from using night-only data is higher (Table 3, roughly 0.8) than the  $R^2$  obtained from using the PAR threshold, which includes night, and deep daytime data (Table 4, roughly 0.6 to 0.7). The reduced correlation coefficient is likely because the PAR threshold introduces additional data points, which introduce greater variability. It is also possible that the broader

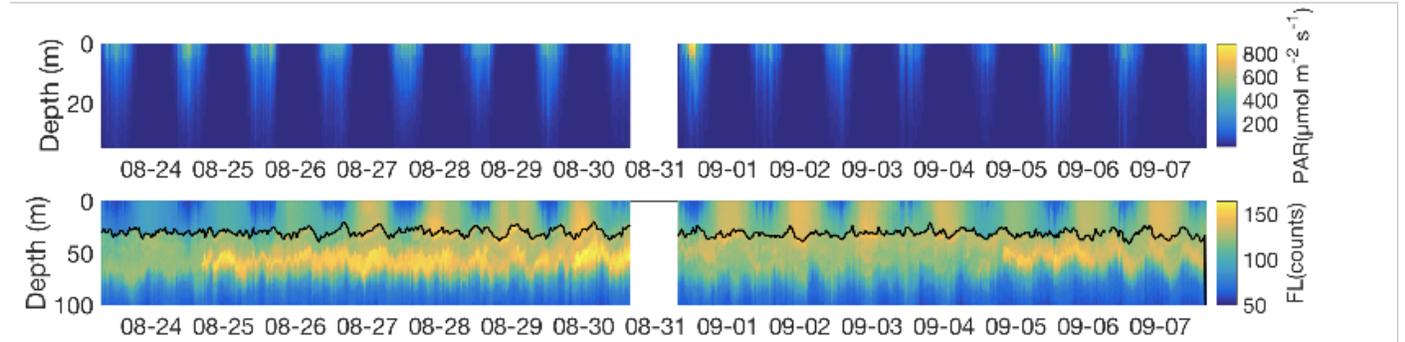
range of points included, may encompass different water masses with different communities or physiological states that have different FL to Chl<sub>a</sub> relationships. For example, the deep values (with low temperature as seen on Fig 6e,f) appear to have a lower slope. By using a PAR threshold instead of a night-time only fit, we introduce more deep values that could lower the slope, increase the overall scatter and reduce the correlation. Since the taped dark cast did not show any changes with depth (figure 2), we interpret this as a 'real' effect wherein that deeper community on the cold water has a different FL:Chl relationship. Since we feel this is likely reflecting a physiological or community state, we do not want to recommend that these data are neglected. Particularly because some sensors may not have many match-ups to the night-time casts, and need all the statistical confidence possible. However, if 'your sensor' is from the surface only for example, and did not collect data where the temperature was <8 degrees C, then the night-time only fit may be desirable. Overall however, **we are recommending using the bottle file matches, PAR threshold fitting, with deep values as a dark count, and a linear fit through zero as the chosen method (see table 1 for values).**

#### **Section 4.** At-A-Glance Calibrations Steps for CTD Chl → **Your Sensor (ChlF)** here:

- 1) Subtract **Your Sensor** dark counts . If unavailable, use the dark counts measured by E. Boss and C. Roesler pre-cruise. (<https://docs.google.com/spreadsheets/d/1pIq3ee5EKUv6UKOBZA2zPuuKozN6umKTzRcyVPJ2CHg/edit#gid=1798388318>) or use an average of deep values.
- 2) Match up times and locations of **Your Sensor** with a CTD cast within one hour and one nautical mile respectively. The one meter binned CTD data found here: [https://drive.google.com/drive/folders/15LCjFMU\\_j56uAq\\_Sa05D1fQ16BozszL8](https://drive.google.com/drive/folders/15LCjFMU_j56uAq_Sa05D1fQ16BozszL8). This analysis was completed using the RR1813 SIO CTD version from March 6, 2019 which were reuploaded on September 16.
- 3) For each measurement, match up the density of **Your Sensor** with the density measured by the CTD during the selected profile to correct along isopycnals.
- 4) The NPQ correction approach is not decided yet, so for now select ChlF data where PAR is less than 20  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . If no PAR data are available, use only the night-time values (2 hours past dusk, to dawn).
- 5) Create a scatterplot of ChlF from **Your Sensor** versus the CTD Chl and fit a line through the data, forcing the intercept to pass zero.
- 6) Convert ChlF from **Your Sensor** to calibrated Chl units of Chl<sub>a</sub> in  $\mu\text{g/L}$ .

#### 4A. Calibration of the Wirewalker to the Calibrated CTD FL sensor

The ECO BBFL2SSC-1309 deployed on the Wirewalker during EXPORTS. This sensor was calibrated to the corrected CTD sensor on the Revelle. Figure 11 shows the Wirewalker PAR data and the uncorrected ChlF data during deployments 2 and 3 when it profiled.

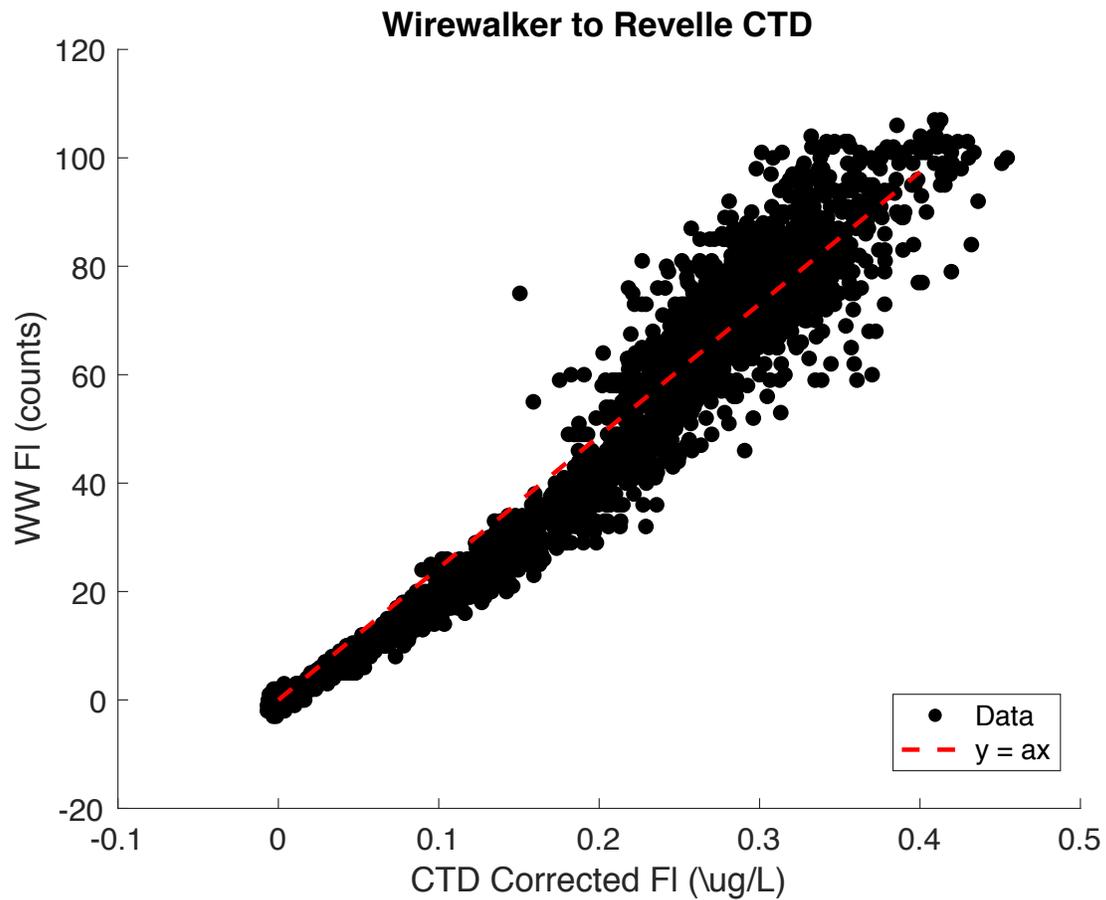


**Figure 11.** Wirewalker PAR (upper) and raw ChlF (lower). The black line in the lower plot represents a mixed layer using a potential density threshold of  $0.1 \text{ kg/m}^3$ . This shows daytime fluorescence values depressed during the daytime within the mixed layer, with some signal below.

The dark value from the beginning of EXPORTS experiment was  $50 \pm 1$  count. The *in situ* dark value (average value deeper than 475 m) was  $53 \pm 1$  counts. This value was also the same when using a 400 m cutoff instead. Since we did not do a cast with the sensor taped, we are using the dark value from the deployments as a reference to subtract from the entire time-series. While we did not directly correct for the contribution of CDOM fluorescence in the chlorophyll channel as discussed in Proctor and Roesler (2010), using the *in situ* dark value removes some of the CDOM influence from the signal.

We matched the WW to the 1 m binned CTD casts by finding a WW cast closest in time to each CTD cast. Then we only kept temporal matches that were within 1 nautical mile and 1 hour. For each CTD-WW cast match we then matched individual data points along isopycnals, which were considered density matches if they were less than  $0.05 \text{ kg/m}^3$  apart. A depth threshold of 500 m was also applied to match the maximum depth of the Wirewalker. This was added to prevent CTD depths greater than the Wirewalker travelled matching to the Wirewalker's deepest value. We then applied the PAR threshold of  $20 \text{ umol photons m}^{-2} \text{ s}^{-1}$  and used the Matlab curve fitting toolbox (figure 12; table 7). A follow up document will discuss the NPQ correction for the Wirewalker.

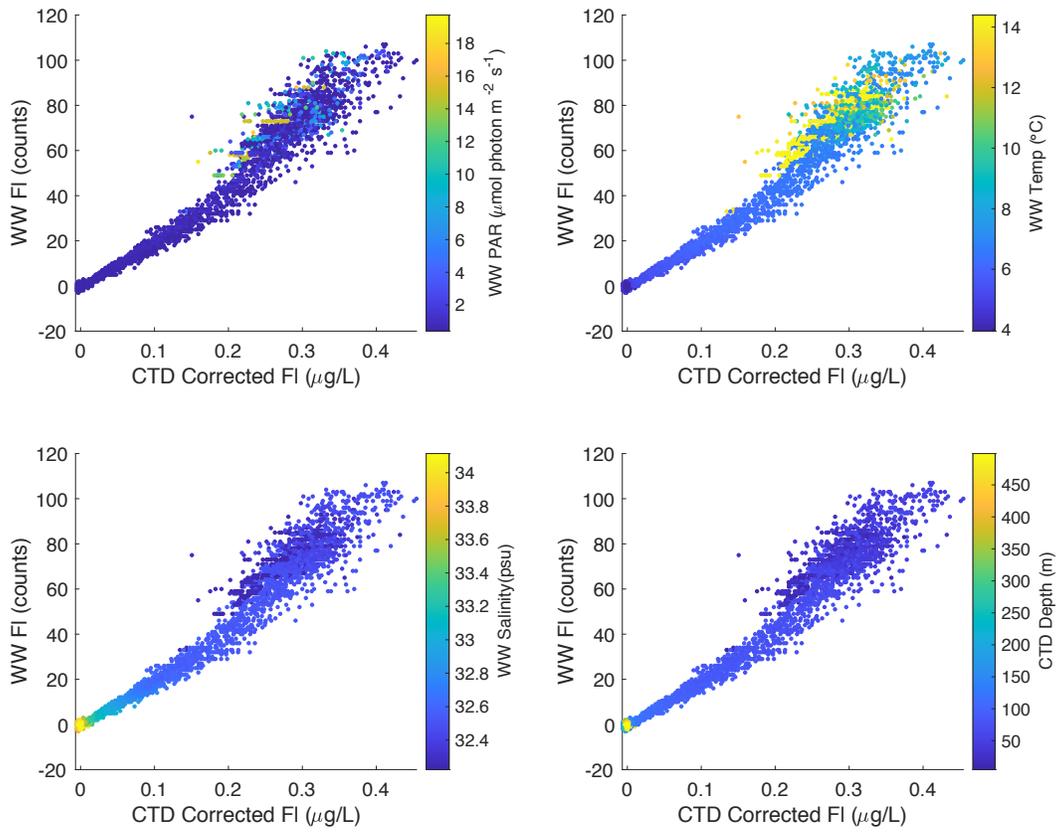
Overall, the calibration had a good fit, but it does appear that the surface values tend to have much more scatter (figure 13). This is possibly due to the density threshold not being strict enough to prevent match-ups that may not be best aligned in physical properties, as the threshold is a tradeoff between number of data points and obtaining a good fit. It is also possible that the warmer, fresher surface water may be a different community from the deeper, colder water. This appears similarly to results from the fits when using a PAR threshold for the CTD sensors (figure 6).



**Figure 12.** Wirewalker ChlF (dark subtracted) compared to the corrected CTD ChlF sensor. Fits computed using the Matlab Curve Fitting toolbox.

**Table 7.** Wirewalker ChlF sensor calibration linear fits. Bold text indicates chosen fit for calibration.

Platform	Method	Equation	R-Squared
Wirewalker	Use calibrated CTD sensor on the Revelle to match WW sensor	<b><math>(243.5 \pm 0.3) * X</math></b>	0.98
		$(245.3 \pm 0.3 * X) + -0.5 \pm 246$	0.98



**Figure 13.** Wirewalker ChlF (dark subtracted) compared to the corrected CTD ChlF sensor colored by the PAR, temperature, salinity and depth, This shows that the increased scatter at the surface, which could be due to misalignment in physical properties observed by the CTD and WW or different phytoplankton communities.

## 4B. Example application- Calibration of the Seaglider to the Calibrated CTD FL sensor

The final result is the following conversion from glider SG219 chlorophyll fluorescence (Fl; counts) to Chl-a concentrations (ug/L):

$$\text{Chl-a (ug/L)} = (\text{Fl} - 42) / 269$$

Matlab code to perform these steps can be found in EXPORTS\_Pacific\_SG219\_fluorescence\_calibration.m.

### 1. Determine dark counts

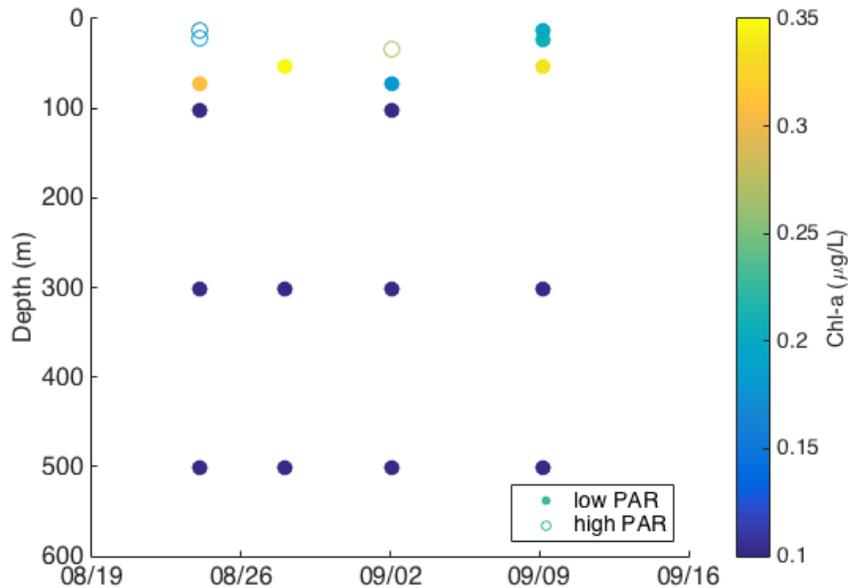
Dark counts were found to be 42 counts by using the median signal below 500 m depth. (The mean value was 42.2 counts). Changing the depth horizon to 400 and 300 m did not change the median value, nor did it change the mean considerably. There was no discernable temporal deviation in this median value. This value is substantially different from the pre-cruise WETLabs calibration of 49 counts.

### 2. Find match-ups to ship-based HPLC measurements

Match-ups were found using data in the [Sally Ride \(SR\) Bottle file, V3.0](#) and those casts designated on the [R2R spreadsheet](#) as for glider calibration (52, 70, 110, and 143). Glider data were considered “close enough” to the SR measurements if they fulfilled all of the following:

- Within 1 nm (using latitude and longitude based on the glider hydrodynamic model depth averaged current (hdm DAC) and the constant scale factors of 1°N = 111.12 km, 1°E = 70.68 km),
- Within 2 m depth,
- Within 0.05 kg m<sup>-3</sup>, and
- Within 1 hour.

A total of 28 match-ups remained, at depths ranging from 10 to 500 m. These casts were conducted near-sunset (4:00–4:30 UTC) and are therefore not considered as “nighttime” values (defined as sunset+2hr to sunrise). However, 24 of the 28 match-ups had concurrent ship-based PAR measurements below the threshold suggested above as subject to non-photochemical quenching (20 μmol photons m<sup>-2</sup> s<sup>-1</sup>). These 24 match-ups were used for the linear regressions. Note that increasing the temporal separation allowed to 2 hours increases the number of matchups to 55, but leads to a slight reduction in adjusted r-squared values and a 8% change in slope.



**Figure 14. Depths and times of the match-ups used in this document between the Sally Ride calibrated CTD chlorophyll fluorescence (colors) and the SG219 chlorophyll fluorescence. Low PAR is <20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; high PAR is at or above this threshold.**

### 3. Perform linear regressions

Linear regressions were done using the Matlab ‘fit’ function between the dark-corrected glider chlorophyll fluorescence and the corrected Sally Ride CTD Chl-a fluorescence measurements (see Matlab code). Fits were performed to linear relationships with and without an additional offset:

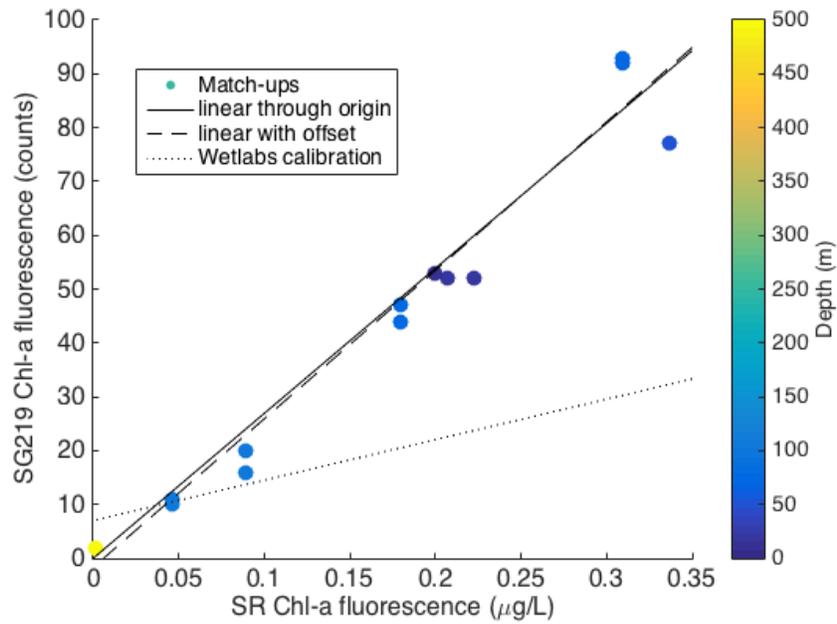
$$\text{Chl-a [ug/L]} = (\text{Fluorescence [counts]} + \text{Offset}) * \text{Scale-factor}$$

The fits are shown in the table below, where the uncertainty is one standard deviation. **This document recommends using a 269 counts/(ug/L) conversion factor** (compare with WETLabs factory calibration scale-factor of 75 counts/(ug/L)).

Scale-factor: conversion from counts to ug Chl-a L<sup>-1</sup> (i.e., divide counts by this)

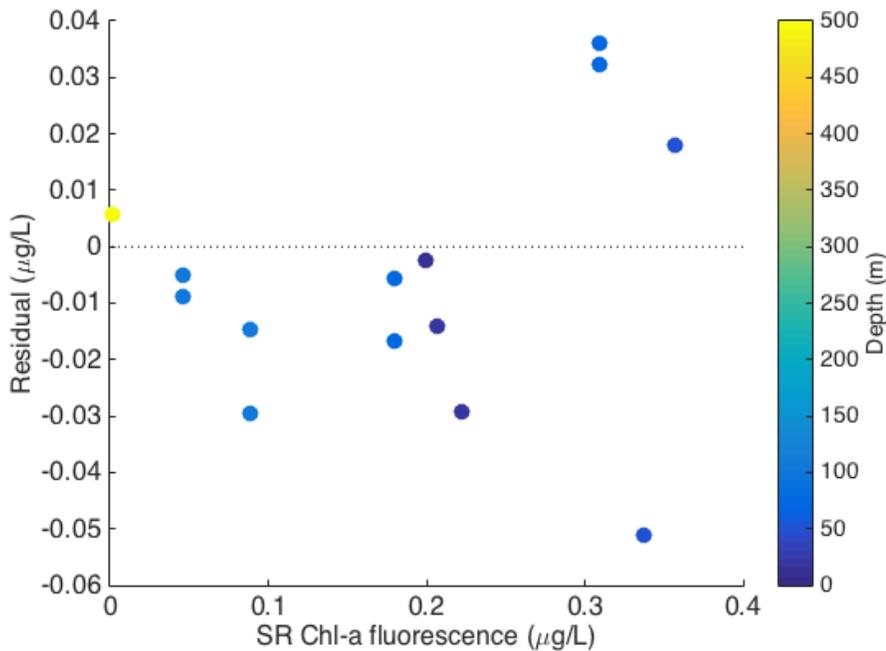
Offset: in units of counts (i.e., linear regression modification to dark counts)

Fit type	Formula	a	b	Adj. r <sup>2</sup>
<b>Linear through origin</b>	<b>Chl = Fl/a</b>	<b>269 +/- 6</b>	<b>N/A</b>	<b>0.978</b>
Linear with offset	Chl = (Fl+b)/a	276 +/- 8	1.7 +/- 1.4	0.979



**Figure 15. Comparison between darkcounts-corrected SG219 chlorophyll fluorescence (counts) and Sally Ride chlorophyll fluorescence (µg/L) at different depths (colors). Fits shown are for a linear fit forced through the origin (solid), linear fit with an offset (dashed), and the pre-cruise Wetlabs calibration.**

Residuals are generally small and never greater than 0.06 mg m<sup>-3</sup> in magnitude.



**Figure 16. Residuals for linear fits through the origin using the recommended calibration (linear through origin).**

#### 4C. Lagrangian Float

The Lagrangian float used a Wetlabs FLNTUS with wiper SN 4992.

**The conversion from Lagrangian Float 92 chlorophyll fluorescence (Fl; counts) to Chl-a concentrations (mg m<sup>-3</sup>) from the start of the record through September 8, 2018 is:**

$$\text{Chl-a (mg m}^{-3}\text{)} = (\text{Fl} - 53) * 0.00181$$

**At the end of the record (December 2, 2018), a second calibration was found**

$$\text{Chl-a (mg m}^{-3}\text{)} = (\text{Fl} - 53) * 0.00255$$

##### 1. Determine Dark counts

The maximum depth of the Lagrangian float was 230 dbar. The median counts below 170 dbar was 53. This is used as the dark counts.

##### 2. Find match-ups to ship-data

The Lagrangian float was calibrated using 5 calibration casts from the *R/V Sally Ride* during the main experimental period and 1 cast from the *R/V Sikuliaq* near float recovery. The table lists the cast information.

Ship	Cast Start time	Minimum distance to float track	Typical Time difference
Sally Ride	15-Aug-2018 02:44:22	638 m	25 minutes
Sally Ride	22-Aug-2018 02:59:13	204 m	20 minutes
Sally Ride	26-Aug-2018 02:44:12	391 m	20 minutes
Sally Ride	01-Sep-2018 02:42:41	509 m	10 minutes
Sally Ride	08-Sep-2018 02:10:12	1078 m	10 minutes
Sikuliaq	02-Dec-2018 16:09:17	1281 m	36 hours

##### 3. Perform Regressions

Fluorescence counts were converted to Chl-a units by comparing with the Chl-a values from the ship's CTD. For *Sally Ride*, the voltages were used and the calibration listed in Table 1 were used. For *Sikuliaq* the CTD Chl-a values were used with a slope of 4 and a zero of 0.01, since a value of 0.03 from Table 1 yielded negative Chl-a values in places. CTD Chl data were linearly interpolated to the potential density of the float measurements and plotted in Figure 17. Only data with PAR values less than 20 were used in the calibrations.

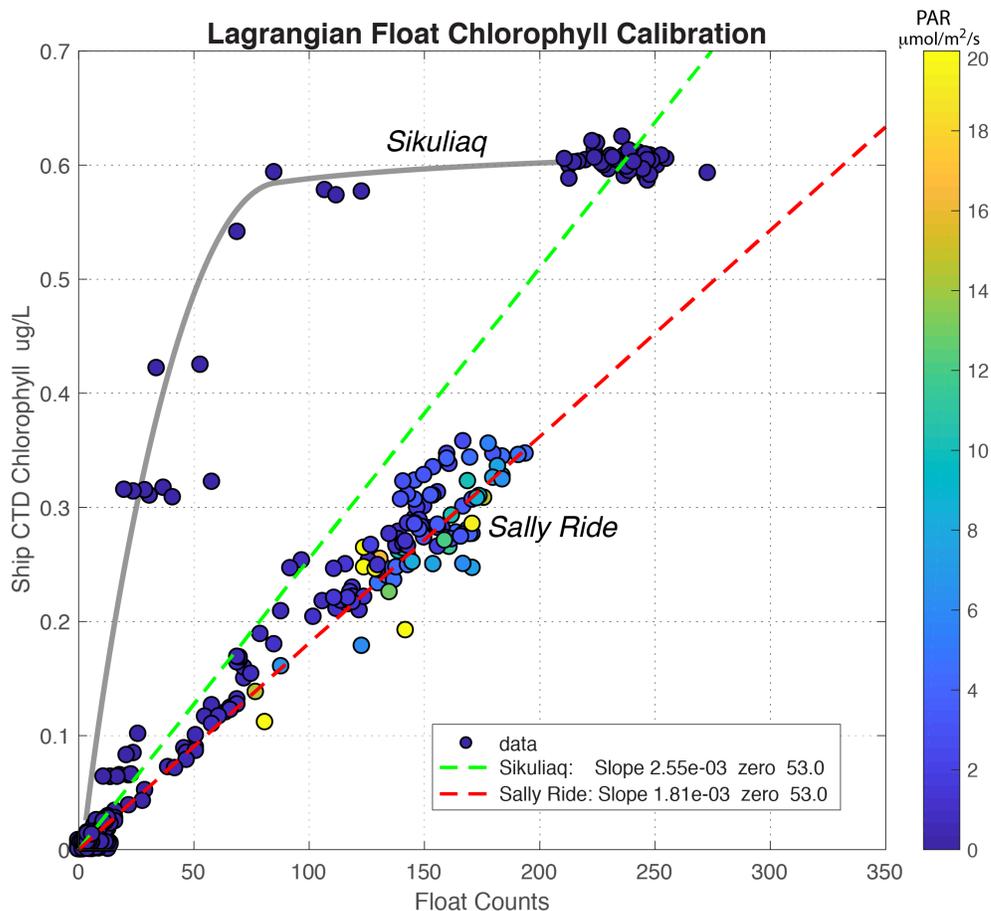


Figure 17. Calibration of Lagrangian float fluorometer. Dots show ship CTD fluorometer data interpolated to the isopycnal of the float fluorometer data. Color is PAR from the CTD. Red line is the calibration for the *Sally Ride* data; Green line is calibration for the *Sikuliaq* data. Thick grey line shows general trend of *Sikuliaq* data.

Two groups of data are apparent in Fig. 17. The lower group is from the *Sally Ride* calibration casts. A linear fit (red dashed line) yields the slopes used in the Lagrangian float Chl-a calibrations.

The upper group is from the *Sikuliaq* calibration cast. These lie above the *Sally Ride* and are nonlinear with a much steeper slope in the pycnocline than in the mixed layer. This may represent different phytoplankton communities in the two depth ranges, or from the large time between the calibration cast and float profiles or from the different shapes of the density and chlorophyll profiles. The suggested calibration (green dashed line) runs through the zero and the mixed layer values and ignores the pycnocline data. These data suggest that there may be a change in the chlorophyll calibration during the 109 days of the Lagrangian float deployment, but do not specify how, when or at what depth the calibration changed. Since this calibration is of low quality, it is not applied to the released Lagrangian float data.

## References

Proctor, C.W. & Roesler, C.S. (2010). New insights on obtaining phytoplankton concentration and composition from in situ multispectral chlorophyll fluorescence. *Limnology and Oceanography Methods*, 8(12), 695-708.

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