The 2008 North Atlantic Bloom Experiment

Calibration Report #4

Calibration of the Chlorophyll Fluorometers on the Knorr CTD and Float 48

Eric D'Asaro
Applied Physics Laboratory, University of Washington
dasaro @ apl.washington.edu
Version 1.3 September 24, 2009

Summary

Comparison of the chlorophyll fluorometers on the *Knorr* CTD and on float 48 with extracted chlorophyll from the *Knorr* water samples show clear depth and time dependences. Accordingly, a linear relationship between chlorophyll and fluorescence is abandoned in favor of a more complex, albeit empirical scheme. Counts from the Knorr CTD fluorometer are converted to chlorophyll using a dark offset and a gain that has dependences on temperature, PAR, depth and yearday. The resulting CTD-fluorometer-determined chlorophyll concentration predicts the bottle concentrations with an error of about 25%. Counts from the float 48 fluorometer are converted to chlorophyll using the same scheme and parameter dependences, but with the gain and offset adjusted so that the float best matches the extracted chlorophyll at the float calibration casts. The resulting float chlorophyll matches the bottles from both the *Knorr* process cruise and *Bjarni* deployment cruises with an error of 30-50%. The higher error for the float calibration is presumably due to the poorer quality of the float-bottle intercomparisons. The calibrated fluorometer data is be included in v5 of the float data and v3 of the CTD data.

1. The fluorometers

Float 48 (Fig. 1) was the primary float in NAB08. It carried a single WetLabs FLNTU (S/N 747) with excitation frequency of 470 nm and detected emission at 700nm as well as a backscatter sensor discussed elsewhere. The unit was ordered with a custom chlorophyll range (0-35 ug/l). It was mounted on the bottom endcap (see Figure), next to the CTD. The FLNTU was equipped with a "biowiper" to control fouling. This was closed during communications, open during sampling modes it was open and operated every 10 samples to wipe the optical windows. FLNTU measurements were made on every data sample, approximately every 50 seconds.

A similar fluorometer was mounted on the CTD package during the *R.V. Knorr* process cruise. Average fluorometer values were compiled for each water sample bottle taken during the cruise and combined with extracted chlorophyll values from the bottle and ancillary data in the *KnorrBottleFilev2.xls* released June 29, 2009. Details are described elsewhere.

3. Calibration of the *Knorr* CTD Fluorometer *[KnorrChlFit2.m]*

Absolute calibration of the Knorr CTD fluorometer is achieved by comparison with the associated water samples. The simplest approach would be a linear relationship between fluorescence and chlorophyll. A scatter plot (Fig. 2) shows a significantly nonlinear relationship and a dependence on time, e.g. data from later in the cruise (red) lies higher on average than data from earlier in the cruise (blue). A more complex relationship is therefore sought.

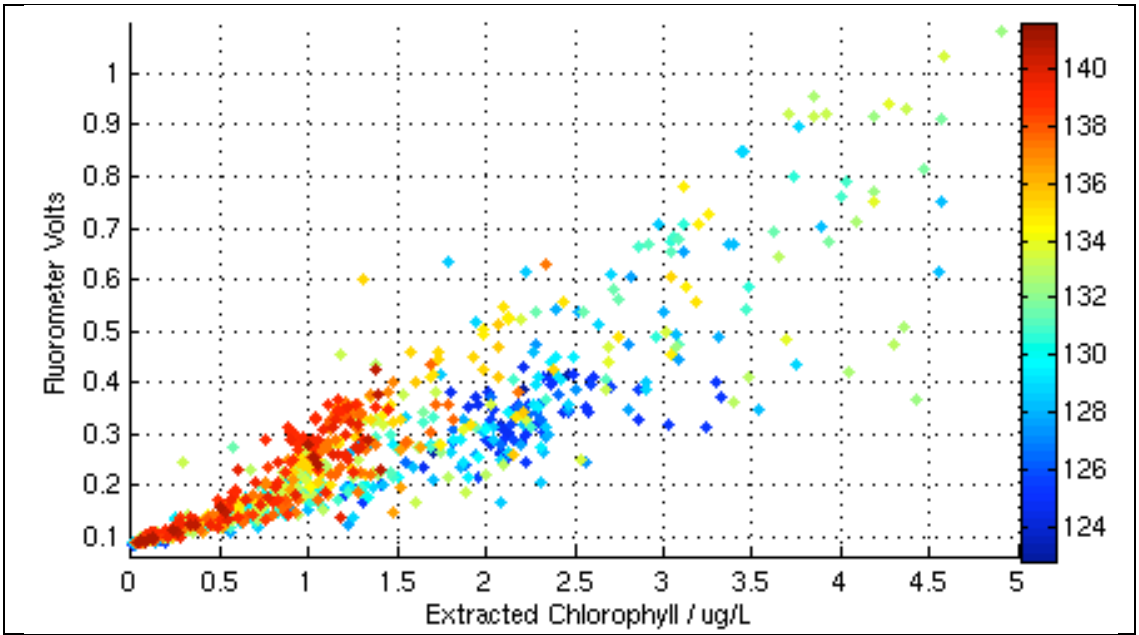

Fig. 2. Scatter plot of raw fluorometer output against extracted chlorophyll for all *Knorr* bottle samples. Color is yearday of 2008.

Let C_X be the extracted chlorophyll [ug/l], F_0 be the fluorometer output [volts] and F_n be chlorophyll value estimated from F_0, with *n* increasing as successively improved estimates are made. The scatter in Fig. 2 increases with C_X, so that it is best to model the slope $S_n = C_x / F_n$ rather than F_n.

The first estimate is linear: $F_1 = (F_0 - F_{dark}) * R_1$, where F_{dark} is the dark value and R_0 is the gain. Note that in *KnorrBottleFilev2.xls* this background has already been subtracted in column 'T'. Fig. 3 shows S_1 as a function of C_X, colored by yearday. A pattern similar to that in Fig 2 is seen; the slope depends on both chlorophyll and yearday.

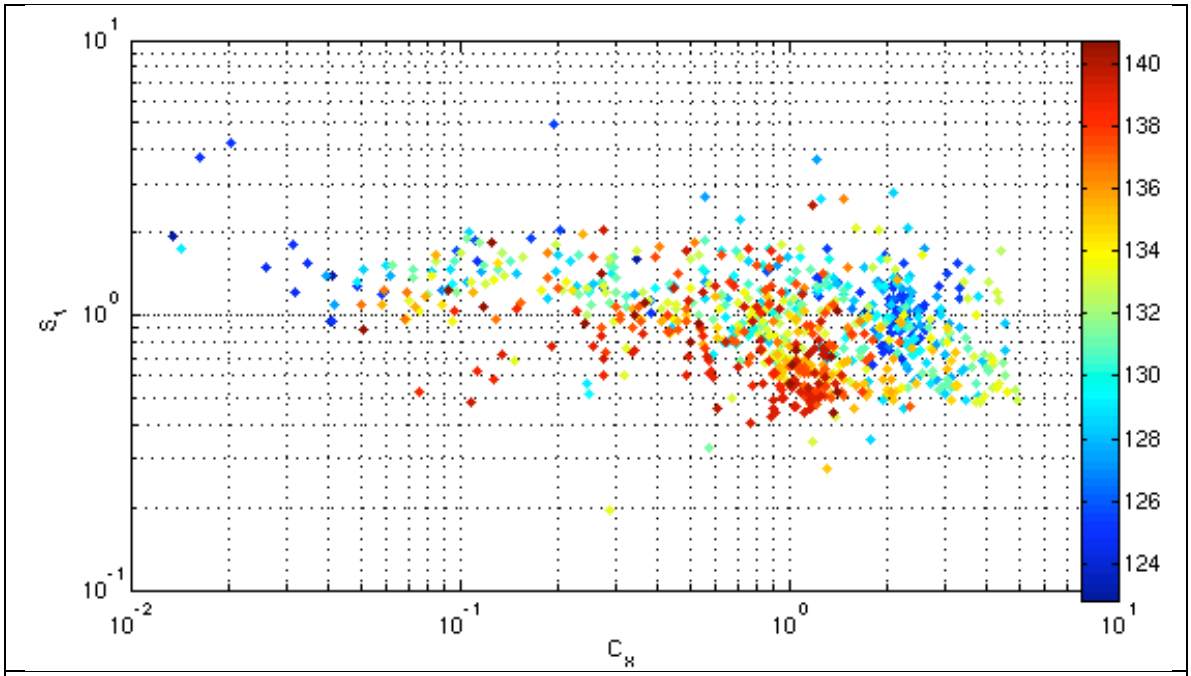

Fig. 3. $S_1 = C_x / F_1$ plotted against extracted chlorophyll and colored by yearday. F_1 is a linear function of the CTD fluorometer output.

The next steps attempt to remove dependences of S on other parameters. The first is a strong dependence on temperature, shown in Fig. 3a (black line), capturing changes in both time and depth. A revised estimate $F_2 = F_1 * R_2$ includes compensation for the temperature effect. The second is a dependence on PAR, shown in Fig. 3b, which crudely captures the effect of fluorescence quenching by ambient light. A revised estimate $F_3 = F_2 * R_3$ includes compensation for the PAR effect. Each of these effects change S by about a factor of 4.

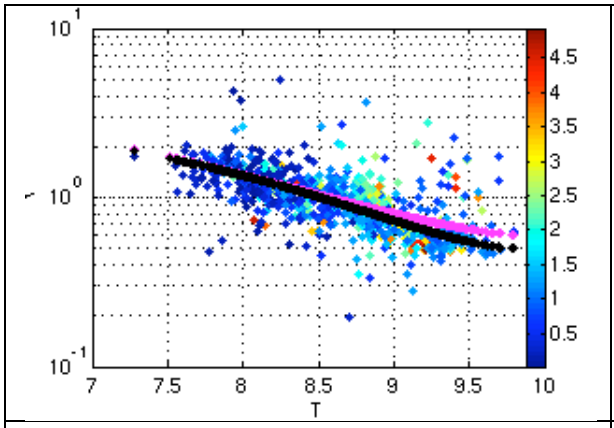

Fig. 3a. S_1 plotted as function of temperature colored by C_x. Magenta circles show a quadratic polynomial fit to all data; black circles are fit only to points closer than 0.3 to magneta line and define R_2.

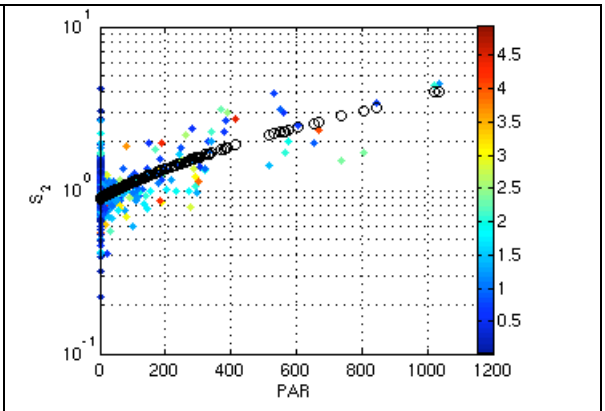

Fig. 3b. S_2 plotted as function of PAR colored by C_x. Black circles show cubic fit to values with PAR>1 and define R_3.

Two smaller effects are shown in Fig. 4. Fig. 4a shows a weak dependence of S_3 on depth. A revised estimate $F_4 = F_3 * R_4$ includes compensation for the pressure effect. Fig. 4b shows a weak dependence on S_4 on yearday. Fig. 4b shows a weak dependence of S_4 on depth. A revised estimate $F_5 = F_4 * R_5$ includes compensation for the yearday effect. Each of these effects change S by about 30%. A weak variation of F_5 with C_x remains, but no correction is applied.

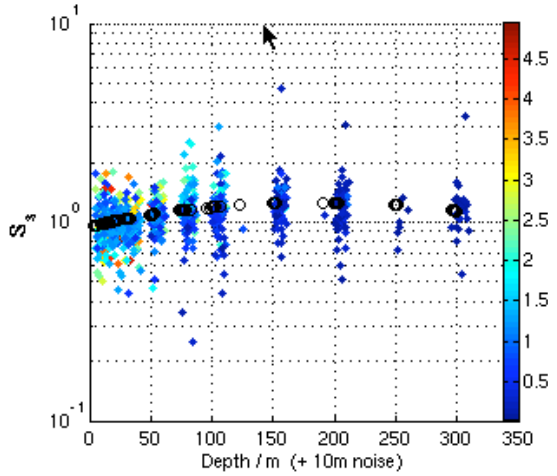

Fig. 4a. S_3 plotted as a function of depth and colored by C_x. Black circles show the fit of a quadratic polynomial that defines R_4. Depth is plotted with 10m of random Guassian noise added in order to visually spread the points.

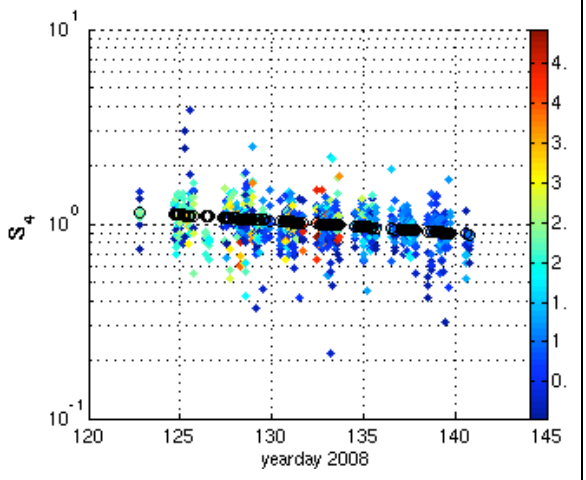

Fig. 4b. S_4 plotted as a function of yearday and colored by C_x Black circles show the fit of a linear function that defines R_5.

Fig. 5 shows the final calibration of the *Knorr* CTD fluorometer in terms of extracted chlorophyll. Compared to Figs. 2 and 3, the scatter is reduced, the relationship is more linear and there is no dependence on yearday. More quantitatively, the standard deviation of S_n has been reduced from 0.45 (i.e. 45% error) for S_1 (linear fit) to 42%, 32%, 26.9% and 25.4% by successively including corrections for T, PAR, depth and yearday respectively. A significant fraction of the residual error may be due to analytical errors in the chlorophyll extractions, estimated at 10%[1]. The final accuracy of the fluorometrically determined chlorophyll should be considerably less than the scatter in Fig. 5. It is conservatively estimated at 20% rms.

Biologically, variations in the S (=chlorophyll/fluorescence) ratio can reflect changes in community structure or in the functioning of the photosynthetic mechanisms of the same community with changes in temperature, light, nutrients or life cycle. None of these details are resolved here; the scheme is only empirical. In particular, it is not

[1] Unesco (1966), Determination of photosynthetic pigments in Seawater. Report of SCOR-UNESCO working group #17.

clear whether the strong dependence on temperature is a real dependence or a convenient proxy for depth and time variations due to other factors.

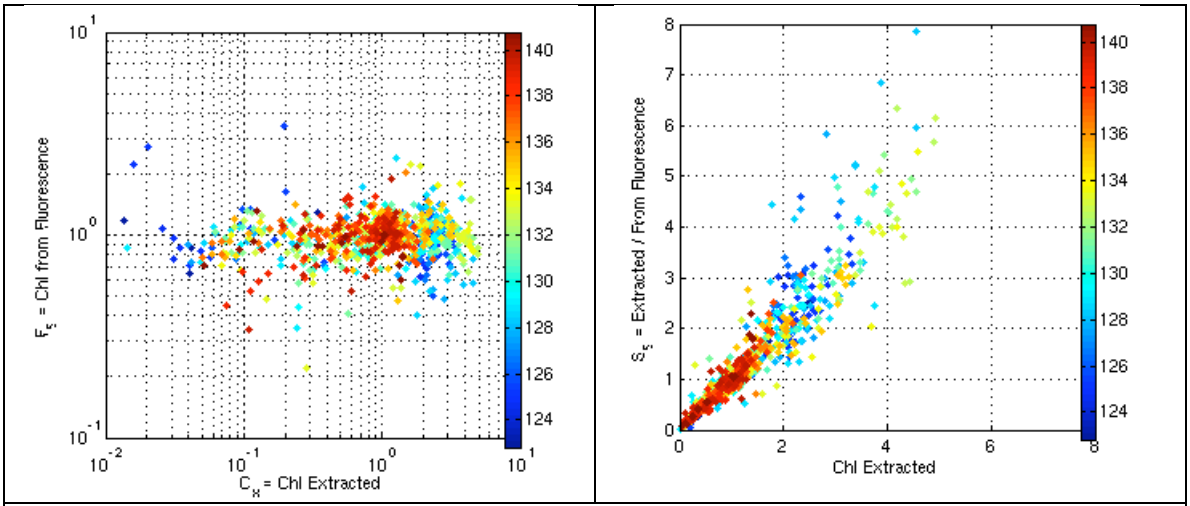

Fig. 5. Final calibration of Knorr CTD fluorometers corrected for temperature, PAR, depth and yearday, plotted as a ratio (left) and a scatter plot (right), and colored by yearday.

5. Calibration of Float 48 Fluorometer *(KnorrFloatChlCal2.m)*

The fluorometer on float 48 is calibrated using the scheme developed for the *Knorr* CTD fluorometer. We assume that the dependences of S_n on temperature, PAR, depth and yearday computed from the CTD data will apply equally well to the float data, so that only a gain and offset for the float fluorometers will be computed. The 10 float calibration casts are used to compute these two coefficients and to check that the calibration dependences computed for the CTD are also approximately valid for the float.

Fig. 6 shows a typical calibration cast with fluorometer profiles for the CTD and the float and extracted chlorophyll data points. The float calibration is based on matching the float fluorometer to the bottles by interpolating in potential density. Comparison of the center and right panels clearly shows that interpolation in potential density is better than interpolation in depth. A complete set of calibration cast plots is included in the Appendix.

The first step in the calibration is to linearly adjust the float 48 FLNTU fluorometer counts F_0 (variable *flntu.chl_raw* from float data release v4, March 25, 2009) to fit the bottle data from all 10 float calibration casts using $F_1 = (F_0 - F_{dark}) * R_1$. Fig. 7 (left) shows S_1. The variability is larger than for the CTD data, due to the spatial and temporal separation between the float and bottles. F_{dark} was chosen to make S_1 near 1 for low values of C_x; it is very close to the minimum value of F_0 in the data. R_1 was chosen to make S_1 near 1 for high values of C_x. It is 1.8 times larger than the value used to make *flntu.chl* in float data release v4.

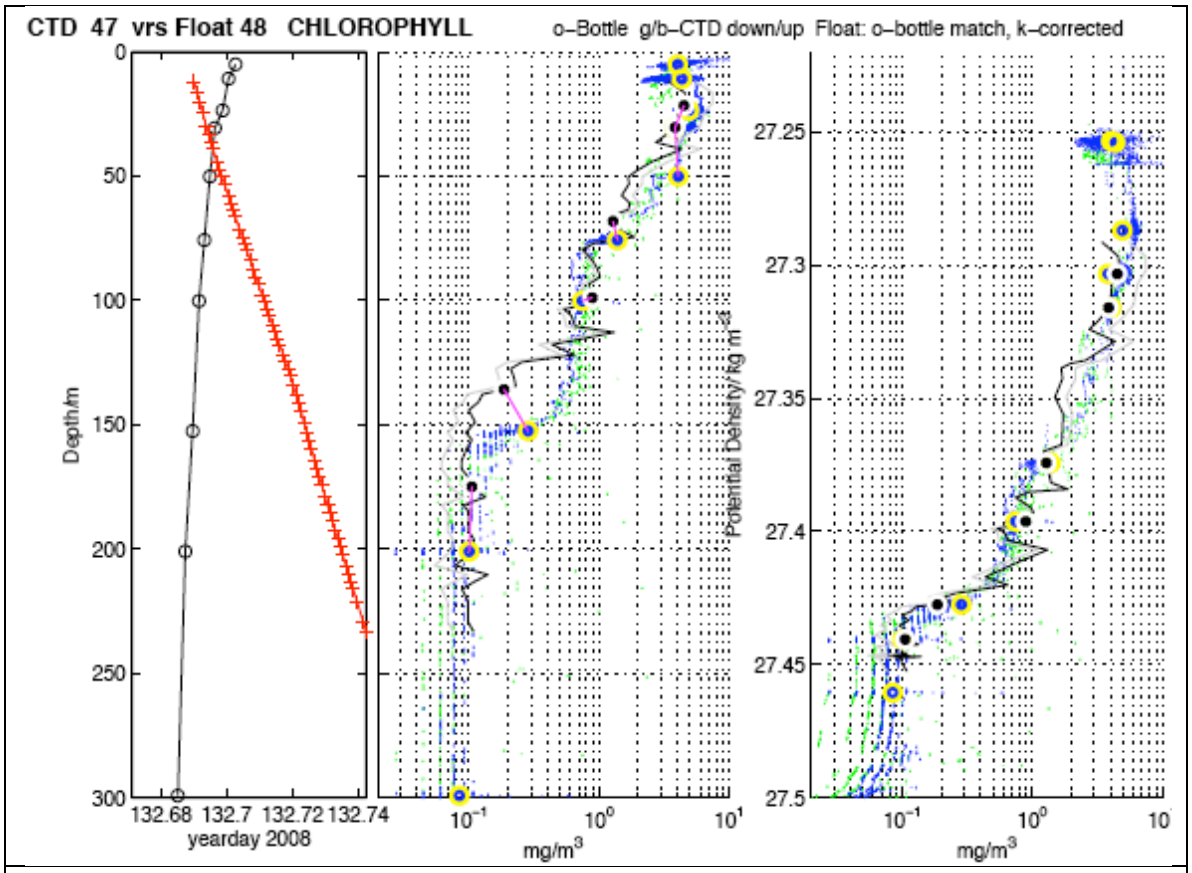

Fig. 6. Typical float calibration profile. Left: depth-time for CTD (black with bottles) and float (red with samples). Center: Chlorophyll profiles plotted against depth. Calibrated CTD fluorometer data from downcast (green) and upcast (blue) median filtered over 15 points with every 7th point plotted. Bottle extracted chlorophyll plotted as blue/yellow circle. Calibrated float fluorometer data: grey for slope and offset only (i.e. F_1), black for full calibration (i.e. F_5). Black/white circles plot float data associated with each bottle, interpolated using potential density. Magenta lines link float and bottle samples. Right: same as center but plotted against potential density.

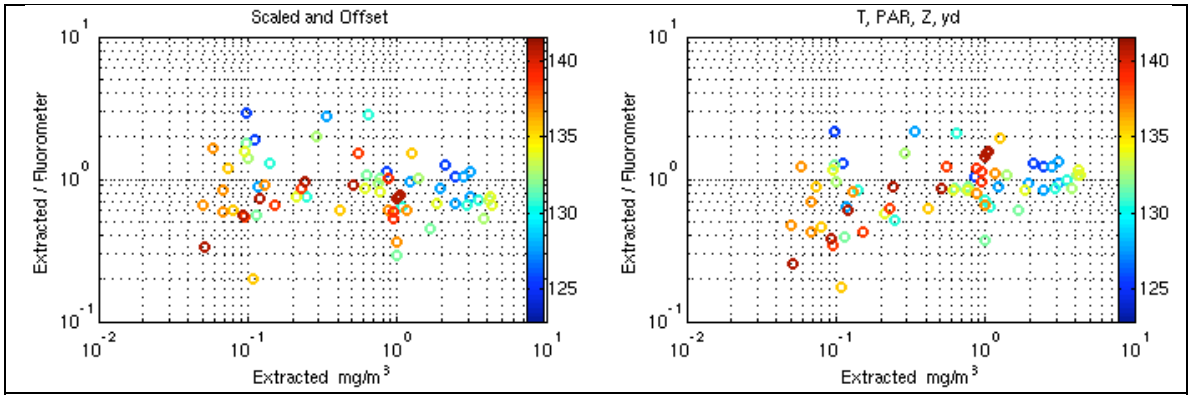

Fig. 7. Float 48 fluorometer scaled values, F_n/C_x plotted against extracted chlorophyll for initial F_1 (left) and final F_5 (right) calibration at all *Knorr* float calibration stations.

The additional corrections to F_1 are identical to those used for the CTD data, so that F_2, F_3, F_4 and F_5 correspond to adding corrections due to temperature, float PAR, depth and yearday, respectively. The 4 panels of Fig. 8 show that the float/bottle comparisons qualitatively support each of these corrections.

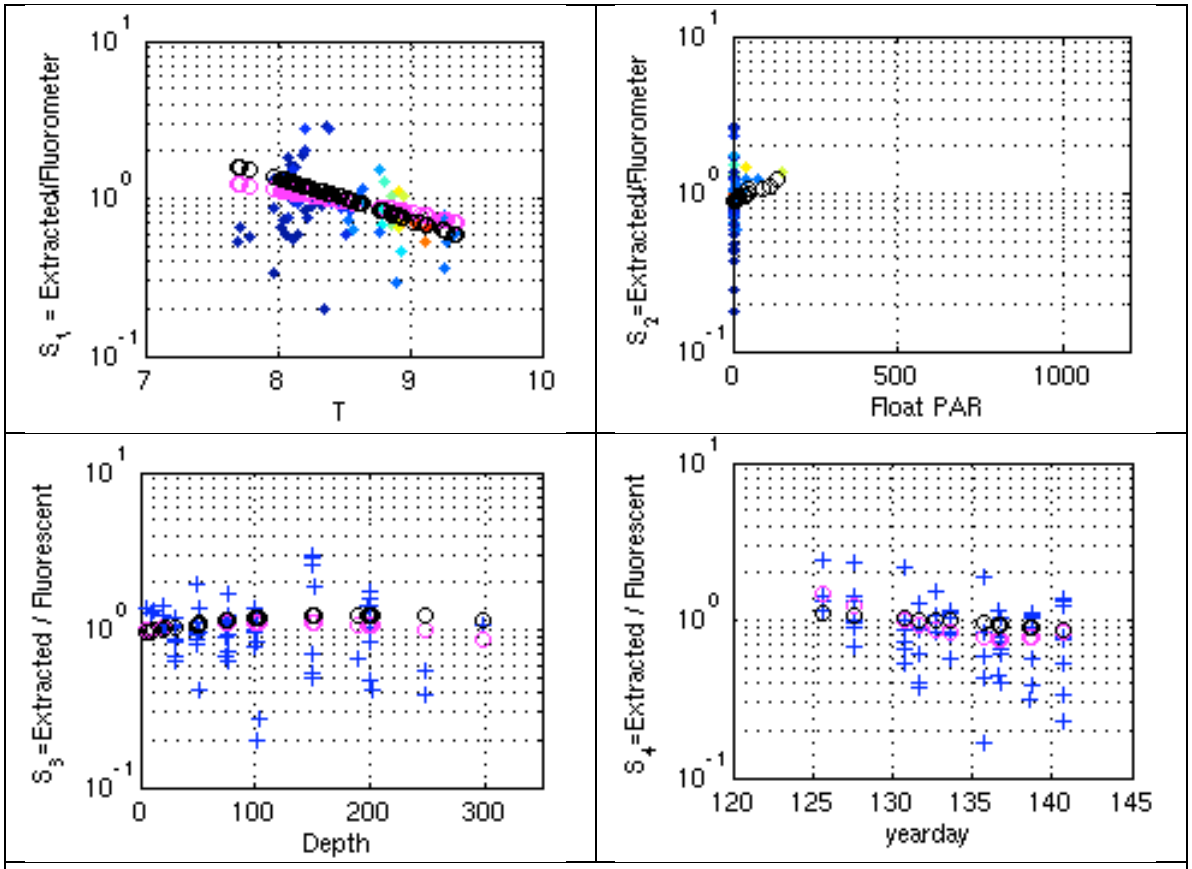

Fig. 8. Dependences of float 48 fluorometer on parameters as in Fig. 3 and 4 with same scales. Magenta circles are polynomial fits to this data; black circles are the polynomical fits from the CTD data. These are used to correct the float data.

Scatter plots of the final, F5, corrected fluorometer data are shown in Fig. 7 (left) and in Fig. 9. The standard deviation of S_5 is 43% for all data and 32% for values of C_x>1, compared to 55% and 24% for S_1. The corrections only slightly reduce the difference between the fluorometer and bottles, even though they clearly remove some of the biases. This may reflect the use of corrections chosen using the CTD data and thus not tuned specifically to the float calibration casts. Using the CTD data, however, seems appropriate both because the number of CTD/bottle comparisons (2013) is larger than the number of float/bottle comparisons (67) and because the CTD/bottle comparisons are more accurate. The accuracy of the float fluorometer calibration should be comparable to those of the CTD fluorometer calibrations.

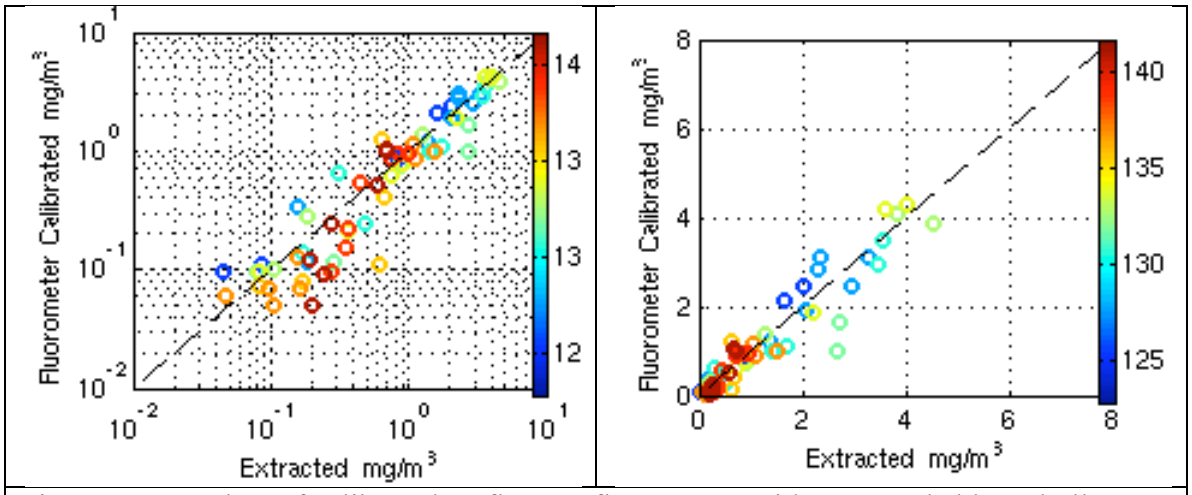

Fig. 9. Scatter plots of calibrated F_5 float 48 fluorometer with extracted chlorophyll.

6. Comparison at Float Deployment *(BjarniChl.m)*

Additional comparisons between the float 48 ISUS measurements, calibrated as above, and bottle measurements are made using data from the R.V. Bjarni Saemundsson deployment cruise. Fig. 10 shows comparisons from the three casts taken close to float 48; cast 152 taken just before deployment, and casts 157 and 158 taken at the time of float profiles. These are also included in the Appendix.

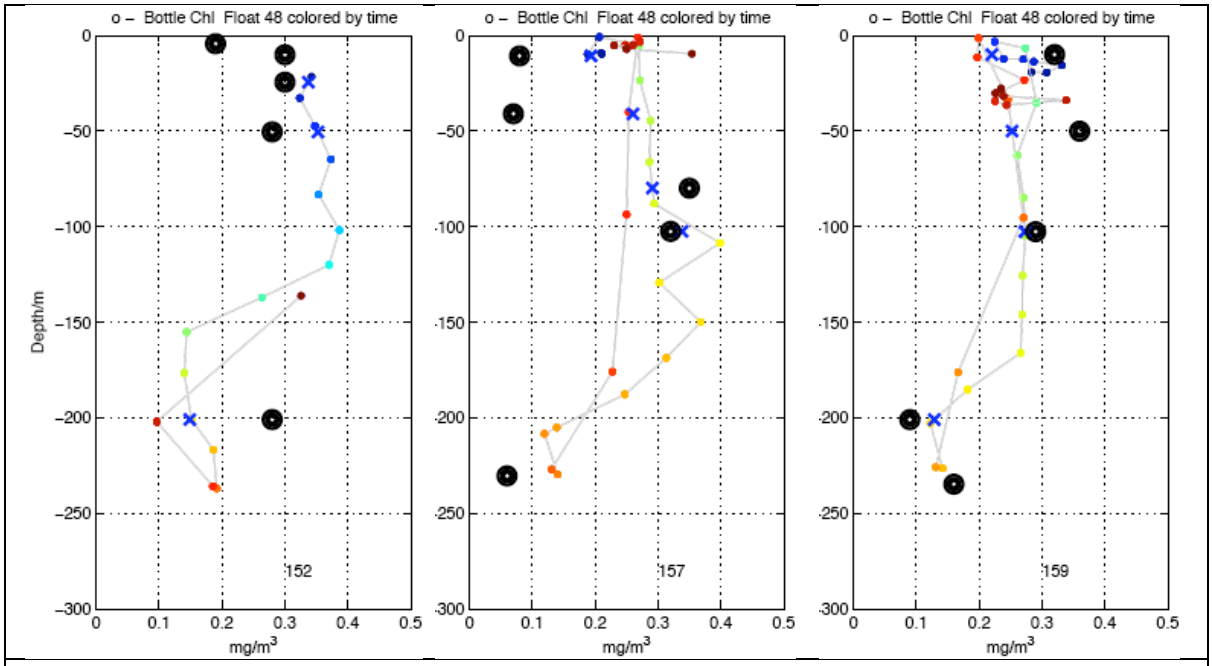

Fig. 10. Comparison of calibrated float 48 fluorometer (lines) and extracted chlorophyll from bottles (o) for float calibration casts from the *Bjarni Saemundsson* deployment cruise.

The values of S_5 for this data (Fig. 11) show a level variability similar to that in Fig. 7 (right) at low values of C_x. The mean value (0.96) indicates that there is no average change in calibration from the deployment to the *Knorr* cruise; the standard deviation (45%) indicates variability which is the same as in the *Knorr* cruise. The calibration developed from the *Knorr* data therefore appears to be valid for the entire float deployment.

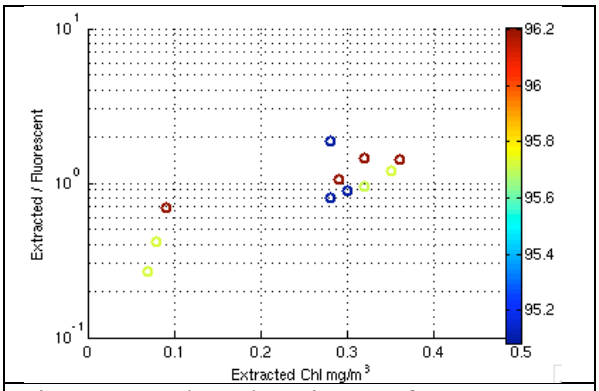

Fig. 11. S_5 plotted against C_x for deployment cruise and colored by yearday.

7. SUMMARY

The functions listed below add calibrated chlorophyll values from the fluorometers to the data structure for float 48 for v5 of the float data release and v3 of the CTD data release.

Float 48: the chlorophyll variables in v5 are:

- *flntu.chl0*– equals *flntu.chl* from v4, renamed here.
- *flntu.chl_raw* – counts from fluorometer
- *flntu.chlcal1* –Counts converted to chlorophyll [ug/l] using an offset and scale only. F_1 in this document.
- *flntu.chlcal2* – Temperature correction added to *flntu.chlcal1*. F_2 in this document.
- *flntu.chlcal3* –PAR correction added to *flntu.chlcal2*. F_3 in this document.
- *flntu.chlcal4* – Depth correction added to *flntu.chlcal3*. F_4 in this document.
- *flntu.chlcal5* – Yearday correction added to *flntu.chlcal4*. F_5 in this document.
- *flntu.chl* – Final calibrated value [ug/l]. Same as *flntu.chlcal5*.
- *flntu.chl_ugkg* – Final calibrated value converted to µg/kg.

The float 48 calibration routine is:

```
function newflntu  =  FlntuCorrect(flntu, licor, floatID)

% FlntuCorrect - Calibration routine for NAB float48 Chlorophyll Fluorometer
%
%  Version: $Id: FlntuCorrect.m 232 2009-08-31 21:58:46Z eric.rehm@gmail.com $
%
%  Author: Eric D'Asaro (U. Washington Applied Physics Lab)
%
%   flntu  =  FlntuRecal(flntu, licor)
%
```

```
%   INPUT:
%   flntu       Input v4 of float 48 flntu structure
%   licor       Input v4 of float 48 licor structure
%   floatID     ID of float, just to make sure we don't apply to Float 47
%
%   OUTPUT:
%   flntu       struct containg metadata (flntu.meta), raw data, and
%               calibrated data (flntu.chl, flntu.bb, flntu.bbp)
%
%   REFERENCES:  The 2008 North Atlantic Bloom Experiment
%                Calibration Report #4
%                Calibration of the Chlorophyll Fluorometers
%                on the Knorr CTD and Float 48
%                Version 1.3 September 24, 2009

% Get Float ID
% Make sure floatID is a string
if (isnumeric(floatID))
   floatID = num2str(floatID);
end

% No correction for NAB08 Float.
if (strcmp(floatID,'47') == 1)
   return
end

% Calibration constants %%
L = [4.37108056e-02 7.36829758e-10 -8.77565475e-06 -1.45352849e-02
   1.88527075e-01 -4.41554690e-08 3.25393752e-03 9.94098415e-01
   -4.71271045e-01 2.28282041e-03 9.49522658e-01 0.00000000e+00
   7.31184833e-01 8.92741651e-01 0.00000000e+00 0.00000000e+00];

Offset = 49.3350125945;
Scale = 0.007146;

%%%%%%%%%%%%%%%%%%%%
L22 = L(:,1);
L3 = L(:,2);
L4 = L(1:3,3);
L5 = L(1:2,4);
flntu.par = interp1(licor.yd,licor.par,flntu.yd,'nearest','extrap'); % Get PAR into flntu

% Correction factors
R2 = polyval(L22, flntu.T(:,1)-9);
R3 = polyval(L3, flntu.par);
R4 = polyval(L4, flntu.z);
```

```
YD = flntu.yd; % Do not extrapolate yearday correction
YD(YD<122) = 122;
YD(YD>142) = 142;
R5 = polyval(L5, YD-133);

% Save Original (V4) calibration
flntu.chlcal0    = flntu.chl; % initial calibration from v4

% Offset and Scale
flntu.chlcal1 = (flntu.chl_raw-Offset)*Scale; % Offset and Scale

% CTD corrections to float Chlorophyll
flntu.chlcal2 = R2.*flntu.chlcal1; % Add Temperature
flntu.chlcal3 = R3.*flntu.chlcal2; % Add PAR
flntu.chlcal4 = R4.*flntu.chlcal3; % Add Z
flntu.chlcal5 = R5.*flntu.chlcal4; % Add yd
flntu.chl     = flntu.chlcal5; % final cal ug/l  =  mg/m^3

% fraction by weight ( mg/m^3 ) / ( kg/m^3 )  =  mg/kg
flntu.chl_ugkg = flntu.chl./(1000.+flntu.Sig0(:,1))*1000; % ug/kg close to usual units

% Done
newflntu = flntu;
```

Knorr CTD: the chlorophyll variables in v3 are:

- *ctd.chl* – v2 chlorophyll value. It is deleted from v3 data.
- *ctd.chl0*– equals *ctd.chl* from v2, renamed here.
- *ctd.chl_raw* – volts from fluorometer
- *ctd.chlcal1* –Counts converted to chlorophyll [ug/l] using an offset and scale only. F_1 in this document.
- *ctd.chlcal2* – Temperature correction added to *ctd.chlcal1*. F_2 in this document.
- *ctd.chlcal3* –PAR correction added to *ctd.chlcal2*. F_3 in this document.
- *ctd.chlcal4* – Depth correction added to *ctd.chlcal3*. F_4 in this document.
- *ctd.chlcal5* – Yearday correction added to *ctd.chlcal4*. F_5 in this document.
- *ctd.Chl* – Final calibrated value [ug/l]. Same as *ctd.chlcal5*.
- *ctd.Chl_ugkg* – Final calibrated value converted to μg/kg.

The Knorr CTD fluorometers calibration routine is*:*

```
%%%%%%%%% Calibration routine for NAB KNORR CTD Chlorophyll Fluorometer
% Input v2 of ctd data
function newctd=CTDCalFunction(ctd)

% Calibration constants   %%%%%%%%%%%%%%%%
L=[4.37108056e-02  7.36829758e-10  -8.77565475e-06  -1.45352849e-02
1.88527075e-01  -4.41554690e-08  3.25393752e-03  9.94098415e-01
-4.71271045e-01  2.28282041e-03  9.49522658e-01  0.00000000e+00
7.31184833e-01  8.92741651e-01  0.00000000e+00  0.00000000e+00];

Offset=0.087; Scale=9.2294;

%%%%%%%%%%%%%%%%%%%%

L22=L(:,1);
L3=L(:,2);
L4=L(1:3,3);
L5=L(1:2,4);

% Correction factors
R2=polyval(L22,ctd.T1-9);
R3=polyval(L3,ctd.PAR);
R4=polyval(L4,ctd.z);
R5=polyval(L5,ctd.yd-1-133);

% Offset and Scale
ctd.chlcal1 =(ctd.chl_raw-Offset)*Scale;  % Offset and Scale

% CTD corrections to float Chlorophyll
ctd.chlcal2=R2.*ctd.chlcal1;  % Add Temperature
```

```
ctd.chlcal3=R3.*ctd.chlcal2;  % Add PAR
ctd.chlcal4=R4.*ctd.chlcal3;  % Add Z
ctd.chlcal5=R5.*ctd.chlcal4;  % Add yd
ctd.Chl=ctd.chlcal5;  % final cal  ug/l = mg/m^3
% fraction by weight  ( mg/m^3  ) / ( kg/m^3 ) = mg/kg
ctd.Chl_ugkg=ctd.Chl./(1000.+ctd.Sig0(:,1))*1000;  % ug/kg  close to usual units

%  adjust old values in structure
ctd.chl0=ctd.chl;  %  initial calibration from v4
ctd=rmfield(ctd,'chl');  % avoid confusing names

newctd=ctd;

%%%% END Calibration routine for NAB KNORR CTD Chlorophyll Fluorometer
```

APPENDIX - KNORR CALIBRATION PLOTS

APPENDIX - BJARNI CALIBRATION PLOTS