The Great Wetstar Fluorometer Mystery - why are they reading so high?

Rich Pawlowicz and Randall Lee,

Department of Earth and Ocean Sciences,University of British Columbia,
Vancouver, Canada, V6T 1Z4, email: rich@ocgy.ubc.ca

Oct 2, 2002

Abstract

A number of Wetstar fluorometers purchased/rebuilt and/or calibrated in 2002 appear to have a greatly increased sensitivity compared with older instruments of the same nominal range (reading about a factor of 4-5 higher than the older instruments and 7 times higher than extracted samples). The older instruments read about a factor of 1.5-2 higher than extracted chla values. The difference is systematic and we suggest that some kind of problem with the factory calibration is the cause.

Background

We began monthly bio/physical sampling in the Strait of Georgia in April 2002 using an SBE-25 with various sensors. After the third (June) survey, our Wetstar fluorometer (#417, calibrated Jul 98) flooded through a cracked connector and was destroyed. We used a borrowed fluorometer (#841, owned by J. Dower, UVictoria, calibrated June 2002) for the July survey. #417 was rebuilt and used for Aug and September cruises. Both #841 and #417 were used on other cruises as well during these months.

On comparison with extracted ("bottle") chla values measured on a Turner 10AU, we found that measured and Wetstar-derived chla values were reasonably consistent for the first three months, but differed by what appeared to be an order of magnitude for the following surveys (it was difficult to be more accurate because of sampling issues).

In addition, the fluorescence signal actually saturated at 5V on a number of casts, not only during our work but also during other cruises this summer, something not seen before in these waters using instruments of the same nominal sensitivity.

Possibilities were:

1. Extracted values were incorrect. However, the extracted values were entirely consistent with previous experience in this area. and an order-of-magnitude increase phytoplankton concentrations seemed pretty unlikely.

2. An increase in fluorescing organic debris. However, the protocol for extracted chla actually measures this factor and the relative ratios were entirely reasonable and consistent with past experience in these (and other) waters.

3. The 'in-vivo'/extracted comparison suddenly changed due to a change in the age and/or species mix of the phytoplankton population. This is certainly possible but it seemed a suspicious coincidence that this would occur at the same time we changed instruments. In addition, although some changes are expected an order of magnitude change in this comparison seems suspiciously large.

4. A CTD malfunction related to the destruction of #417 (perhaps the A/D circuitry was compromised?).

5. A problem in the instrument set-up or in the conversion from voltages to "nominal" fluorescence.

6. A systematic problem in calibration by Wetlabs (systematic because both 417 and 841 seemed to be providing consistent results, and both had been calibrated this year).

CTD malfunction/setup error ruled out

First, we switched fluorometers and CTDs, comparing Strait of Georgia casts taken by different instruments (set up independently by different users). Very similar fluorescence profiles were found, apparently

Fluorometer	*Cal. Date*	*Vo*	*V50**	*Noise*	*FScale V*
#830 (Digital01)/counts	4 Sept'02	0.047/56	1.435/1211	0.423/0.743	5.001/4095
#245 (Digital#2)/counts	5 June'02	0.051/58	1.425/1189	0.531/0.77	5.508/4095
#417 (CTD)	23 July'02	0.048	3.007	0.872	5.482
" " "	1998	0.098	3.072	0.23	5.009
#841 (Uvic)	29 May'02	0.06	3.028	0.833	NA
#626 (Roger)	23 Jun'00	0.068	2.944	0.155	4.89981
#715 (IOS)	29 Sep'01	0.053	3.147	0.313	5.2586
EcoDFLSB−115	10 May'02	188	2178(V25#)		4095

*V50= voltage equivalence to 50 ug/L chlorophyll−a
#V25 = counts equivalence to 25 ug/L chlorophyll−a

Figure 1: Calibration dates and coefficients

ruling out a set-up problem and/or a CTD circuitry malfunction.

Laboratory testing procedures

We then decided to do tests in the lab, intercomparing available instruments. During the September cruise (which actually took place late August) 25 litre carboys were filled with Strait of Georgia water from locations near the fluorescence peak at two stations, covered in opaque plastic and/or a towel and stored overnight on ice. The next day a test rig was set up in the lab, consisting of a gravity-feed from a carboy about 1m above the bench down a single hose which was then split in two to pass through two fluorometers, whose analog output was monitored on a pair of multimeters.

After these initial tests it was noted that chla values decreased slightly as the carboy emptied; possibly because the water was stratifying. In the final series of tests the water was periodically stirred to prevent this. In addition, the possibility that the fluorescence induced by the Wetstar would affect values for extracted samples was handled by creating a 3-way split in the feed hose and drawing un-fluoresced samples. The final test runs were also done in the dark, and the delay between sampling and laboratory testing was reduced to about an hour to avoid any problems associated with longer delays. These efforts (other than the stirring) did not appear to noticeably degrade or improve the results.

Voltages (and later, digital counts) were converted to nominal chla using the formula

$$C(\mu g/l) = SC * (V_{output} - V_{blank}) \qquad (1)$$

where V_{blank} was taken from the calibration sheets (Figure 1), and SC was computed so that the calibration voltage taken from the cal sheets (around 3V for the 75 $\mu g/l$ range) would give a chla value of 50, the stated nominal chla equivalence of the copro solution for Wetstars, or 25, the stated equivalence of the calibration solution for the ECO-DFLSB.

Intercomparison of "2002" instruments

For this first test 4 fluorometers were available. Two were "digital Wetstars" (a new #830 and a rebuilt #245, obtained spring 2002 for eventual use in a ship flow-through system), with a nominal range of 150 $\mu g/l$. The other two, both having a nominal range of 75 $\mu g/l$ were the rebuilt #417 and the brand-new #841. Various pairs were taken in order and the mean and range of the analog output voltage estimated "by eye".

The range of fluctuations was observed to be about $\pm 10\%$ of the mean value (Figure 2), and it appeared that the carboy had stratified somewhat as

Fluorometer	St5 avg(var)	S4 avg(var)	Ebay1 avg(var)	Ebay2 avg(var)
#830	20.6 (4.6)	63.1 (4.5)	45.1 (1.9)	30.4 (0.6)
#245	21.8 (2.2)	60 (6.0)	NA	27.7 (1.2)
#417	17.8 (1.7)	55.8 (5.4)	39.7 (5.9)	31.3 (2.6)
#841	15.8 (0.5)	56.3 (4.9)	44.5 (4.0)	31.0 (1.5)
#626	NA	NA	9.6 (0.6)	7.7 (0.04)
#715	NA	NA	NA	6.1 (0.5)
Eco	NA	NA	NA	4.4 (0.5)
Turner (lab)	2.6	6.9	5.4 (0.2)	4.25 (0.25)

Comparison of Chlorophyll-a estimates (mg/m^3) from Wetstar fluorometers and Laboratory analysis.

Figure 2: Nominal chla results for 4 tests against natural waters from the Strait of Georgia. #830 and #245 are "digital" Wetstars. The ECO-DFLSB is serial number #115. Wetstars #626 and #715 are the "older" instruments, the first 4 are the "2002" instruments. Turner values are extracted chla measured using standard protocols for water samples. The first two tests were run in Late August, and the others at different times in September.

nominal chla values decreased with time by 10-20% as the carboy emptied. However, although voltages varied by instrument the nominal chla values were quite consistent. For carboy S5 nominal values were in the range of 16-22 $\mu g/l$ (Figure 2), and for carboy S4 56-63 $\mu g/l$. Extracted chla values were 2.6 and 6.9 respectively (differing by a factor of about 8). The lab-measured Wetstar chla values matched those found from the relevant CTD profiles obtained during the previous day.

Thus it appeared that a) it was not a CTD setup or circuitry problem, b) it was not a problem specific to a particular serial #, with the caveat that all instruments tested had been calibrated in the April-June time-frame.

Intercomparison with older instruments

However, we were still left with the problem that the pre- and post-rebuild values measured by #417 were wildly different. We then made attempts to borrow fluorometers that had not been calibrated recently. First we obtained #626 (owned by R. Pieters, UBC, and calibrated Feb 2000). Another carboy of surface water was obtained from a sailboat in English Bay (part of the Strait of Georgia) and another set of tests done, this time stirring the carboy to prevent stratification problems. The results of this (Figure 2) showed that the "2002" fluorometers consistently read in the range 40-45 $\mu g/l$, compared with an extracted value of 5.4 (again a factor of 8 difference). However, #626 gave a reading of only 9.6 $\mu g/l$ (a factor of about 4.4 smaller than the other fluorometers, but about 1.8 times greater than extracted value).

The size of fluctuations was a bit of an issue, and we attempted (unsuccessfully) to do a calibration against the spinach extract used to calibrate the Turner 10AU; but the small quantities available made it difficult to use in the test rig we had and results were not trustworthy.

We then borrowed #715 (owned A. Pena, IOS, and calibrated Sept 2001), and also recovered an ECO-DFLSB (#115, calibrated April 2002) which had been moored in the Strait over the summer. Although it had been moored slightly below the fluorescence maximum comparison with CTD data at this location from all surveys tended to confirm the factor of about 5 change in Wetstar fluorometer chla values, and did *not* show any sudden increase in chla.

A final set of tests were run in which all 7 fluorometers were intercompared using another sample of English Bay surface water (see Figure 3). The Wetstars were rigged in pairs on the gravity-feed hose as before. Each pair consisted of one of the digital fluorometers (this time logged to a computer) and one of the analog fluorometers whose voltages were read by eye from a multimeter. In addition, the shutter was removed from the ECO-DFLSB and it was inserted into the mouth of the carboy at the conclusion of the run and its readings also logged to computer (it was not clear in advance if its readings would be biased by the plastic walls of the carboy but

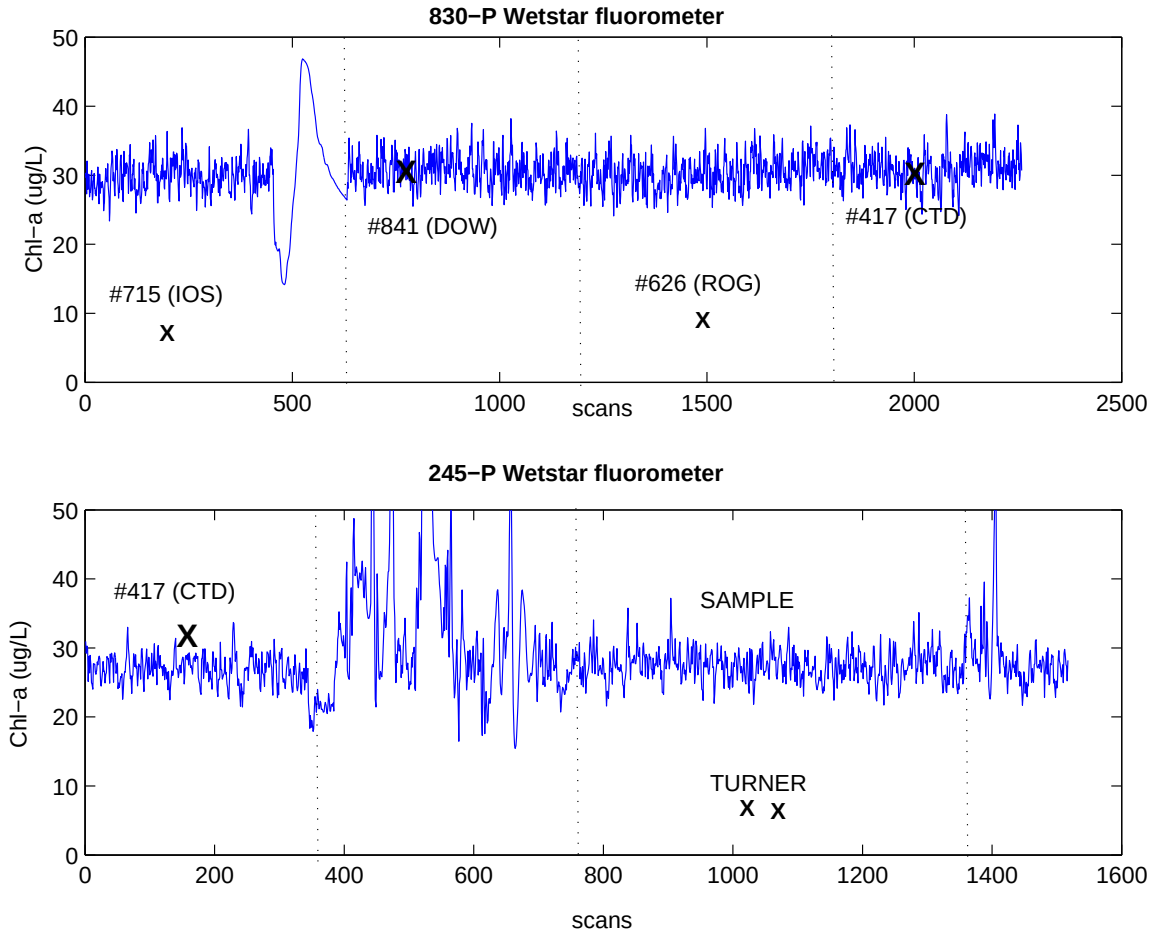

Figure 3: Comparison of various analog Wetstars against the digitals. Large excursions occur when analog fluorometers are changed in the test rig.

the results appear reasonable).

The "2002" Wetstars gave consistent readings in the range 28-31 $\mu g/l$ (Figure 2), compared with "older" Wetstar readings in the range 6.1-7.7 $\mu g/l$, ECO readings of 4.4 $\mu g/l$, and extracted values of 4.25 $\mu g/l$, thus suggesting that:

1. "older" Wetstar chla were about 1.5 times greater than extracted chla.

2. "new" Wetstar chla were about 7 times greater than extracted (and hence 4-5 times greater than "older" Wetstars.

3. ECO-DFLSB was the same as extracted chla.

Now, how does this relate back to pre-rebuild #417? A student project in Feb 2002 (in Barkley Sound, on the West Coast of Vancouver Island) compared fluorescence values against the Wetstar (mounted on an SBE-19), and found (for extracted chla in the range of 0-1 $\mu g/l$) that the Wetstar measured about 2-2.5 higher than actual values. This suggests that the pre-flood #417 was scaled similar to the other "older" Wetstars.

5. Conclusions

Using natural waters we have found that 4 Wetstar fluorometers purchased/rebuilt and/or calibrated since April 2002 give consistent readings which are about a factor of 4 or 5 higher than the consistent readings from two Wetstar fluorometers purchased/calibrated in 2000 and 2001. The older Wetstars seems to read about a factor of 1.5-1.8 higher than extracted chlorophyll, and this seems to match a (rather noisy) estimated factor of about 2.5 found for pre-flood #417 in the spring (in another ecosystem). An ECO fluorometer purchased/calibrated this year

seems to give values consistent with extracted chla. The variation in these scale factors appears to arise partly from sample to sample variability and may be due to changes in the size and species composition of the phytoplankton within the Strait of Georgia, but the sudden apparent increase in Wetstar derived chla in CTD surveys, coinciding with the use of a different unit, is not otherwise apparent in mooring or water sample time series.

Thus it appears that the calibration procedure used by Wetlabs for the Wetstar fluorometers has changed substantially sometime between September 2001 and April 2002. It would be useful to resolve this issue because at this point comparisons between different instruments are not possible, and also because at their current setting the instruments are saturating unexpectedly.

Acknowledgements

John Dower (UVictoria), Angelica Pena (IOS), and Roger Pieters (UBC) lent us their Wetstar fluorometers.. Shannon Harris (UBC) helped with the chla analysis and the operation of the Turner 10AU. Phil Whittle (UBC) attempted the fluorometercalibration in Feb 2002. Ian Walsh (Wetlabs) offered suggestions.