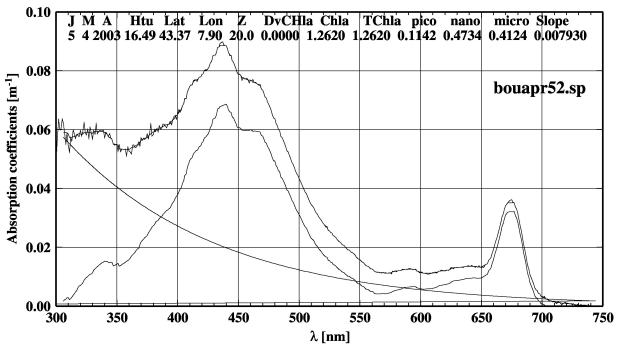
BOUSSOLE Particulate Absorption Measurements

Comparison between the results from two spectrophotometers over a year (December 2010 to November 2011).



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Introduction

The "**BOU**ée pour l'acqui**S**ition d'une **S**érie **O**ptique à **L**ong term**E**" project (BOUSSOLE), can be translated from French as the “buoy for the acquisition of a long-term optical series." It involves monthly servicing cruises to an offshore site between Nice and Corsica where a buoy dedicated to radiometric measurements acquires data continuously. During these visits, the activities are dedicated to the buoy servicing and to the acquisition of complementary data such as vertical profiles of radiometric quantities, phytoplankton pigments (HPLC), particulate absorption (filter pad technique), total suspended matter etc…(Antoine et al., 2006). These latter three parameters are obtained thanks to *in situ* sampling of seawater at 11 depths throughout the euphotic layer using rosette and CTD deployments.

The present work focuses on the comparison of the filter pad particulate absorption data obtained from two different spectrophotometers. With the acquisition of a new Perkin Elmer lambda 850 spectrophotometer at the LOV, it was important to ensure continuity in the measurements done in the frame of the BOUSSOLE time series, before passing from one older instrument, which is not used for BOUSSOLE measurements anymore since November 2011 to a new and more sensitive one.

Material and methods

2.1. Sample collection and storage

Seawater samples were collected from the BOUSSOLE site at 11 depths between the surface and 200m (5, 10, 20, 30, 40, 50, 60, 70, 80, 150, 200m) using a rosette sampler equipped with eleven 12L Niskin bottles. 2L of seawater were filtered onto 25mm GF/F Whatman filters (0.7µm particle retention).

The filters were immediately placed in polycarbonate petri slides which were covered with aluminium paper and placed in liquid nitrogen then at -80°C until analysis.

The studied cruises cover the yearly seasonal patterns, ranging from cruise 105 (December 2010) to 117 (November 2011).

2.2. Spectrophotometric analysis

Perkin Elmer Lambda 19 (L19) spectrophotometer

Acquired in 1993, it is a dual path spectrophotometer, equipped with 2 detector modules. The first is a standard detector and the second comprises a 150mm integrating sphere lined with barium sulphate. This sphere only allows for absorption measurements of the filter in front of the sphere. Antoine et al. (2006) describe in detail the particulate absorption protocol on this instrument. The instrument settings are summarized in Table 1:

Lamps:	Deuterium + Tungsten	**PMT slit:**	2nm
Range:	800-300nm	**Number of cycles:**	1
Data interval:	1nm	**Smooth**	0nm
Scan speed:	240nm/min	**Ordinate mode**	A
Lamp change:	319.20		

Table 1: Summary of the settings for the Lambda19 spectrophotometer

Perkin Elmer Lambda 850 (L850) spectrophotometer

Acquired in 2010, it is also a dual path spectrophotometer and its specifications include 2 detector modules: the first is a standard detector and the second comprises a 150mm integrating sphere lined with barium sulphate and with a device for measurement of filter pad absorption inside the sphere. However, in order to maintain a similar protocol as with the L19, the filters are placed in front of the sphere and not inside it. The instrument settings are summarized in Table 2:

Lamps:	Deuterium + Tungsten	**PMT slit:**	2nm
Range:	800-300nm	**Number of cycles:**	1
Data interval:	1nm	**lens**	Mode C
Scan speed:	266.75nm/min	**CBM**	10% with open iris
Lamp change:	319.20	**Data collection**	Mode A

Table 2: Summary of the settings for the Lambda850 spectrophotometer

The instrument and data processing software is Perkin Elmer UV WinLab 6.0.3.0730.

Before installing the integrating sphere for filter pas measurements, the spectrophotometer is switched on with the standard detector module so to carry out calibration verification. The calibration is then verified with the integrating sphere module in place.

Measurement protocol

a) Preparation:

Blank filters are left to soak in distilled water for 1 hour prior to analysis.

The laboratory is always maintained in fairly dark conditions (shutters closed, no direct lights) to avoid any stray light affecting the measurements but also to prevent any pigment light-degradation in the samples.

The spectrophotometers are switched on at least 1 hour before analysis.

Using forceps, a water saturated blank filter is placed over the opening of the integrating sphere. The adherence of the damp filter should be sufficient to keep it in place.

An “autozero” is carried out and when this is complete, two measurements of the blank filter are made in order to visualize the baseline and noise. This should vary around the zero value, with a 0.02 AU standard deviation for the lambda19 and 0.003 for the lambda850. If these conditions do not occur, the settings and the filter are checked and an “autozero” and 2 blank measurements are repeated until the results are satisfactory. In some cases it may be necessary to recalibrate the spectrophotometer (with the standard detector module) to recover optimal results.

b) Scanning the samples:

The samples are maintained in a cooler with an ice-pack while waiting to be analysed. They are placed at room temperature for defrosting for maximum one minute before being placed in the spectrophotometer.

Using forceps, the sample filter is placed in front of the integrating sphere opening using existing moisture in the filter for adherence. The same filter is placed first in the lambda19, then in the lambda850.

Only one scan is carried out per instrument in order to avoid any potential degradation of the pigments in the sample.

Once the second scan is complete the filter is immediately placed in a 10mL Falcon tube with 3mL of 100% methanol for pigment extraction and subsequent HPLC analysis (according to the method described in Ras et al., 2008).

Data processing

For subsequent quantification purposes, the diameter of the filtered area is reported for each filter. However in this particular study, this information is not used.

In this study, the individual raw files were extracted in the form of ".sp" or ".asc" files for the lambda19 and lambda850 respectively. Due to the variable hydration level of the filters, a spectrum offset can be observed. This was corrected for by subtracting the average value of the signal between 799 and 789nm.

The spectrum from the Lambda 19 and Lambda850 were then compared for each sample and a regression analysis was carried out for each cruise, thus allowing for the identification of outliers.

Results

Table 3 summarizes the amount of data that were analysed during this study.

Dates	Cruise Number	Number of casts	Number of samples	Number of blanks	Number of data points
10-13 December 2010	105	4	35	4	17533
18-21 January 2011	106	1	9	4	6513
12-15 February 2011	107	3	32	6	18926
3-6 March 2011	108	2	20	4	12024
25-29 March 2011	109	2	21	4	12525
27-29 April 2011	110	3	30	5	17535
18-20 May 2011	111	3	33	6	19539
16-18 June 2011	112	3	33	6	19539
11-13 July 2011	113	3	32	5	19,038
15-17 August 2011	114	3	33	6	19539
13-15 September 2011	115	2	22	4	13026
18_21 October 2011	116	1	11	2	6523
15-18 November 2011	117	3	31	6	19038

Table 3: description of the particulate absorption dataset obtained between December 2010 and November 2011.

1.1 Blank samples (wet GF/F filters)

In this study, noise ranges were defined as 6 times the standard deviation of the blank measurements.

The lambda850 spectrophotometer presents a 10-fold higher sensitivity (average noise 0.0043 AU) than the lambda19 (0.029 AU).

The “zero” value for all cruises varies on average around -0.0007 AU and -0.0017 AU for the lambda850 and lambda19 respectively. While the lambda850 presents average blank values between 0.0004 and -0.0030 (0.0034 AU amplitude), the lambda19 presents a significantly wider range of blank values between 0.0097 and -0.0139 (0.0236 AU amplitude). Comparison has been made with a few blank spectrum analysed during 2008, 2009 and 2010 (Table4). Results indicate that the noise is slightly higher in 2011 than in 2008, suggesting the effect of lamp aging. However the performance and therefore the quality of the lambda19 data have not been significantly altered since 2008.

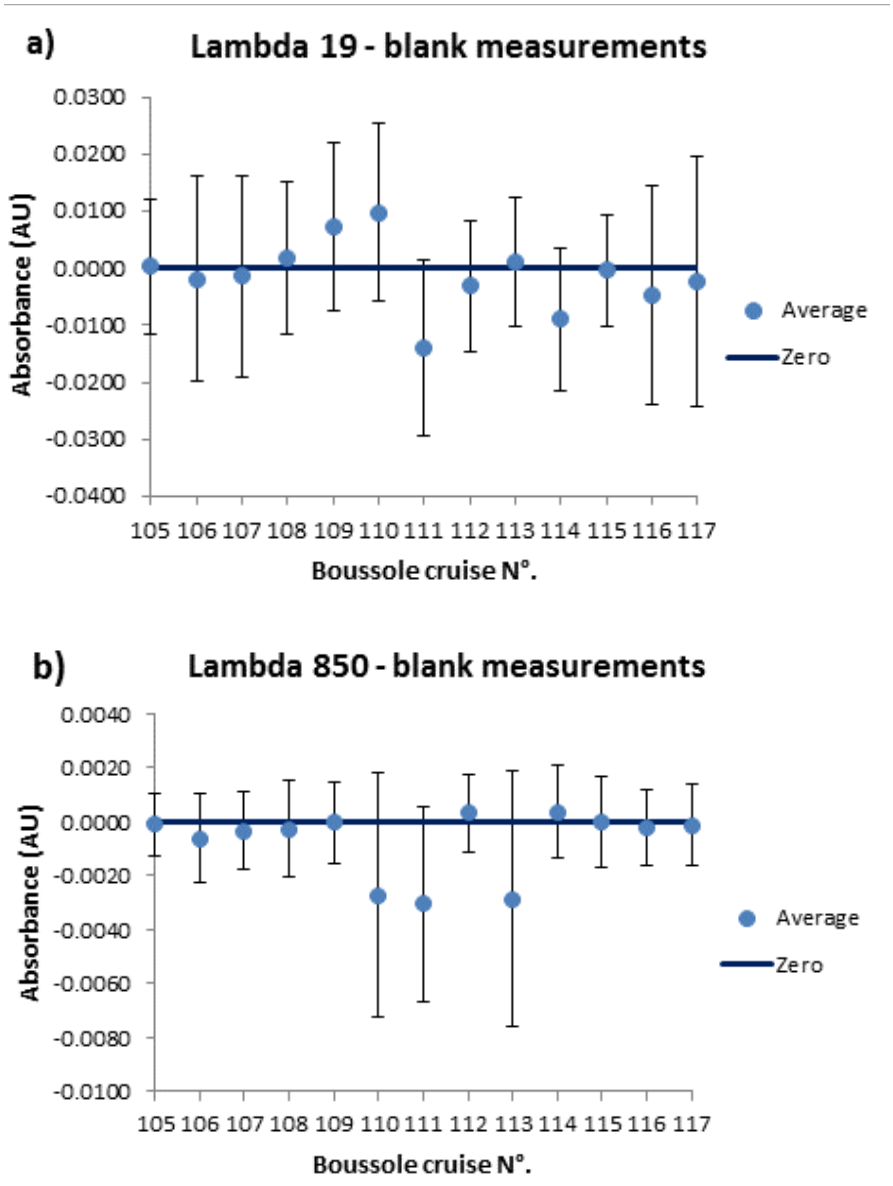


Figure 1: Average blank measurements for the different BOUSSOLE cruises in this study. The range of the error bars represents the noise range defined as ±3 times the standard deviation of the blank measurements.

	Noise (AU)	« zero » value (AU)
Average (2011)	0.030	-0.002
BOUSSOLE102 (2010)	0.029	0.003
BOUSSOLE092 (2009)	0.026	-0.007
BOUSSOLE075 (2008)	0.017	-0.002

Table 4: Blank measurements and noise values (6 times the standard deviation) obtained in 2008, 2009 and 2010, from the lambda19 spectrophotometer.

Figure 2 illustrates the difference in amplitude generally observed between the 2 instruments, where the highest variability for the lambda19 is essentially encountered between 300 and 400nm and between 700 and 800 nm. The lambda850 also presents a slight increase in variability within these ranges. Although it is not very significant this variability should be regularly monitored in order to detect eventual lamp aging.

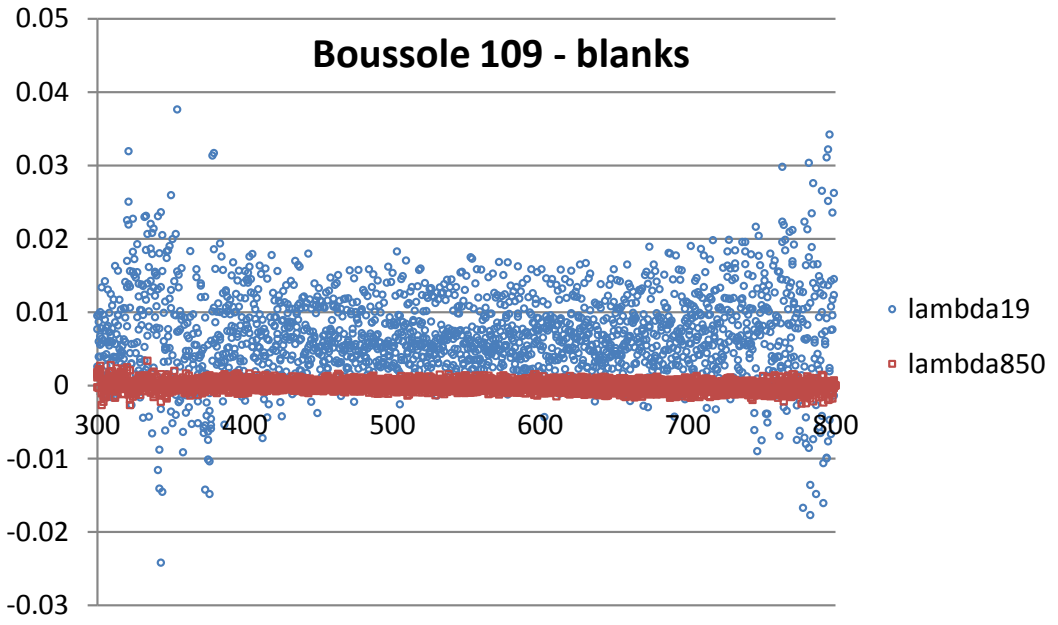


Figure 2: Corrected blank measurements (in AU) from the BOUSSOLE 109 cruise, carried out on the lambda19 (blue circles) and lambda850 (red squares) spectrophotometers.

1.2 Seawater samples

In a general overview of the dataset, regression analyses were carried out and compared for each cruise in Table5. On average, the slope varied around 1.008, while the intercept was close to zero. The average determination coefficient varied around 0.986. The regression sum of squares is particularly high during the spring bloom period from March to June (cruise 109-112).

lambda19= f(lambda850)	slope	intercept	r2	residual sum of squares	regression sum of squares
105	1.021	-0.0007	0.987	0.79	61
106	1.007	0.0007	0.978	0.62	28
107	1.017	-0.0009	0.984	1.41	85
108	1.007	0.0011	0.981	0.65	34
109	1.009	-0.0006	0.997	0.76	233
110	1.038	0.0023	0.994	1.46	249
111	1.004	-0.0010	0.994	1.11	177
112	1.019	-0.0008	0.994	1.04	170
113	1.005	-0.0018	0.994	0.81	142
114	1.019	0.0003	0.991	0.85	92
115	0.958	0.0005	0.989	0.52	45
116	0.978	-0.0064	0.979	0.41	19
117	1.024	0.0010	0.961	1.42	35

Table5: Summary of the statistics resulting from the regression analysis between Lambda 850 and Lambda19 data for each individual cruise over the whole studied period: slope, blank, determination coefficient r^2, residual sum of squares (error) and regression sum of squares.

Note that cruise 117 shows the lowest value of r^2, with a corresponding high value of residual sum of squares, pointing to a larger range of error between the lambda19 and lambda850 data. This could be partly due to the fact that four lambda850 spectra during this cruise presented decreasingly negative values between 325 and 300 nm, as illustrated in Figure 3. These samples were deep samples from 3 different CTD casts. For the moment, no clear explanation has been found for this observation.

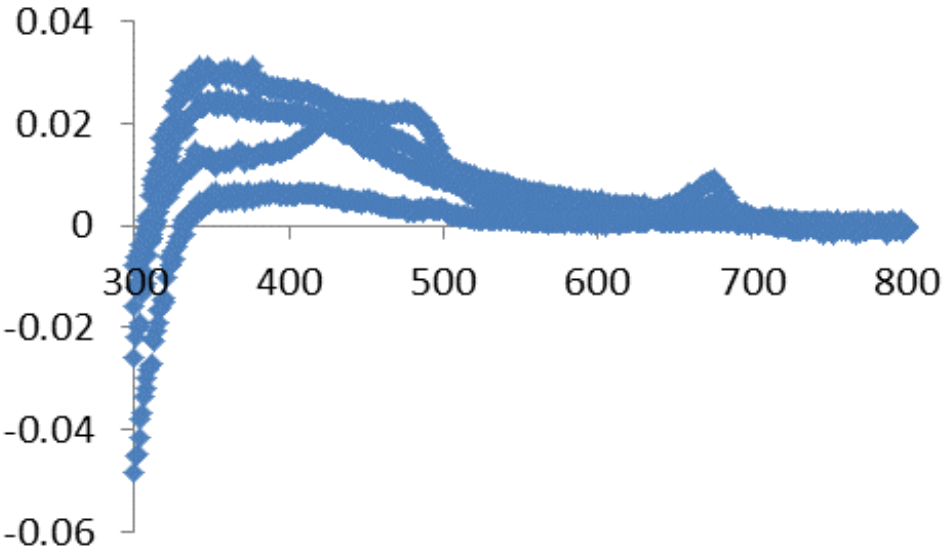


Figure 3: Cruise 117 deep samples from 400m and 200m (CTD1), 150m (CTD2) and 80m (CTD3) presenting strongly negative values between 325 and 300nm.

When the signals at 676nm from the 2 spectrophotometers are compared over the whole cruise period, most of the data can be found in between the 90% confidence intervals. As illustrated in figure 4, only the data from cruise 114 (august 2011) present points that are outside of these limits. They are not particularly associated to a certain depth range. Nevertheless, the general trend shows a good

determination coefficient of 0.976, with a slight overestimation of lambda19 values relative to the lambda850 data (slope= 0.97).

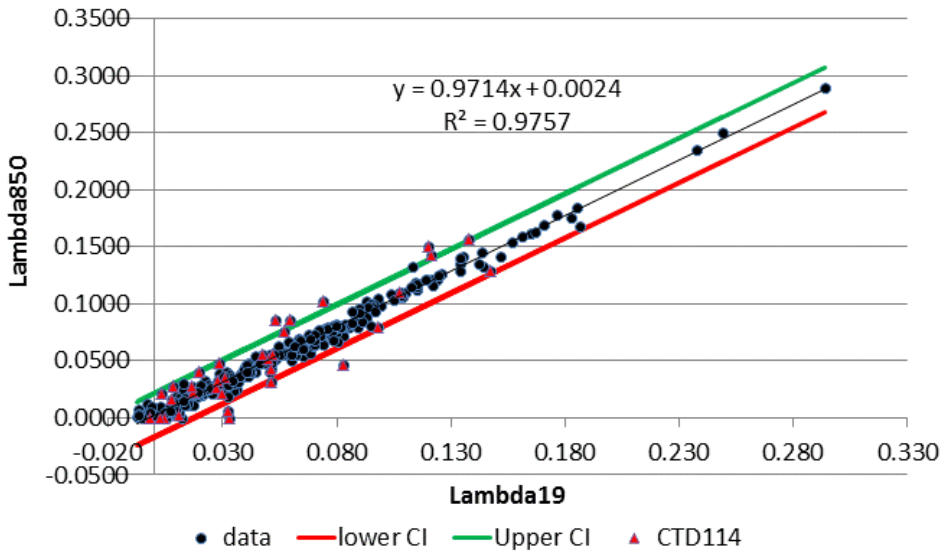


Figure 4: Comparison between the data points at 676nm for lambda850 as a function of lambda19 over the whole studied period. The red and green lines represent the 99% lower and upper confidence intervals respectively. The blue dots represent the whole dataset, while the red triangles represent the cruise 114 data.

As a case study, Figure 5 illustrates the comparison in the results for 3 samples from CTD01 during cruise 109 (28 march 2011) collected at different depths (5, 50 and 150m) while spring bloom conditions prevailed. The surface sample presents the highest concentrations (equivalent to 1.5 mg Chla.m^{-3}, Figure 5a). The TChla profile obtained from HPLC analysis points to typical bloom conditions at the surface, rapidly decreasing below 30m. The 50m sample is therefore at an intermediate level, where the phytoplankton biomass varies around 0.34 mg Chla.m^{-3}. At the 150m sample, there is hardly any phytoplankton biomass present, with values around 0.026 mg Chla.m^{-3}.

For the 5m sample the determination coefficient is the best for this sample. The strong absorption signal tends to reduce the differences observed and therefore the effect of the noise for the lambda19. The slope of the regression line is close to 1.

As the concentration in particles reduces, the effect of the lambda19 noise becomes more important (figures 5c and 5e). The slope of the regression line (figures 5d and 5f) tends to decrease, pointing to a general overestimation of lambda19 data as the Signal to Noise ratio decreases.

In Figure 6b the signals at 665nm for both instruments are plotted on the same depth profile as for TChla (Fig. 6a). This wavelength is considered to be proportional to the amount of TChla in the sample. Regression lines between the two instrument signals at 665nm and the TChla concentration (Fig. 6c) show very similar slopes. However, it is the determination coefficient for the lambda850 that is slightly better (R^2 0.98 for lambda850, 0.96 for lambda19).

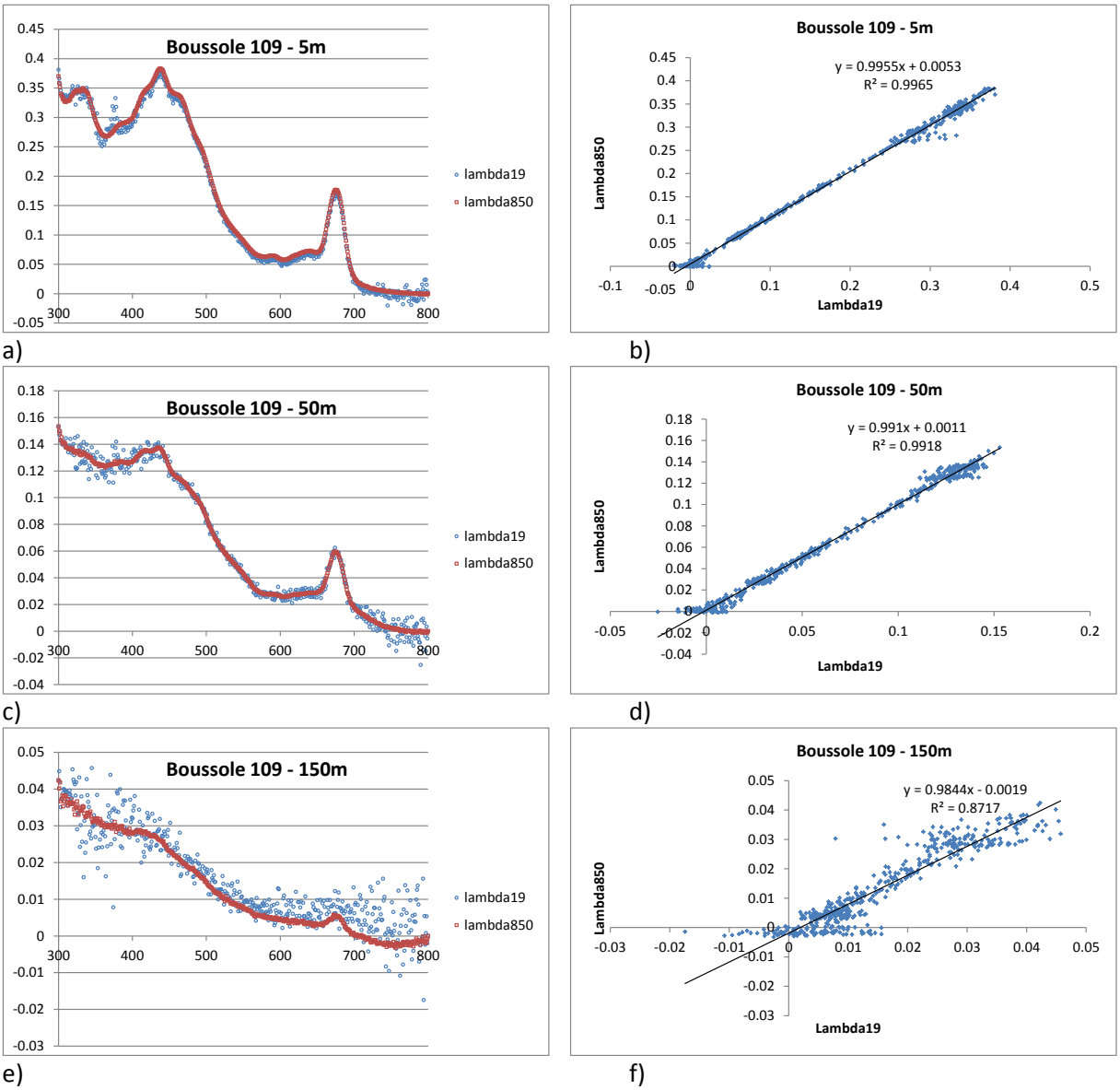


Figure 5: Summer oligotrophic conditions: CTD01 from the 109 cruise (28 March 2011). a), c), e) : Absorption spectra for both instruments at 5m, 50 and 150m respectively. b), d), f): Regression line for Lambda850 as a function of Lambda19 at 5m, 50m and 150m respectively.

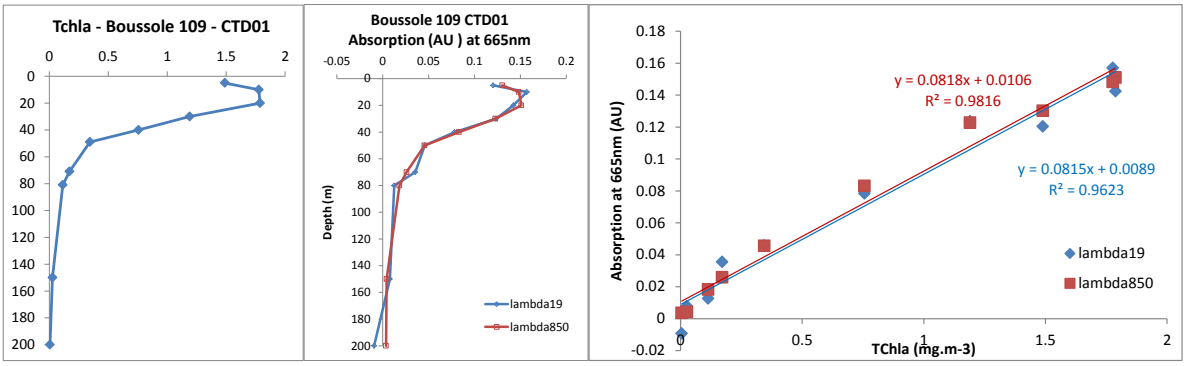


Figure 6: Spring bloom conditions. Depth profiles for a) Tchla ($mg.m^{-3}$) and b) 665nm signal for both instruments for the CTD01 profile of cruise 109 (28 March 2011). c) Distribution of the 665nm absorption signal for both instruments as a function of TChla ($mg.m^{-3}$).

These results suggest that for the high concentration samples, the equivalence between the two spectrophotometers is good (surface sample: slope = 0.99, R^2=0.996). However, due to the important noise effect for the lambda19, its results for low concentration (deep) samples appear to be less reliable with a slope pointing to a slight overestimation for the lambda19 data and a larger dispersion of the data points (deep sample: slope = 0.98; R^2 = 0.87).

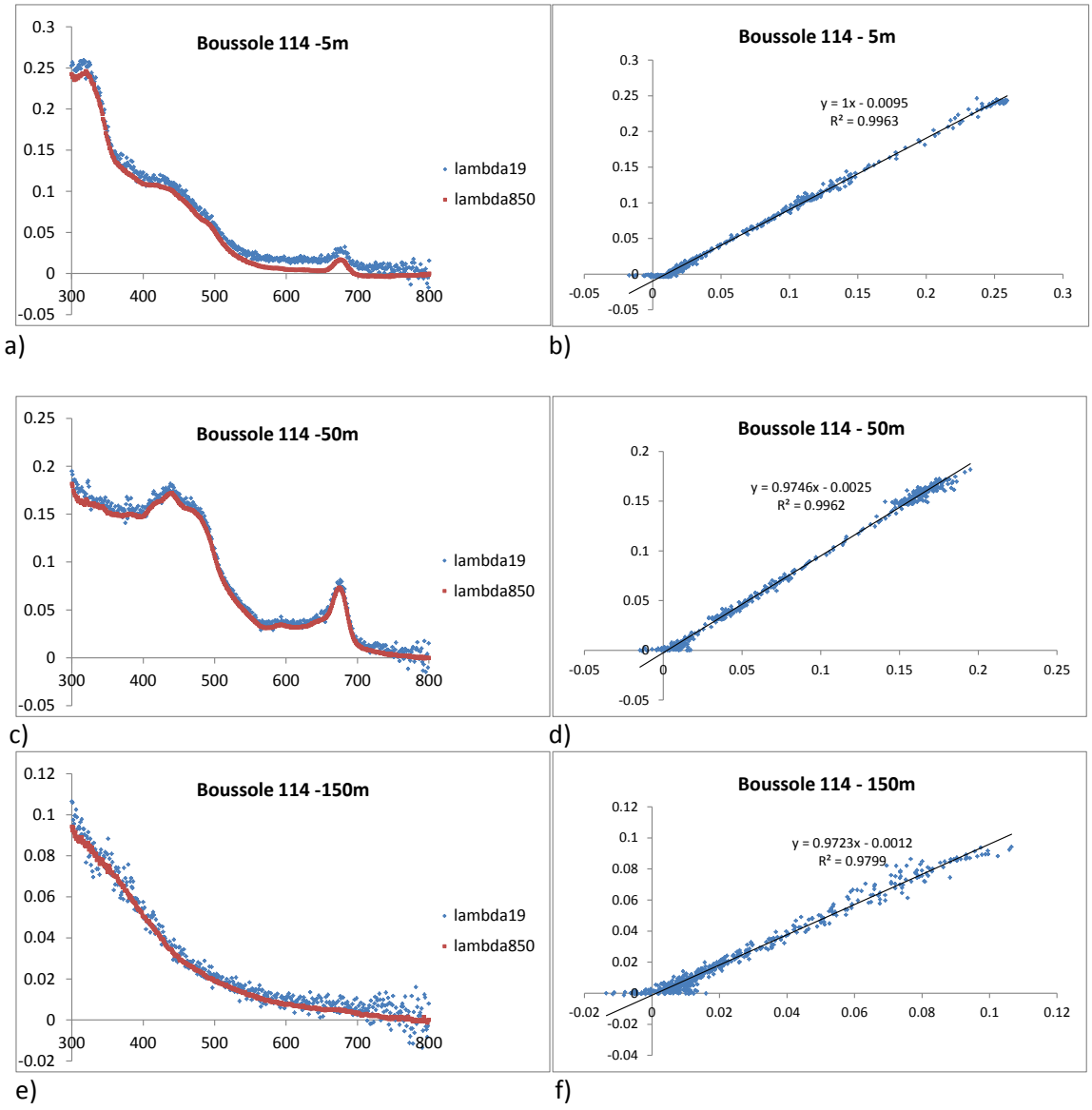
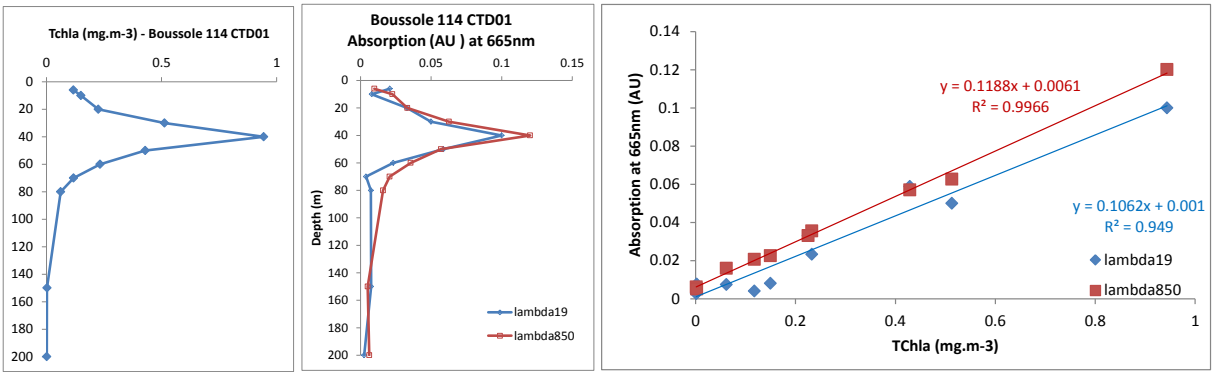


Figure 7: Summer oligotrophic conditions: CTD01 from the 114 cruise (15 August 2011). a), c), e) : Absorption spectra for both instruments at 5m, 50 and 150m respectively. b), d), f): Regression line for Lambda850 as a function of Lambda19 at 5m, 50m and 150m respectively.

Another contrasting time of the year is the summer oligotrophic period. Results from August 2011 are presented in figure 7. These results show that the results for the sample from the DCM at 50m, are slightly overestimated for the lambda19 (surface sample: slope = 0.97, R^2=0.996). The surface sample, although characterized by a low phytoplankton biomass presents high values of detrital matter which strongly absorbs towards the UV. The slope and determination coefficients are similar to the surface sample from the 109 cruise (slope = 1, R^2 = 0.996). The important noise effect for the lambda19 is once again visible for the deep 150m sample, and as for the 109 cruise, results appear to be less reliable with

a slope pointing to a slight overestimation for the lambda19 data and a larger dispersion of the data points (deep sample: slope = 0.972; R^2 = 0.97).

In Figure 8a, the surface sample presents low TChla concentrations typical of oligotrophic conditions (equivalent to 0.117 mg Chla.m-3) and a DCM can be observed at 40m depth. In Figure8b the signals at 665nm for both instruments are plotted on the same depth profile as for TChla. Regression lines between the two instrument signals at 665nm and the TChla concentration (Figure 6c) show a larger difference than for the 109 cruise samples, with higher absorption values for the lambda850. As observed above, the determination coefficient for the lambda850 is slightly better (R^2 0.99 for lambda850, 0.95 for lambda19).



a) b) c)

Figure 8: Summer oligotrophic conditions. Depth profiles for a) Tchla ($mg.m^{-3}$) and b) 665nm signal for both instruments for the CTD01 profile of cruise 114 (15 August 2011). c) Distribution of the 665nm absorption signal for both instruments as a function of TChla ($mg.m^{-3}$).

Discussion and conclusions

A general assessment of the dataset reveals several points:

- The necessity to clean the lambda19 dataset as there are a number of irregular data points that must be eliminated. These points are generally found between 300 and 400nm or between 700 and 800nm. Some are merely outliers, others present values of 9.9999 in the raw data files.
- On both instruments the 800nm signal sometimes showed abnormally high values. For this reason the average range of 10 values used as a reference to correct the whole signal rather covers 799 to 789nm.
- The slope of the curve and the dispersion of points in a comparison between the spectra of the 2 instruments can be variable according to the season and to the absorption range.

The main problem encountered with the lambda19 is the strong noise in the measurements, especially at the extremities of the spectral range. This significantly affects the data when the particle concentrations are low. Moreover the number of outliers between 300 and 400nm and between 700 and 800nm is important. In some cases, it was observed that these outliers could cause an important offset for the whole spectrum, when the average value between 799 and 789 is used as a reference. This instrument's performance has probably degraded with time. The inside of the lambda19 integrating sphere, for example, today shows important signs of deterioration. However, such degradation is also typical of the visible lamp aging. Therefore the first steps in trying to improve the performance of the lambda19 would be to change both the visible and UV lamps, as well as carry out a renovation of the

inside of the sphere. For data processing of the lambda19 data already collected, a preliminary cleaning process of the spectrum should be carried out before the routine data processing.

To conclude, results suggest that the continuity between the two generations of spectrophotometers can be made without having a significant offset between the two datasets. Obviously, the new lambda850 instrument shows a much stronger stability and sensitivity, with a noise level which is approximately 10 times lower than for the lambda19. This leads to a clear improvement for the measurements of low concentration samples. Therefore if an offset might be observed between the two instruments, it would be for the low concentration deep samples. In the summer period the phytoplankton biomass is low at the surface but there remains an important component of detrital matter which therefore reduces the impact of instrumental noise. This study also points to lower accuracy and precision values at the extremities of the spectra (300-400nm and 700-800nm) from the lambda19, thus implying that precaution should be taken if using data from the lambda19 within these ranges data previous to 2011.

Acknowledgements

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