AN INTER-COMPARISON OF COLOURED DISSOLVED ORGANIC MATERIAL AND PHYTOPLANKTON ABSORPTION COEFFICIENTS: IMPLICATIONS FOR MERIS DATA VALIDATION.

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ABSTRACT

Understanding differences between laboratories in the measurement of absorption coefficients of phytoplankton, detrital and coloured dissolved organic material is important if we are to accurately validate MERIS data. Phytoplankton absorption coefficients (a_{phyto}) and the absorption coefficient of coloured dissolved organic material (a_{ys}) were determined by three laboratories on natural seawater samples taken from three stations off Plymouth Sound between 50 20.02 °N, 4 10.02 °W, during a second inter-comparison exercise (PlymCal 2). The absorption coefficient of an artificial CDOM sample composed of a coloured humic acid salt suspended in buffered MilliQ water was also determined by the three laboratories. For the natural seawater samples, good agreement was found between a_{phyto} at 442 nm at three stations where a_{phyto} varied between 0.066 and 0.145 m^{-1}. Over MERIS bands (412 to 680 nm) there was a 14 % difference between laboratories and the standard deviation was small (mean of all labs at MERIS bands ~0.003 m^{-1}). The range in coloured dissolved organic material absorption coefficient at 442 nm was from 0.1 at Station L4 (50 15 °N, 04 12.5 °W) when there was a 6 % difference between laboratories to 1.0 m^{-1} at Barnpool (50 21.3 °N, 04 10.1 °W), where there was a 48 % difference between laboratories. For the artificial CDOM sample the difference was 13 % at 442 nm and 20 % over MERIS bands.

Using a_{phyto} and a_{ys} values at Station L4 for the three laboratories during PlymCal 2, above water surface reflectance and water leaving radiances were calculated and then SeaWiFS OC4 switching band ratios was applied to calculate corresponding SeaWiFS Chlorophyll a (*Chla*) concentrations. The results showed that the variation in a_{ys} at L4 produced on average an 11 % variation in *Chla* and that the variation in a_{phyto} resulted in an 18 % variation in *Chla*. The variation in *Chla* using *in situ* absorption coefficients at L4 was less than the accuracy of SeaWiFS *Chla* using the OC4 algorithm (~35 %), which suggests that once methodologies have been fixed, the between laboratory variation in a_{phyto} and a_{ys} at L4 is acceptable and will not significantly affect the validation of MERIS data.

1. INTRODUCTION

Satellite sensors such as CZCS and SeaWiFS have provided enhanced global distributions of oceanic chlorophyll a (*Chla*) biomass both spatially and temporally. CZCS provided qualitative information on the global distribution of phytoplankton [1], whereas SeaWiFS yielded quantitative information of *Chla* distributions in Oceanic waters where the main absorbing constituent is phytoplankton [2]. The determination of *Chla* by SeaWiFS is based on blue to blue green band ratios [3,4], but this method has limited application in coastal regions, as it cannot distinguish between other major absorbing constituents such as coloured dissolved organic material and detrital organic material. Although coastal regions are only 10 % of the surface area of the global ocean [5], they account for ~30 % of the global primary production [6]. There is an obvious need to accurately determine *Chla* concentrations in coastal waters and this may be achievable with the new generation advanced satellite sensor MERIS, which has higher spectral and spatial resolution than previous satellite sensors [7]. Data from MERIS coupled with *Chla* Case 2 water algorithms [e.g. 8, 9, 10, 11] have the capability of differentiating between the main Case 2 water inherent optical property (IOP) constituents and yielding very high resolution spatial and temporal coverage of *Chla* biomass in coastal zones [12].

Before MERIS *Chla* products can be quantitatively used, data validation is required using *in situ* measurements. Historically a number of approaches have been proposed for the determination of the absorption coefficients of the main IOP's in seawater [13, 14, 15] and consensus is needed within the marine optics and remote sensing community on protocols for the determination of these parameters if accurate validation of MERIS products is to be achieved. For the determination of the absorption coefficient of phytoplankton these include *in situ* measurements using the reflecting tube absorption meter [16], the integrating cavity absorption meter [17], optico acoustic methods [18], modelling approaches based on the apparent optical properties of water [19] the filter transfer technique [15] and the filter pad method [20]. The differences between these methods can be > 40 % [21], which only adds to the uncertainty in "ground truthing" satellite data. Considerable progress has been made in documenting methodologies and fixing protocols for Case 1 [22] and Case 2 waters [23, 24, 25]. Once protocols have been standardised, inter-comparison exercises are necessary to understand the potential variation in parameters between laboratories and to assess whether these differences will significantly affect the accuracy of MERIS data validation.

In this paper measurements of *in vivo* phytoplankton and coloured dissolved organic material absorption coefficients are inter-compared using the same methodologies by different laboratories to assess whether *in situ* data from different laboratories can be used to validate MERIS observations. The results of the inter-comparison are then subjected to a sensitivity analysis to assess the effect of between laboratory variations in the major absorbing components of seawater on *Chla* concentrations derived from satellite algorithms.

2. METHODS

2.1 In vivo phytoplankton absorption coefficient

2.1.1. PlymCal 2: Three laboratories participated in an inter-comparison held at Plymouth Marine Laboratory from 11 to 14th June 2002; The Institute for Coastal Research (GKSS), Nowegian Institute of Water Research (NIVA) and Plymouth Marine Laboratory (PML). Samples were taken from the following stations: L4, 50 15 °N, 04 12.5 °W; Mayflower 50 21.7 °N, 04 10.3 °W (MF) and Barnpool 50 21.3 °N, 04 10.1 °W (BP) using CTD (type) and Niskin bottles. Samples from each site were transferred to 25l carbuoys and transported back to the laboratory. Three replicate samples were filtered onto 25mm GFF filters from the three stations and three blanks per station were prepared by passing an equal volume of filtrate from the seawater at each station that had passed through the 0.7 µm GFF filters. All filters were flash frozen in liquid nitrogen and subsequently stored in liquid nitrogen. Both GKSS and NIVA samples were transported back to their respective laboratories on dry ice before being re-stored in liquid nitrogen or at – 80°C and analysed.

2.1.2. Methodology. The method of Tassan and Ferrari [14] were used to determine phytoplankton absorption coefficients with some modifications. The "Autozero" of the instrument was made with free entrance ports, using high-grade spectralon plates at the exit ports and the baseline flatness was at least ±0.004 A units. The bleaching solution concentration was 3.33% NaClO, (0.1% active chlorine) and the application of the NaClO was made using Tassan and Ferrari [26]. The disappearance of the peak at 675 nm in the bleached sample and the concave shape of the OD spectrum near to 440 nm was used as evidence of complete filter bleaching [23]. 5 ml of MilliQ was re-filtered through the treated GFF filter to remove any residual NaClO [27]. Blank filters were also bleached and re-filtered using the same procedure. Care was taken to ensure that both sample and blank filters did not dry out since dry filters adversely affect the optical density of the sample. For the determination of phytoplankton absorption coefficients, GKSS used a Perkin Elmer Lambda 19 spectrophotometer with a 60 mm spectralon integrating sphere. NIVA used a Perkin Elmer Lambda 40P with a 20 mm integrating sphere (Labsphere RSA-PI-20) and PML used a Perkin Elmer Lambda 800 spectrophotometer with a 60 mm spectralon integrating sphere.

2.1.3. Data processing. In Case 1 waters a zero offset from the baseline may occur which is presumed to be the product of scattering throughout spectrum. Hence a spectral region is identified where phytoplankton absorption is assumed to be negligible (typically 750 to 800nm) and the scattering observed is due to non-phytoplankton material. In Case 2 waters scattering by particles ≥ 750 nm is not negligible since scattering and absorption by detritus increases with decreasing wavelength [14]. The experimental and data processing methods of Tassan and Ferrari [14; equations 11 to 14] were used to convert the measured absorbance of the filter-retained particles into the equivalent particle suspension

absorption. τ is defined as the ratio of (1-Tsd)/(1-Tsp) where Tsd is the transmission of diffuse light through the filter and Tsp is the transmission of parallel light. The following routine was used to calculate τ:

$$\tau = 1.171 - 0.2615 * \alpha + 0.00013 * \alpha * \alpha \qquad (\textit{Equation 1})$$

where α is the absorption in transmission mode either of the pigmented or de-pigmented sample. Four measurements were made for each pigmented and de-pigmented sample (two transmission and two reflectance [14]). The instrument baseline for the integrating sphere was recorded every four samples. An exponential curve was fitted to the detrital absorbance which was then used to offset the particulate absorbance to baseline. High suspension absorbance leads to increasing errors when applying pathlength wavelength corrections - β [23] and few β values have been reported for Case 2 waters [28]. β was set to 2, following Roesler [29] and MERIS protocols [24], which is based on the assumption that for GF/F filters the diffuse absorption of a sample is twice the volume of the absorption coefficient.

2.2 Coloured dissolved organic material absorption coefficient

Three laboratories participated in the PlymCal 2 CDOM inter-comparison; GKSS, NIVA & PML.

2.2.1 Artificial samples. A 1% solution of Aldrich humic acid sodium salt was prepared and aliquots of the standard were added to pre-filtered MilliQ to give an absorption coefficient of 0.2 m^{-1} at 442nm. The absorption spectra of the humic acid salt was measured immediately after preparation. Three replicate samples were prepared for shipment to each laboratory.

2.2.2 Natural Seawater samples. Samples were collected from two sites; L4 and Barnpool, and were prepared at PML. Prior to sampling the all glass filtration apparatus was washed in 10 % HCl, rinsed three times in MilliQ and fired at 450°C for 4 hrs to remove any residual DOC contamination. 2 * 125 ml sub-samples of seawater were filtered through 0.2 µm Polycarbonate filters at a vacuum pressure of 120 mm Hg and then discarded. A third 125 ml sub-sample was filtered and the filtrate was collected in 125 ml amber glass Winchester bottles with PTFE lined screw caps. Three replicate samples were prepared for each laboratory and the samples were stored in polystyrene containers surrounded by ice packs and shipped to the respective laboratories within 36 hrs of filtration. The samples were analyzed at approximately the same time three days later.

2.2.3 Sample blanks. 125 ml of MilliQ was filtered into clean Winchester bottles for use as reference blanks.

2.2.4 Methodology. Samples were allowed to warm to the same temperature as the blank since temperature differences between reference water and sample can lead to strong spectral absorption features [30]. An air vs air baseline was run and recorded to check instrument performance which was spectrally flat, with a variance < 0.0005 A units. Details of the spectrophotometers used are given above. Samples were run in 10 cm cuvettes in dual beam mode with no integrating sphere. Each empty cuvette was placed in the spectrophotometer and scanned relative to air to check the cleanliness of the cuvette. An autozero was performed from 350 to 800nm with a cuvette filled with MilliQ water in sample cell and nothing in the reference cell and the spectrum was recorded. The MilliQ was then discarded and the cuvette was rinsed three times with 5 to 10 ml of the next sample and the scan was repeated. A MilliQ scan was run between every three samples to check the stability of the instrument.

2.2.5 Data processing. The MilliQ spectra was subtracted from the sample spectra. No scattering offset correction was performed. The spectral absorption coefficient of the coloured dissolved organic matter was calculated from the measured absorbance as follows:

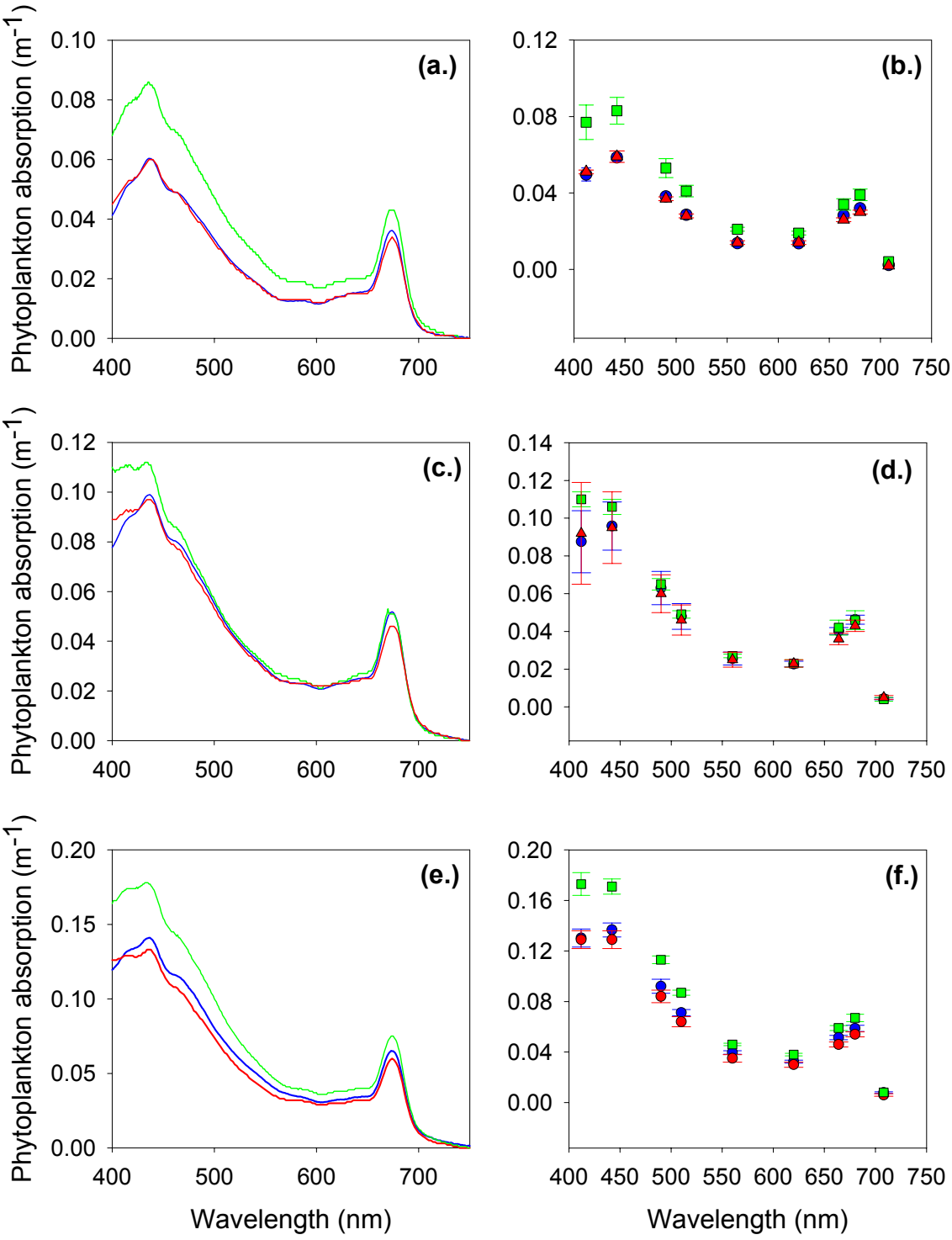
$$a_{ys}(\lambda) = 2.303 * \frac{A_{ys}(\lambda)}{l} \qquad (\textit{Equation 2})$$

where A_{ys} is the measured absorbance (relative units) and l is the cuvette pathlength (m).

3. RESULTS

3.1 In vivo phytoplankton absorption coefficient

Fig 2. Comparison of phytoplankton absorption spectra (m^{-1}) at L4 (a. & b.), Barnpool (c. & d.) and Mayflower (e. & f.).LAB 1 - red lines and triangles; LAB 2 - green lines and squares, LAB 3 - blue lines and symbols.

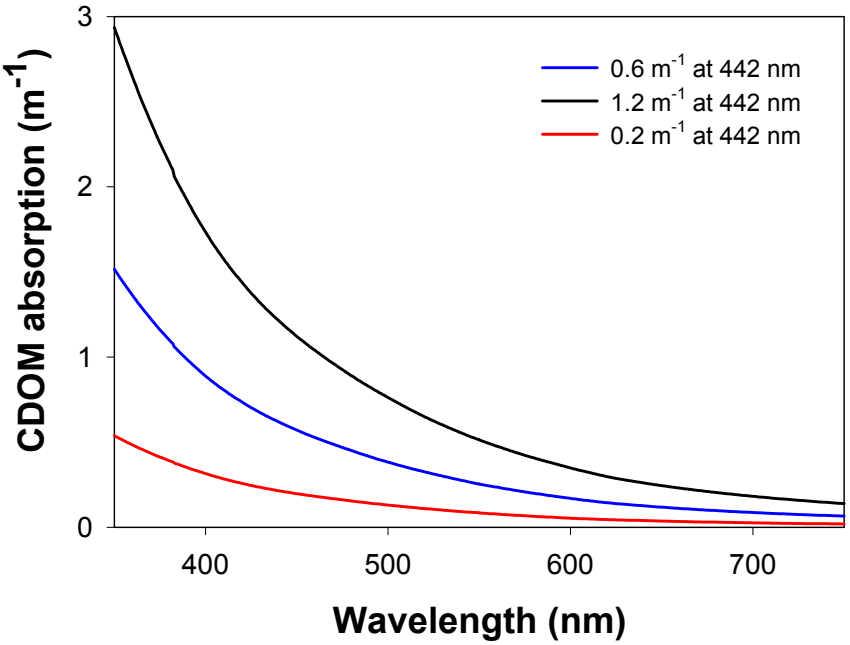


3.1.1. PlymCal 2. Phytoplankton absorption coefficients at 442 nm varied from 0.066 m^{-1} at L4 to 0.145 m^{-1} at Mayflower. For all stations the difference between laboratories was small ~14 % over MERIS bands (412 to 664 nm). In general LAB 2 data was 18 % higher than the LABS 1 & 3 data (Fig 2 a, c & e). For the L4 sample, there was a 20 % difference between laboratories over MERIS bands (Fig 2 a & b). The difference between LABS 1 & 3 was 2 % and whereas the difference between LABS 1 & 2 was 28 %. For Barnpool, the difference between Labs was 5 % over the MERIS bands (Fig 2 c & d). The variation between LABS 1 & 3 was 3 % over MERIS bands and between LABS 2 & 3 was 4 %. The Mayflower samples exhibited the highest phytoplankton absorption the difference between Laboratories was 16 % over MERIS bands and 17 % at 442 nm (Fig 2 e & f). In general all laboratories showed a low standard deviation; the highest was found at Barnpool at 412 and 442 nm (Fig 2d).

3.2 Coloured dissolved organic material absorption coefficient

3.2.1 Artificial samples. By varying the concentration of humic acid salt in MilliQ, we were able to produce a solution with a known optical density at 442 nm which could be used to mimic CDOM concentrations found in natural seawater (Fig 3). For the humic acid solution with an absorption coefficient of 0.2 m^{-1} at 442 nm, the difference between the labs was 20 % over MERIS bands and 13 % at 442 nm (Fig 4 a & b). The difference between Lab 1 & 2 was 6 % and between Lab 1 & 3, 18%. The standard deviation was generally low, but for some labs it increased in the red end of the spectrum (Fig 4b).

Fig 3. Varying concentrations of humic acid solution.



3.2.2 Natural Seawater samples. The difference between laboratories for MERIS band centres 412 to 560 nm was 22 %. The variation was lower at station L4 (10 %), where the absorption coefficient at 442 nm was 0.1 m^{-1} (Figs 4c & d), and higher at Barnpool (43 %), where the absorption coefficient was 1.0 m^{-1} (Figs 4e & f). For the L4 sample, the standard deviation for all labs was small (Fig 4d). For the Barnpool sample, LAB 3 showed higher variation in standard deviation than the other laboratories, indicating large differences in CDOM concentration between samples (Fig 4f). For the L4 sample at MERIS band 442 nm there was only a 1% difference between laboratories. For the Barnpool sample LABS 1 & 2 exhibited a difference of 10 %, whereas the difference between LABS 1 & 3 was > 50% (Fig 4 e & f).

4. DISCUSSION

4.1 In vivo *phytoplankton absorption coefficient*

The filter pad method has become the standard method of determining phytoplankton absorption coefficients and over the past thirty years methodologies have diverged. During PlymCal 2 the same methodologies were used by different laboratories and the difference between them was 14 %. Other inter-comparison exercises have found similar differences [~10 %; 21]. The difference between laboratories could be related to differences in data processing especially the way in which τ is calculated. Roesler [30] defined two main sources of error for the determination of particulate absorption coefficients using this technique. The first is methodological, which includes variability in optical density of blank filters, filter moisture content and blank filter preparation. During PlymCal 2 these were minimized by using the methods outlined in Tilstone and Moore [25]. The second is due to differences in data processing and analysis; Roesler [30] observed that the pathlength wavelength correction (β) produced the highest source or error. Different filter pad loadings lead to large variations in phytoplankton absorption coefficients [30]. In this study errors due to the β factor were minimized by using the same filtration volume and setting the β value to 2. Other differences in data processing could include the derivation of τ. In the PlymCal 2 inter-comparison all laboratories calculated τ using equation 1. However, some laboratories defined α as the absorption in transmission mode either of the pigmented or de-pigmented sample as follows::

$$\alpha = \log_{10}\left(\frac{1}{st}\right) \qquad (\textit{Equation 3})$$

where st is the sample transmission. LAB 1 calculated α from equation 3 and used an offset correction at 750 nm. Using data from one laboratory (LAB3), we compared absorption coefficients derived from the two methods of calculating α but the difference was not significant We found that filter reflectance and sample transmission measurements caused the variation in absorption coefficients between laboratories, which was largely due to differences in instrumentation and possibly due to differences in integrating sphere configuration and / or baseline variation between the different spectrophotometers.

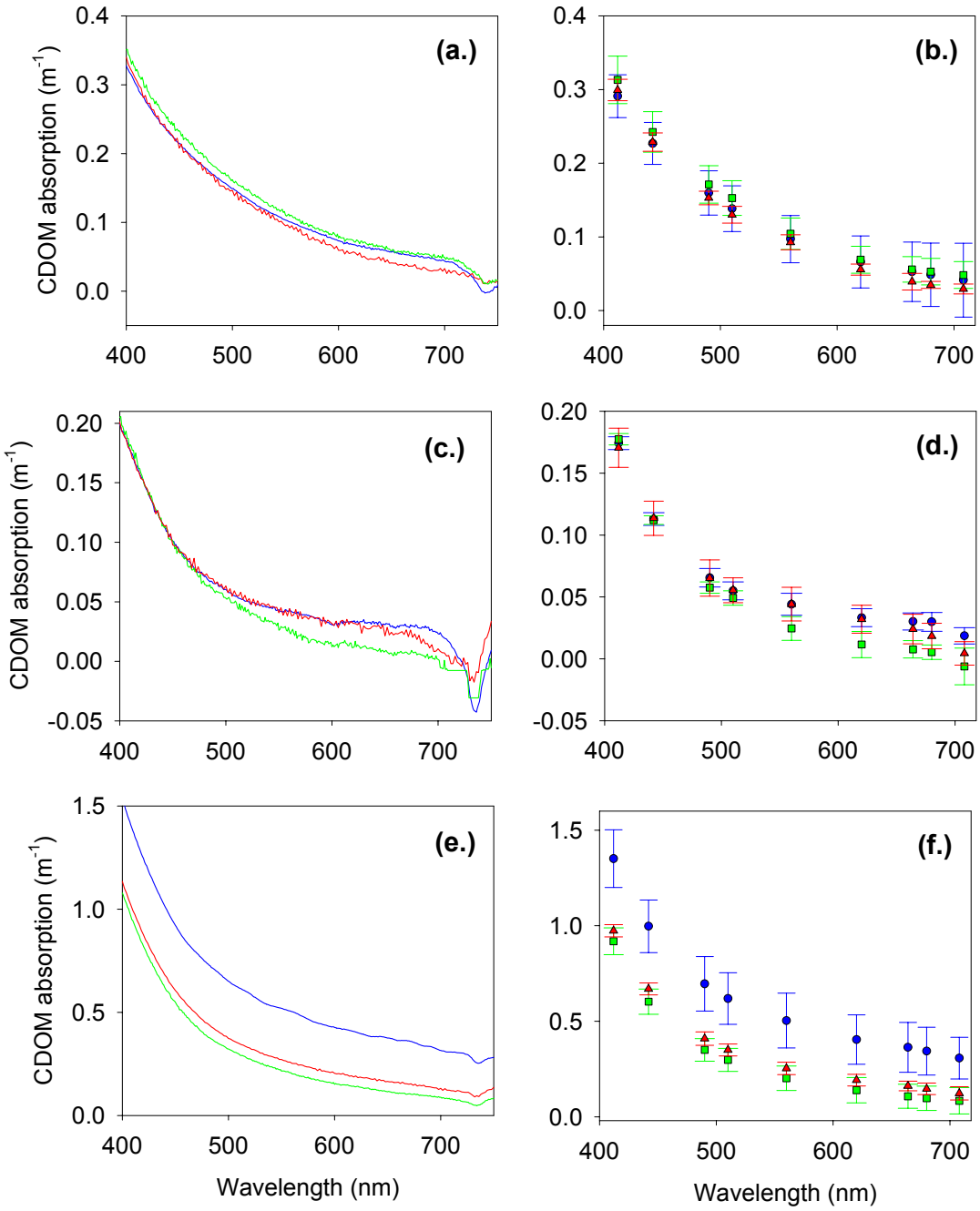
4.2 The inter-comparison of Coloured dissolved organic material absorption coefficient

CDOM absorption coefficients exhibit a larger variation between measurements than in vivo phytoplankton absorption coefficients [21]. Once protocols have been defined for the determination of the absorption coefficients of CDOM, the main sources of error include differences in instrumentation, variability in MilliQ water quality, variability in absorbance intensity due to cleanliness of cuvettes, temperature offsets and differences due to CDOM degradation. For the L4 and humic acid samples, the difference observed between laboratories was acceptable, but for the Barnpool sample the difference was high. Compared to fresh samples run on the day of sample collection, L4 and the humic acid salt exhibited a difference of 17 & 15 % respectively at 442 nm, but for Barnpool the difference was 54 %, indicating high degradation of CDOM. Although care was taken during the inter-calibration to eliminate methodological differences between laboratories, and even though the samples were in the same insulated packaging, LABS 1 & 2 samples were transported whereas the LAB 3 samples were kept in the laboratory. Differences in ambient conditions during shipment may have led to variations in CDOM degradation as indicated by the large standard deviation for LAB 3 (Fig 4f) which would account for the differences between laboratories at Station Barnpool.

4.3 Implications for satellite Chlorophyll algorithms: A sensitivity analysis.

The SeaWiFS switching band ratio OC4 algorithm was developed for Case 1 waters where the accuracy is ± 35 % [31]. The accuracy of OC4 in Case 2 waters is low since it does not have the capability of distinguishing between CDOM, detrital and phytoplankton absorption. By using *in situ* absorption coefficients to recalculate water leaving radiances, nL_w and then using OC4 to calculate *Chla* concentrations, we can assess the effect of inter-laboratory variations in a_{phyto} and a_{ys} on validating satellite *Chla*. If the variation in the resulting *Chla* is < 35 %, we can be confident that between laboratory variation in *in situ* measurements will not adversely affect the validation of MERIS *Chla*

Fig 4. Comparison of CDOM absorption coefficients for humic acid solution (a.) & (b.), L4 (c.) & (d.), Barnpool (e.) & (f.). LAB 1 - red lines and triangles; LAB 2 - green lines and squares, LAB 3 - blue lines and symbols.



products. From *in situ* values of a_{phyto} and a_{ys} above water reflectance spectra (R) was calculated following the methods of Gordon et al. [32].

$$R = \ell_1 * \left(\frac{b_b}{b_b + a}\right) + \ell_2 * \left(\frac{b_b}{b_b + a}\right)^2 \qquad \textit{(Equation 4)}$$

Where ℓ_1 = 0.0949 and ℓ_2 = 0.0794, a is the total absorption of detrital, phytoplankton, CDOM and water and b_b is the backscattering coefficient. Absorption coefficients for detrital (a_d), phytoplankton (a_{phyto}) and CDOM (a_{ys}) were taken from *in situ* values measured by each laboratory at station L4

during PlymCal 2. The absorption of pure water (a_w) was taken from Pope and Fry [33]. During the inter-calibration a_d varied by 30%, a_{phyto} varied by 20% and a_{ys} varied by 10%. The backscattering coefficients at every wavelength were calculated from:

$$b_b(\lambda) = b_{bw}(\lambda) * 0.5 + b_{bp}(\lambda) \qquad (Equation\ 5)$$

where b_{bw} is the backscattering coefficient of water, which was taken from Morel [34] and b_{bp} is the backscattering of particles, which was taken from measurements made at L4 during the inter-calibration. From the above water reflectance spectra, the normalized above water leaving upwelling radiance, nL_w was calculated from:

$$nL_w = \frac{R}{Q} * f_0 * \left(\frac{1}{(1 - rR)} \right) \qquad (Equation\ 6)$$

where Q is the ratio of upwelling radiance to upwelling irradiance towards the zenith. Q varies between 4 and 5 and we used a value of 4.55 to calculate nL_w, f_0 is the mean extraterrestrial solar irradiance which was taken as 189.45 W m^{-2} and r is the water-air reflectance for totally diffuse irradiance (~0.48) [35]. Having calculated nL_w, *Chla* concentrations were calculated using the SeaWiFS OC4 switching band ratios for the following combinations:

- a_d was fixed at its minimum value measured at station L4 by each of the three laboratories and a_{ys} and a_{phyto} were varied through their maximum and minimum values.
- a_d was fixed at the mean value measured at station L4 by each laboratory and a_{ys} and a_{phyto} were varied through the maximum and minimum values.
- a_d was fixed at its maximum value measured at station L4 and a_{ys} and a_{phyto} were varied through their maximum and minimum values.

Table 1. Chlorophyll values (mg m^{-3}) calculated from the inter-laboratory variation in a_d, a_{phyto} & a_{ys} *using SeaWiFS OC4.(Rows variation due* a_{ys}*, columns variation due to* a_{phyto}*).*

a_d min	a_{phyto} min	a_{phyto} mean	a_{phyto} max
a_{ys} min	1.65	1.68	1.94
a_{ys} mean	1.49	1.52	1.59
a_{ys} max	1.37	1.40	1.59
a_d mean	a_{phyto} min	a_{phyto} mean	a_{phyto} max
a_{ys} min	1.81	1.85	2.12
a_{ys} mean	1.63	1.66	1.72
a_{ys} max	1.50	1.52	1.72
a_d max	a_{phyto} min	a_{phyto} mean	a_{phyto} max
a_{ys} min	1.98	2.02	2.24
a_{ys} mean	1.78	1.81	1.85
a_{ys} max	1.62	1.65	1.85

The results are summarised in table 1. When a_d was fixed at its minimum value and a_{ys} was varied, *Chla* concentrations varied by 12 % and by varying a_{phyto} *Chla* then varied by 17 %. When a_d was fixed at its mean value and a_{ys} was varied, *Chla* concentrations varied by 11 % and when a_{phyto} was varied this caused an 18 % difference in *Chla*. When a_d was fixed at its maximum value and a_{ys} and a_{phyto} were varied, *Chla* varied by 9 % and 18 % respectively. The total variation in a_{ys} between laboratories (~10%) caused an 11 % variation in *Chla* and the total variation in a_{phyto} (~20%) produced an 18 % variation in *Chla*. In both cases this was < 35 %, which suggests that the variation in a_{ys} and a_{phyto} between laboratories at L4 is acceptable for MERIS validation.

5. CONCLUSIONS

In an inter-laboratory comparison using the same methods to determine *in vivo* phytoplankton absorption coefficients, a_{phyto} and coloured dissolved organic material absorption coefficients, a_{ys} on natural seawater samples, there was good agreement between laboratories. For a_{phyto} the difference was 14 % between laboratories and for a_{ys} the difference was 22%. The difference between laboratories increased under increasing concentrations of CDOM. It was found that a humic acid salt dissolved in MilliQ could be used as a CDOM standard to check the instrument performance of each laboratory. For a humic standard solution with an absorption coefficient of 0.2 m^{-1}, the differences between laboratories was 20 % over MERIS bands.

When a_{phyto} measurements determined by each laboratory on seawater samples from station L4 were used to calculate SeaWiFS *Chla* using OC4, there was an 18 % difference in *Chla* concentrations. Similarly, the variation in a_{ys} between laboratories at station L4 produced an 11 % variation in SeaWiFS *Chla* . It is concluded that the differences observed between laboratories on natural seawater samples, does not have a significant effect on satellite *Chla* retrieval and can therefore be used to accurately validate MERIS data.

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