Anonymous Referee #2

GENERAL COMMENT

This manuscript reports the occurrence of a shoulder at 410-415 nm in the absorption spectra of both DOM and POM in subsurface waters of a basin scale transect along the Eastern Atlantic Ocean. The authors also found this absorption shoulder in a few samples from Santa Barbara Basin and New Caledonia in the Pacific Ocean. Breves et al. (Ocean Dynamics 53, 86-97, 2003) have already described the presence of this shoulder in subsurface waters of the Indian Ocean and hypothesised that it was linked to a pronounced fluorescence emission of the samples at about 660 nm when excited at 420 nm. This would mean that the absorption shoulder at 410-415 nm and the red fluorescence maxima recurrently found in previous works (e.g. Broenkow et al., Journal of Marine Research 43, 875-891, 1985) belong together and that it should be a pigment degradation product.

The innovation of this manuscript is the concentration of CDOM by solid phase extraction. However, the authors use the isolates just to study its absorption and fluorescence properties to link the absorption at 410-415 nm to the fluorescence emission at about 650-670 nm because they do not performed direct fluorescence measurements of the water samples. Unfortunately,a the authors do not explore state-of-the-art high resolution molecular magnetic resonance or mass spectroscopy techniques that would probably allow testing their hypothesis that cytochrome c is behind the absorption shoulder.

Author comment: Here we do not agree. This paper is part of a special issue, and concentrates on the absorption and fluorescence of some specific samples from the depth of 200 m. In addition the sophisticated analysis of these samples were done and are presented in other papers (see e.g. Flerus et al. and Hertkorn et al.). We examined these samples with high-resolution molecular techniques but were not able to identify specific molecules in these samples that would give indications for the molecular nature of the pigment degradation products. With the current samples and available techniques it was not easily possible to isolate the desired molecule and perform a molecular identification.

In the present form, the manuscript looks just like a confirmation that the absorption shoulder at 410-415 nm also appears in more places than those reported by Breves et al. (2003), reinforcing the hypothesis, already posed by those authors, that it is an ubiquitous feature of the global ocean.

Authors comment: In the authors view the absorption shoulder was only shown once for some sample from the Arabian Sea, only the deep red fluorescence was seen as ubiquitous, not the absorption. Also the connection between the 410nm absorption shoulder and the red fluorescence at 650 nm was speculated in the Breves et al. papers, there was no final proof. So, we do not indent to confirm the observation of the absorption shoulder in the Arabian Sea but to show that the same (or similar as long we do not know the nature of the absorption) shoulder can be found in other areas. To the best of our knowledge this has not been done before. In addition we proof the direct connection between the absorption at 415 nm and the fluorescence at 650 nm, by making both measurements with the same sample and providing a fluorescence

excitation spectra that fits well to the absorption shoulder.

To represent a significant advance in knowledge, this manuscript should either:

i) relate the shape and intensity of the absorption shoulder to the salinity (S), temperature (T) and apparent oxygen utilization (AOU) of the samples. If the absorption shoulder is a respiratory pigment, a significant correlation should be observed with AOU once the effect of water mass mixing is eliminated. Maybe the authors should try a multiple linear correlation with S, T and AOU. The manuscript by Carlson et al. (Deep-Sea Research II 57, 1433–1445, 2010) is a good example on how to proceed with this. In addition, the fluorescence maxima at Ex/Em 370 nm/420 nm that the authors found in their matrices are related to bacterial respiration (Romera-Castillo et al., Applied and Environmental Microbiology, 77: 7490–7498). Therefore, a significant linear relationship should also be observed with the fluorophore peaking at Ex/Em 410-415 nm/650-670 nm.

Authors comment: We do not agree here. If one believes our results, meaning that the 415nm absorption shoulder and the deep red fluorescence originate from the same molecule/substance than the hydrographic conditions at which the molecule is found is described in the papers of Broenkow and co-workers and Breves et al. for many other areas. So, this is not a new aspect, and from these papers it is clear that it occurs in the oxygen minimum zone. Our results from the depth profiles and the relevant CTD data would only confirm this for the Atlantic. This confirmation for the shoulder is seen by the reviewer as being not sufficient for a publication, so why should it be sufficient to confirm the hydrographic condition under which the molecule is found in the Atlantic. For many other aspect like proposed for the AOU the current sample density is far too low, also, this could much better be done via fluorescence profiles,

or ii) expand the observations by considering the extensive dataset produced by Nelson et al. (Geophysical Research Letters 37, L03610, 2010) using a WPI UltraPath liquid waveguide spectrophotometer;

Authors comment: This is envisaged for another paper, we thought that presenting our data of the fluorescence absorption connection etc, and the proposed results from Norm Nelson's data will be too much for a single paper.

or iii) resolving the chemical structure of the chromophore absorbing at 410-415 nm.

Authors comment: Resolving the chemical structure is not an easy tasks. If this would be easy we would have done so. One intention of the paper was to inform the community about this very special molecule in the OMZ and increase the interest on its necessary identification.

SPECIFIC AND MINOR COMMENTS

Page 10699, lines 14-19. I do not agree with the statement that absorption measurements are mostly performed in the UV region of the spectrum and in the surface layer. Normally, absorption spectra are recorded from 250-300 nm to 600-700 nm and there are recent studies measuring CDOM in subsurface waters (e.g. Nelson et al., Marine Chemistry 89, 273– 287, 2004; Geophysical Research Letters 37, L03610, 2010).

Authors comment: Ok, but still the number of studies on CDOM from subsurface water is much lower than that for surface water, this is meant by mostly.

Page 10701. A map showing the location of the sampling stations would be quite useful.

Authors comment: This iwill be shown in the other papers of the special issue. We will add these as references in the M&M

Page 10703. Fluorescence spectra should be corrected for inner filter effects, which usually produce wavelength shifts of the Ex/Em maxima.

Authors comment: The EMM were measured to show the occurrence of the specific em/ex at 410/650 nm. Inner filter effect were not corrected but would not alter the drawn conclusion. We did not intend to provide absolute fluorescence numbers.

Page 10717. Please, replace “absolute absorption at 370 nm” by “absorption coefficient at 370 nm”.

Authors comment: OK

Page 10720. The absorption spectra presented in Figure 5 are not normalised to the absorption coefficient at 380 nm. Note that the units of the Y-axis are 1/m. The efficiency of the PPL cartridges to isolate the CDOM absorbing in the visible should be tested and compared with the efficiency to isolate DOC and DON. The amount of CDOM absorbing in the visible could be estimated by integrating the absorption coefficient between 380 and 600 nm.

Authors comment: OK, this will be corrected!

Page 10721. The contour lines of the EEMs should be labelled.

Authors comment: We only intend to show the specific Em/Ex peaks. A labelling is not needed for this. Also, we choose the contour lines to show the specific EM/EX at 650/415 nm and the lines are not in a constant order. We feel that adding labels would be confusing.

Anonymous Referee #1

General comments This manuscript presents evidence for the occurrence of a pigment like absorption peak at around 410 nm in subsurface ocean waters, which superimposes the overall exponential shaped absorption spectrum of colored dissolved organic matter (CDOM). They find this peak to be widespread within their set of samples from the Atlantic and a few samples available to them from the Pacific and additionally can trace a similar signal in the particulate absorption from depths with low phytoplankton abundance. Fluorescence spectroscopy on DOM isolates reveal that the absorption in this region also results in a fluorescence at wavelengths between 650-680 nm. They conclude that these signals originate from a “bacterial respiration pigment”. I find the dataset interesting but consider the work in its current form as unfinished. Much more could be obtained from this data with some modeling of the absorption spectra and plotting of the data with other parameters (oxygen, T, or S), along the lines of what is published in Breves et al 2003. I was not originally aware of the Breves paper, but after reading it I find that this BG submission does not really provide anything new on the origins of this material, rather just shows that it can also be found in the Atlantic. If the authors expand on their data analysis and calibrate the fluorescence signals they would be able to directly compare the data they have

collected with the earlier work in the Arabian Sea. Additionally it would be good to see auxiliary data. What did the water column temperature, salinity and oxygen profiles look like? At least for the stations where profile CDOM samples were taken. Finally, I like the idea that it may represent cytochrome c and am sure there is some link to redox conditions. It would be a great idea to discuss this in the Discussion rather than look at latitudinal patterns, which I do not quite know how to interpret. In its current condition I do not find the manuscript suitable for publication, but recommend that the authors expand the data analysis on what seems like an interesting phenomenon that many of us have not seen in our own datasets, possibly due to storage problems.

Author comment: please see comments to Reviewer 2.

Some specific comments

1. I find the paper would benefit from a clearer distinction from the Breves et al 2003 paper. Rather than focusing on Broenkow et al 1992 which is quite vague. Why not present the conclusions of Breves et al 2003 and lead on to how this work will build on it? Another paper that seems to be very relevant is Broenkow et al 1985, although it does not seem that they are able to distinguish clearly between the two emission peaks you find (surface vs 200m at 650 and 670 nm).

Author comment: We did not focus on Broenkow et al. 1992 alone but on the work done by Broenkow and co-worker, which is the basis for the knowlegde of the deep red fluorescence. In our opinion Breves et al. 2003 did not give a proof that the 415nm absorption shoulder and the specific red fluorescence are related. They only showed an absorption shoulder of a sample and a fluorescence emission spectrum. With this paper we are giving a much stronger evidence that these features are connected, by providing measurements with the same sample and showing that the fluorescence excitation spectra (representing absorption) fits well to a proposed absorption. (I agree that it would be good to show a modeled absorption spectrum and compare this with the excitation spectrum.)

2. I find it intriguing that some many earlier studies have not found similar peaks in the absorption spectra. One possibility is that the signal is degraded during storage (Breves et al 2003) and therefore may disappear below the detection limit of most standard spectrophotometers quite quickly. This should be discussed rather than just saying that all other studies have been on surface water samples (which is not true).

Author comments: another possibility is that it is simply overlooked! If you measure cdom absorption down to 300 nm and plot the whole data in a graph, showing the highest values at 300 nm, as the upper axis limit, you will hardly see the shoulder, only if you zoom into the specific region you will see it.

3. It would be good to see how the CDOM measurements compared between the two instruments used PSICAM and the LWCC approach). It seems that some samples were measured on both (fig 3) but it is unclear which is which? I am convinced that the absorption peak is not an instrumental artifact but this could be made more convincing. Lines 164-172.

Authors comment: Theres are several papers that show that LWCC and spectrophotometer

measurements agree, And, there is a paper that showed that PSICAM and spectrophotometer agree (Röttgers & Doerffer 2007). I do not think it is necessary to show a direct comparison of LWCC and PSICAM. I can include a sentence that we have never seen differences between the two approaches. The lwcc has a wider wavelength range, so I prefer to show those data when available, for the first cruises no LWCC was available.

4. It is unclear how the fluorescence intensities were adjusted to compile the data from different measurements with different signal amplification into one EEM. The treatment of the fluorescence spectra in general was quite poor. There are several issues such as intensity calibration (what are the units?), instrument spectral bias correction and inner filter effects, which are basically ignored. This makes the data presented here impossible to compare with other studies using other instruments. This is especially relevant for your discussion where the position of maxima are slightly different between samples (can occur due to inner filter effects) and studies (due to instrumental effects). See Murphy et al 2010 for some of these issues Environ. Sci. Technol. 2010, 44, 9405–9412. For example the greatest fluorescence intensity should be form excitation below 300 nm. This is apparent once you correct for inner filter effects.

Authos comment: The M&M part does read like this „For all measurements pure methanol was used with the same scan speed, band width, and amplification settings for a subsequent subtraction of Raman and Rayleigh scattering. Internally recorded light intensity of the excitation was used to correct for spectral variation in the excitation light spectrum. Further corrections for fluorescence re-absorption or other quenching were not applied. When possible the fluorescence was normalized to the measured absorption at 415 nm.“ We did only not correct for the ex and em absorption/re-absorption as stated. We did not intend to give calibrated values, because here we were only interested for the qualitative difference. I do not think that the missing correction would alter the results e.g. the exact position of the 650 nm emission, nor the conclusion drawn by it. The three separated EMM measurements of a single sample were scaled to the amplification value of the PMT before combined. We will add this to the M&M.

5. There is repeated use of “absolute” and “specific” which I think is incorrect and needs revising. How does the “absolute absorption” (e.g. 147) or fluorescence (e.g. line 204) differ from just the absorption values?

OK!

6. Breves et al 2003 carried out an analysis of this absorption peak by modeling the absorption spectra using an exponential and a Gaussian. I suggest that a similar approach should be used here. This would allow you to isolate the signal from this ab- sorption peak and plot with other parameters or just look at vertical profiles. See their Figure 11. This would also help you with comparing with the particulate absorption

Yes, this can be done!

7. I suggest you try to include a spectrum of cytochrome c (absorption and fluores- cence). It will make your arguments more convincing in section 4.4. In particular there might also be a dependency on whether it is oxidized or reduced which may give rise to some of the correlations with low oxygen conditions (shown in earlier work, but why not here (if you have the oxygen data from the CTD?)).

Author comment: We were looking for optical data on cytochrome. One do find published spectra of either absorption or fluorescence, but nothing that could be reproduced. Although there are several papers (some are cited) of bacterial absorption that is thought to be cytochrome, but no in vivo or in vitro cytochrome absorption nor fluorescence data are yet available to us. Due to differences in oxidized and reduced form and the variety of cytochromes it is not easy to just order cytochrome and do it in the lab yourself. However, I agree that such spectra in the paper would be helpful.

Minor details Line 32. Include reference for " a large proportion of is refractory".

OK!

Line 32-34. Rephrase this sentence. Possibly expand. Try to cover too many issues in one sentence.

OK!

Line 36. "suitable" seems like the wrong word to use. Line 39. "accessed" should this be accessed.? Line 41. Drop "e.g."

OK!

Line 44. " mostly performed in UV rather than at visible". I don't agree. The majority of studies measure CDOM absorption at least between 300 and 650 nm, so well into the visible range.

OK!

Line 46-47. I agree that many studies have focused on surface water due to remote sensing, but there are also several works covering deeper CDOM samples. E.g. Del Castillo, Coble Deep-Sea Research II 47 (2000) 15631579. Nelson et al. Deep-Sea ResearchI45(1998)931âA T 957.StedmonMarkagerLimnol.Oceanogr.,46(8),2001, 2087–2093. Nelson et al. / Marine Chemistry 89 (2004) 273–287. These are missing from the discussion on the origins of CDOM.

OK, this is correct, I will include them in the discussion. However, only Nelson DRS 45, does show spectral data. If you check carefully the shoulder might be visible in the spectrum of 120 m (fig. 1) but stated being below the detection limits.

Line 49. Either it degrades or it is refractory.

OK!

Line 50. Have you considered other studies on the origins of CDOM. For example rs above dicuss this. Also Steinberg et al have a range of peaks being produced by organisms Mar Ecol Prog Ser 267: 45–56, 2004, and Norman et al (Deep-Sea Research II 58 (2011) 1075–1091) show the presence of shoulders on CDOM absorption spectra.

author comment: Yes, there are other shoulders in the CDOM but mainly in the UV part of the spectrum by e.g. MAAs. There is even one in the paper of Steinberg in the VIS. But this paper is about experimental work not natural samples. I know that in Trichodesmium blooms one can easily detect MAAs in the CDOM spectrum.

Line 51. The last sentence on fluorescence appears as a bit of an add on. As a lead on to the next section on red fluorescence it would be worthwhile expanding on this some explaining that a

fraction of CDOM also fluoresces.

OK!

Line 54. Start with “Previous studies have shown than”.

OK!

Line 67-68 What wavelengths was Broenkow measuring at. I could not find the ones for fluorescence or light attenuation.

Authos comment: Please see Broenkow et al. 992, page 419, or Broenkow et al. 1985 table 1. Transmission was measured at 480 nm with a 1m path length., fluorescence with different setting, for chla EX: 336- 582, Em >667 nm.

Line 119. How about reporting the efficiency of the CDOM extraction? In fig 5 legend you mention normalized spectra, but in the figure the absorption coefficient is plotted. Is this right? Could the procedure really extract everything apart from a little at around 450 nm, and did it add absorption at 370 nm?

Authors comment: This is wrong and will be corrected. Only the absorption of the extract is corrected to fit to the original spectrum. Small deviation in the spectra can be due to a couple of errors, the deviation at >380 nm is e.g. due to artifact in the PSICAM measurements.

Line 153. “Linear deflection” what do you mean? Breves et al 2003 refer to it as a Gauss addition which seems easier to follow.

OK, meant is the deflection from an exponential increase to a more linear relationship, without showing a pronounced shoulder. This will be re-written.

Line 178. DO you have a reference for the heterotroph/detritus absorption spectra from deep ocean?

No, this is also very rarely measured.

Line 198. This also shows no dependency on instrument right?

No, wavelengths accuracy of the instrumentation is usually quite good (better than 2nm), and is checked in our lab very often.

Line 217-220. Or inner filter effects could cause this difference.

Right!

Line 245-247. This sentence seems too speculative.

What do you mean by too speculative? I just said „it is possible“ and we are seeing this „in all major ocean“, which is proofed here, right!. You mean it is too speculative to say it is typical?

Line 259. The absorption spectrum of cytochrome c also has peaks just above 500 nm which would also fit with your excitation spectra Fig 7b.

Yes, like other heme molecules as well.

Line 276. Could also be explained by the lack of instrumental and inner filter correction.

Authos comment: Instrumental error were corrected, but sure this could be due to inner filter effects.

Line 282. What is the “absorption efficiency”?

Author comment: The excitation spectrum is giving information on the absorption characteristics of the fluorophor for a specific emission. It does not give absolute values in terms of an absorption coefficient, but shows at which wavelength the light is absorbed most efficiently.

Line 341-343. This sentence seems strange. Consider rephrasing.

OK!

Line 386-389. This is pushing it a little. Seems to ambitious and I am not quite sure how this can be related to rates.

Author comments: The idea is: when you measure the concentration of the chromophor in short time intervals, e.g. days or weeks, and would find differences that are not related to a change in water mass (for sure there are difference between geographic positions), you might be able to interpret this in several directions. E.g. if you find difference in CDOM absorption in the same water mass, this is usually interpreted for a reduction by photobleaching or production from the biota, and you can calculate a rate, right. As long as we do not know what the molecule is, this is speculative, sure, but is it forbidden to be speculative here.