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# Indications for a ubiquitous dissolved pigment degradation product in subsurface waters of the global ocean

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## Abstract

Measurements of light absorption by chromophoric dissolved organic matter (CDOM) from sub-surface waters of the tropical Atlantic and Pacific showed a distinct absorption shoulder at 410–415 nm, indicating an underlying absorption of a pigment. A similar absorption maximum at ~410 nm was also found in the particulate fraction and is usually attributed to absorption by respiratory pigments of heterotrophic unicellular organisms. The CDOM absorption shoulder was described earlier in the Indian Ocean at 600 m depth and was related to a “deep red fluorescence” found in the same depth, i.e. in the oxygen minimum zone (Breves et al., 2003; Broenkow et al., 1983). In our study, fluorescence measurements of pre-concentrated DOM samples confirmed that the absorption at ~410 nm was related to a specific fluorescence at 650 nm. The absorption characteristic of this specific fluorophor was examined by fluorescence emission/excitation analysis and this showed a clear excitation maximum at 415 nm (in methanol) that can explain the absorption shoulder in the CDOM spectra. The spectral characteristics of the substance found in the dissolved and particulate fraction did not match with those of chlorophyll *a* degradation products (as found in a sample from the sea surface) but can be explained by the occurrence of respiratory pigments from heterotrophs. Combining the observations of the “deep red fluorescence” and the 410 nm-absorption shoulder suggests that there are high concentrations of a pigment degradation product (cytochrome *c*) in DOM of all major oceans. Most pronouncedly we found this signal in the deep chlorophyll maximum and the oxygen minimum zone of tropical regions. The origin, chemical nature, turn-over rate, and fate of this molecule is so far unknown.

## 1 Introduction

Dissolved organic matter (DOM) in the ocean forms the largest reservoir of reduced organic carbon in the ocean. It consists of various, mainly small molecules (<1000 kDa),

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a large proportion of which is refractory. The overall distribution of DOM in the world ocean is determined by autochthonous and only partly allothonous sources, by photodegradation near the sea surface, and particulate matter re-mineralization in deeper waters. Measurement of DOM light absorption is one method to describe the biogeochemistry and hydrography of different oceanic regions and water masses. In combination with fluorescence characterization it is suitable to get an insight into the chemical nature and origin of DOM (Stedmon et al., 2003). Chromophoric and fluorescent moieties of the DOM (CDOM and FDOM, respectively), represent only a small part of the DOM pool, but can easily be accessed by optical methods. The absorption of CDOM in the aquatic environment has a characteristic spectral shape, with a strong absorption in the UV to visible range, which decreases exponentially towards higher wavelengths. The slope of this decrease is used e.g. to characterize CDOM sources (e.g. Vodacek et al., 1997) and to describe its molecular composition (e.g. Helms et al., 2008). Because of the generally low CDOM absorption in oligotrophic regions, the measurements are mostly performed at UV rather than at visible wavelengths. Commonly, samples are measured near sea surface where dissolved organic carbon (DOC) concentration is highest and CDOM absorption can be retrieved by satellite remote sensing. Therefore most scientific studies addressed CDOM absorption features at the sea surface. However, due to photo-degradation at the surface, CDOM absorption is higher below the surface mixing zone, and often reflects the hydrographic origin of a water mass, as a major proportion of DOM is refractory and degrades only slowly. A pronounced shoulder in the CDOM absorption spectrum can usually be found only in the UV (~270 nm) due to high concentrations of aromatic structures in DOM. Also its fluorescence emission originates from chromophors absorbing in the UV (<400 nm, Coble 2007), fluorescing in the UV to shorter VIS wavelengths. A previous studies showed that a CDOM absorption spectrum from mesopelagic waters (600 m) of the Arabian Sea had an absorption shoulder in the visible wavelengths at around 410 nm (Breves and Reuter, 2000; Breves, 2001; Breves et al., 2003). This observation was related to a chlorophyll *a*-like fluorescence observed in the same depth (Breves, 2001;

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Breves et al., 2003). A red fluorescence in subsurface waters was also reported earlier from the Pacific (Broenkow et al., 1983, 1985, 1992; Lewitus and Broenkow, 1985). The authors concluded that this “deep red fluorescence” is a global feature found in all major oceans and at all latitudes, and that its maximum is associated with the oceanic oxygen minimum zone (OMZ). Chlorophyll or some of its degradation products in phytoplanktonic particles are thought to be a possible molecular precursor. To our knowledge it is still unresolved whether the red fluorescence occurs in the particulate and/or dissolved fraction. Moreover, the exact molecular nature of this phenomenon is also unknown. Breves et al. (2003) concluded from sample filtration and a specific fluorescence emission at ~650 nm, which appeared in samples from mesopelagic depth when excited with light of 420 nm, that it is most likely in the dissolved phase. This contradicts earlier results (Broenkow et al., 1992) which often found a close correlation of the “deep red fluorescence” with light attenuation (mainly scattering by particles). It is possible that the fluorescence at 650 nm emits from the chromophor that absorbs at 410 nm, and that this fluorescence is collected by common in situ chlorophyll fluorometers. Hence, the observation of this absorption shoulder in the visible spectral range and the “deep red fluorescence” in the subsurface waters are most probably linked. If this is true, the observed “deep red fluorescence” provides evidence that this specific chromophor/fluorophor occurs globally and throughout the water column, its optical characteristic provides evidence that it is of pigment origin and unique among other chromophoric/fluorescent DOM molecules in the subsurface ocean. The aims of the work presented here are (1) to give evidence that the absorption at 410 nm and the fluorescence at 650 nm are originating from the same molecule, (2) to clarify whether it is found in either the dissolved or the particulate fraction, and (3) to investigate the distribution of this chromophor/fluorophor in the water column and on a latitudinal transect in the Atlantic Ocean proofing hypotheses by Broenkow et al., (1983, 1985, 1992) about its global and depth distribution.

## 2 Materials and methods

### 2.1 Sampling

Several oceanographic cruises were conducted to collect samples for measurements of particulate and CDOM absorption, and for DOM extracts. The first cruise (ANT XXIII/1, RV “Polarstern”, October/November 2005) covered a transect from Germany to South Africa crossing the Atlantic Ocean along the African continent. During this cruise CDOM samples were mainly taken from the surface and 200 m depth. On 5 additional stations the entire water column was sampled. DOM extracts were collected from the surface and once from 200 m depth. On a second transect cruise (ANT XXV/1, RV “Polarstern”, November 2008) which covered the same region, DOM was extracted throughout the water column across the Atlantic. Two other cruises were used to collect single samples for CDOM and particulate absorption measurements from the surface and from 80–200 m depths: (1) in the West-Pacific offshore of New Caledonia (VALHY-BIO, RV “L’Alis”, March/April 2008), and (2) in the Santa Barbara Channel (California, North-East Pacific, RaDyO, RV “Kilo Moana”, September 2008).

### 2.2 Light absorption measurements

The light absorption of dissolved (CDOM) and particulate matter,  $a_{\text{cdom}}$  and  $a_{\text{p}}$ , respectively, was measured using a point-source integrating cavity absorption meter (PSICAM) as described in Röttgers et al. (2007) and Röttgers and Doerffer (2007). For this, the sample absorption was measured before and after a filtration through 0.2  $\mu\text{m}$ , and the absorption of the filtered fraction was subtracted from the sample absorption to obtain particulate absorption. During the RaDyO cruise the absorption by CDOM was measured additionally using a 2 m path length liquid core waveguide capillary cell (LWCC), as e.g. described in Miller et al. (2002). All PSICAM and LWCC measurements were done shortly after sampling (<2 h) onboard of the ship, using ultrapure water from a water purification system (MilliQ, Gradient, Millipore) as the reference.

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Measurements were done at least in triplicates and averaged, and the absorption coefficient,  $a$ , was calculated as  $a = -\ln T/L [m - 1]$ , where  $T$  is the transmission and  $L$  the corrected optical path length of the capillary cell, as provided by the manufacturer (WPI Inc.). CDOM filtration was done using cleaned glass filtration units. After rinsing with 50–100 mLs of the sample, a first filtration was performed through pre-combusted GF/F filters (Whatman) under low vacuum ( $<100$  mbar) to avoid breakage of phytoplankton cells. A second filtration through  $0.2 \mu\text{m}$  (washed GWSP filters, Millipore) was done under vacuum of 200–300 mbar. Prior to absorption measurements, filtered samples were adjusted to the same temperature as the reference purified water to minimize temperature effects. However, slight temperature difference as well as the difference induced by the difference in salinity between sample and reference water were individually corrected, using instrument-specific correction coefficients obtained from measurements of concentrated NaCl solutions. Light absorption (350–700 nm, 2 nm resolution and slit width) of the methanol DOM extracts were measured back in the lab against pure methanol as a reference in a Lambda 850 spectrophotometer (Perkin Elmer) using 1 cm path length quartz glass cuvette.

### 2.3 DOM extraction

DOM was extracted from 5 l filtered (GF/F) sea-water samples using solid phase adsorption (1 g PPL cartridges, Varian) according to Dittmar et al. (2008). Solid phase extractable DOC (SPE-DOC) amounted to  $42 \pm 6\%$  of the total DOC ( $n = 138$ ) and  $22 \pm 11\%$  of the DON was solid phase extractable ( $n = 128$ ). For an in-depth description of the extraction method refer to Flerus et al. (2011).

### 2.4 Fluorescence measurements

Fluorescence emission and excitation spectra from the methanolic DOM extracts were recorded with an Aminco-Bowman Serie 2 Luminescence Spectrometer in a 1 cm path length quartz glass fluorescence cuvette. Excitation (Ex) – emission (Em) matrices

(EEM) were recorded for selected samples only using different wavelength ranges and signal amplification in order to amplify low fluorescence features at  $>500$  nm. EEM measurements were done with a resolution of 2 nm, band widths of 4 nm for excitation and emission, and scan speed of  $5\text{--}10\text{ nm s}^{-1}$  for (1) Ex: 250–500 nm, Em: 270–500 nm, (2) Ex: 380–680 nm, Em: 400–700 nm, (3) Ex: 380–480 nm, Em: 550–700 nm. Single emission spectra (270–700 nm) were recorded for several samples for excitation between 250 and 500 nm. Single excitation spectra ( $>350$  nm) were recorded for emissions at 490, 650, 670, and 720 nm. For these single scans the signal to noise level was reduced by a slower scan speed ( $1\text{--}2\text{ nm s}^{-1}$ ), multiple scans ( $n = 5\text{--}10$ ) that were averaged, and a band width of  $8\text{--}6$  nm for the fixed monochromator (i.e. for emission during excitation scans, and excitation during emission scans). 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.

## 3 Results

### 3.1 CDOM absorption

CDOM absorption spectra from the sea surface showed a typical spectral distribution in the UVA to VIS range (380–700 nm), i.e. an exponential decrease with increasing wavelength without pronounced structures. Absolute absorption varied due to water mass differences along the latitudinal transect from north to south. Over a depth profile CDOM absorption was lowest at the sea surface and typically highest in depths of the oxygen minimum zone. Figure 1 shows one example of CDOM absorption spectra of a sample profile taken in the tropics. Note that not only the CDOM absorption

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in sub-surface water is higher, there is also a pronounced absorption shoulder at ca. 410 nm for intermediate depth of 110–800 m. Even at higher depths this shoulder is still observable by a linear deflection of the exponential curve. This “typical” exponential decrease in the CDOM absorption spectra was only found in samples from the sea surface whereas the shoulder was found in nearly all samples from depths below the surface mixing zone (40–100 m, Fig. 2), as well as in depths of the deep chlorophyll maximum (DCM) just below the mixing layer (e.g. 82 m, Fig. 1). It was most pronounced in and below the DCM, up to a depth of 800 m. This corresponds to the typical extent of the oxygen minimum zone in this region. The maximum of the shoulder was found between 110 and 500 m (Fig. 1) where the oxygen minimum occurred. However, sample resolution was too low to determine the exact maximum appearance of the shoulder for each profile. Regular sampling was carried out at 200 m water depth. The highest extent of the shoulder in 200 m was found in samples from the tropics (25° N–25° S), the lowest in samples from higher latitudes (ca. 40° N, data not shown).

During two additional cruises, samples for CDOM absorption from depths up to 200 m were taken to determine the occurrence of this absorption shoulder in other oceanographic regions. A few samples were taken from the shallow Santa Barbara Channel (SBC, Californian coast, West Pacific, bottom depth ca. 200 m) and offshore of New Caledonia (South East Pacific, bottom depth >2000 m). During the SBC cruise, CDOM absorption was measured using a liquid waveguide system in addition to the PSICAM to test two independent methods. In both regions (and with both methods in case of the SBC) a shoulder at 410 nm was found in depths below the mixed layer of the sea surface (Fig. 3), whereas the surface samples showed typical surface CDOM spectra without a visible shoulder (data not shown).

### 3.2 Particulate absorption

The CDOM absorption showed an occurrence of a 410 nm-absorption shoulder in the dissolved fraction (<0.2 μm). Additionally, the particulate absorption was measured for each sample to check whether the same absorption feature occurs in the particulate

fraction ( $>0.2\ \mu\text{m}$ ) as well. At the surface the particulate absorption in the ocean is usually dominated by phytoplankton pigment absorption and in the deep sea by that of heterotrophs (mainly bacteria) and detritus. Surface samples were ignored as the phytoplankton pigment maxima and shoulders would mask the distinct shoulder at 410 nm.

5 On the other hand particulate absorption in the deep sea is very low and, thus, difficult to measure. The PSICAM provides a sensitive method to determine particulate absorption; nevertheless the absorption was often below the detection limit. Significant measurements could only be made for the samples from the SBC and a few samples from the Atlantic Ocean (Fig. 4). The spectra showed an absorption maximum at 410 nm in all samples from 90–200 m, and no pronounced maxima in samples taken from depth  $>2000$  m in the Atlantic Ocean. The absorption spectra from these higher depths showed a typical detritus absorption, i.e. an exponential decrease with increasing wavelength with a slope lower than the slope for CDOM absorption. The absolute particulate absorption at 410 nm was lower than the respective CDOM absorption due to the high concentration of DOM compared to POM (particulate organic matter). However, if the absorption of the shoulder is regarded as an absorption maximum on top of the normal CDOM absorption, the absorption of the underlying pigment in the CDOM fraction is in the same range than that of the similar particulate absorption, at least for samples which possessed higher particulate absorption.

### 20 **3.3 DOM fluorescence and absorption from ANTXXV/1**

During ANT XXIII/1 a DOM extract was collected from a 200 m sample to verify the occurrence of the shoulder in concentrated DOM samples and to further analyze a possible fluorescence associated with the 410 nm-absorption shoulder. This DOM methanolic extract showed the same absorption shoulder at 410 nm than the original water sample (Fig. 5), proving that DOM extraction was a good method to concentrate DOM for a later lab analysis. During ANT XXV/1, a set of DOM extracts from the surface and several depths down to the sea bottom were collected. The absorption

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and fluorescence characteristics for all samples from 200 m and for a few samples from the surface and from higher depths were determined. First, emission-excitation matrices were measured for a few samples but for a large wavelength range (Ex: 250–600 nm, Em: 270–700 nm) as fluorescence measurements of the single ANTXXIII/1 extract had shown a specific fluorescence emission at >600 nm. As the absolute fluorescence is highest in the UV to short-VIS, the matrices were repeated at longer wavelengths with higher detector signal amplification. Figure 6 shows the composite of three matrices of one sample from the sea surface and one from 200 m. There is a strong maximum at 370 nm/420 nm (Ex/Em) in both samples and some minor shoulders in the UV and short-VIS. The surface sample showed a low but distinct maximum at 410 nm/670 nm, whereas the maximum in the 200 m sample was located at 415 nm/650 nm. The relevant emission spectra are explicitly shown in Fig. 7a. The possible absorption related to these emissions were examined by single excitation spectra (Ex: 370–630/–650/–690 nm, for Em: 650/670/720 nm, respectively). The emission wavelength of 720 nm was used to examine absorption features around the emission maxima for each sample. Figure 7b shows the excitation spectra for these two samples (surface and 200 m). The surface sample showed a strong excitation maximum at 410 nm and smaller, rather narrow maxima at ca. 509, 538, 608 and 665 nm. The sample from 200 m depth showed a distinct maximum at 415 nm and smaller, broad maxima at 512 and 552 nm, and no significant features at >600 nm. The excitation maximum at 415 nm most likely corresponds to the CDOM absorption shoulder found at the same wavelength in the extract; as slight shift in wavelength compared to the absorption of the water sample (ca. 410 nm) can be explained by the difference in solvent (seawater vs. methanol). To examine the geographic distribution of the obtained excitation maxima at 415 nm, excitation spectra of each sample were recorded with the same fluorometer settings and amplification, and afterwards normalized to the absorption at 415 nm (Fig. 8). With the exception of one sample from >4000 m, pronounced 415 nm maxima were mainly found in the 200 m samples. Surface samples showed smaller maxima, only pronounced in one sample. The geographic distribution from

north to south is shown in Fig. 9 (samples from  $>20^{\circ}$  S were not available). The CDOM absorption at 200 m showed high values in the highest latitudes (Gulf of Biscay), low values in the subtropics ( $40\text{--}23^{\circ}$  N), and high values in the tropics with distinct maxima north and south of the equator. The relative 415 nm excitation maximum in 200 m depth was low in higher latitudes, steadily increased throughout the subtropics, and was highest in the tropics. Distinct maxima were found on the onset of the CDOM increase/decrease north and south of the equator at ca.  $23^{\circ}$  N and  $20^{\circ}$  S. Samples from the surface showed only small excitation maxima, with higher values at ca.  $23^{\circ}$  N only, however the number of samples was limited.

## 4 Discussion

### 4.1 CDOM and particulate absorption of subsurface waters

The CDOM absorption spectra from subsurface water samples in the Eastern Atlantic Ocean showed a clear absorption shoulder at ca. 410 nm that, in its maximum extent and wavelength position (see Figs. 1 and 2), is similar to the shoulder found previously in the Arabian Sea in 600 m (Breves and Reuter, 2000; Breves, 2001; Breves et al., 2003). The shoulder was absent in samples from the sea surface, most pronounced in depths below the surface mixing layer, i.e. in and below the DCM, and extended into the OMZ. Samples from the deep ocean showed no pronounced shoulder but a clear deviation from the typical exponential decrease of surface samples that can still be an indication of a possible underlying absorption maximum at 410 nm. The shoulder is found as well in sub-surface coastal waters of the Santa Barbara Channel and in offshore waters near New Caledonia, both in the Pacific Ocean. Hence, it is possible that the 410 nm-absorption shoulder is a typical feature of the subsurface CDOM in all major oceans, although our samples are taken mainly in the subtropics and tropics.

When the particulate absorption exceeded the detection limit, spectra from subsurface water samples in the Atlantic Ocean and SBC showed an absorption maximum

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at 410 nm (Fig. 4). The extent of this maximum is in the same range than that of an absorption maximum underlying the shoulder in the CDOM spectra. If the same chromophor in the particulate fraction also occurs in the dissolved fraction, it is likely that particles are the source for the dissolved chromophor. It should be noted that in most cases the particulate absorption of deeper water was below the detecting limit. The particulate fraction in deeper water consists mainly of detrital matter and bacteria, and not many measurements exist for samples from these depths. Surface detritus showed a similar exponentially decreasing absorption as CDOM; this can be assumed for open ocean deep waters as well. Absorption by non-colored marine bacteria (or heterotrophic flagellates, ciliates) usually show a strong maximum of the respiratory enzyme (e.g. cytochrome *c*) which absorbs strongest around 410 to 415 nm (Yentsch, 1962; Morel and Ahn, 1990, 1991; Stramski and Kiefer, 1990). The presented data showed that there is a possible particulate source for the observed chromophor in these depths.

## 4.2 CDOM extracts

The same CDOM absorption shoulder that was found in seawater samples was found in methanolic DOM extracts (Fig. 5) that were taken for later fluorescence analyses. The extraction provided a simple way to concentrate the DOM. The fluorescence analysis was stimulated by the observation of a specific fluorescence at 660 nm (Ex: 420 nm) in the OMZ of the Arabian Sea (Breves, 2000; Breves et al., 2003), which was related to the 410 nm-CDOM absorption shoulder. The fluorescence EEM analysis showed Ex/Em maxima typical for oceanic DOM (e.g. Coble, 1996; Coble et al., 1998) with the strongest one being 340/425 nm. In addition, there are distinct maxima with emission at longer wavelengths, which are not measured in most DOM-EEM studies due to the very low fluorescence signal at these wavelengths (exception: Coble et al. 1998). Samples derived from the surface and from 200 m showed a peak at 410/670 nm (Ex/Em) and at 415/650 nm, respectively (Fig. 6). For the surface sample this peak fit well to the fluorescence of chlorophyll *a* or its degradation products (Peak P, 410 nm/680 nm

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in Coble et al., 1998). The small wavelength shift in the emission and excitation maximum compared to that found by Coble et al. (1998) could be explained by the different solvent (methanol vs. water). The peak at 415 nm/650 nm was to our knowledge not described earlier, neither for surface nor for sub-surface DOM samples. Only a 660 nm-emission (for 420 nm excitation) was observed in situ (Breves, 2000; Breves et al., 2003). Considering the small solvent-dependent wavelength shift and the associated 415 nm-excitation maximum, it is most likely that this 660 nm emission peak is derived from the same common fluorophor.

The excitation spectrum can be used to get information on the related absorption efficiency for each emission maximum. The fluorescence at 650 nm is linked to an absorption maximum at 415 nm, whereas the emission at 670 nm related to absorption at 410 nm. The absorption characteristics of each fluorophor were further analyzed by more precise excitation spectra (Fig. 7). The underlying chromophor for the 670 nm fluorescence at the sea surface showed the characteristic absorption of a chlorophyll *a* degradation product, e.g. pheophytin *a*, pheophorbide *a*, protopheophorbide *a* (which all have the same absorption characteristics, e.g. Jeffrey et al., 2007). The absorption characteristics of the chromophor in the subsurface water were clearly different from these chlorophyll *a* derivatives. First, there was no strong absorption maximum in the red region (650–670 nm), and second the strong Soret maximum was shifted to 415 nm, with the minor maxima being broader and at longer wavelengths (Fig. 7). In conclusion, the specific chromophor/fluorophor of the sub-surface samples was not directly related to that found in surface waters; the surface signal was clearly derived from a chlorophyll *a* degradation product. Hence, the 415 nm-chromophor was not an early degradation product from chlorophyll *a*, as the typical red absorption maxima was missing and the fluorescence was shifted to shorter wavelengths.

### 4.3 Geographic distribution

The specific 650 nm-fluorescence can be used to roughly quantify the 415 nm-chromophor. Therefore excitation spectra (Em: 650 nm) were recorded for each extract

and normalized to the absorption at 415 nm. The normalized excitation at 415 nm was used as an indication for the relative chromophor concentration over the latitudinal transect in the Atlantic Ocean between 50° N and 20° S. In a few surface samples this value was relatively low throughout the transect (Figs. 8 and 9) and the visible maximum related to fluorescence of the chlorophyll *a* degradation product that is still measurable at 650 nm. In samples from the deep sea this excitation maximum was generally low. The strongest 415 nm-excitation were found in the samples from 200 m depth (only samples from the surface, 200 m, and the deep sea were analyzed, so, the 200 m sample might not be representing the maximum of the shoulder in the water column). The absorption at 415 nm showed higher values at 200 m throughout the tropics and lower values in the subtropics. The chromophor concentration was related to this high CDOM absorption, showing the highest concentration in the tropics around the equator.

The results presented here provide strong evidence that the 415 nm-absorption shoulder is related to the fluorescence at 650 nm. This fluorescence most probably can be detected by regular chlorophyll *a* fluorometers, and, as there was no chlorophyll-like fluorescence detectable in subsurface water, the same fluorescence was likely detected earlier as “deep red fluorescence” (Broenkow et al., 1983, 1985, 1992; Lewitus and Broenkow, 1985; their “third” fluorescence maximum), and by Breves and co-workers (Breves and Reuter, 2000; Breves, 2001; Breves et al., 2003). Breves and co-workers reported this fluorescence from the Arabian Sea in the tropical Indian Ocean, and Broenkow et al., (1983, 1985, 1992) from the eastern Pacific Ocean ranging from tropical to temperate regions. Broenkow et al. were one of the first measuring red fluorescence in deeper waters, as those measurements were mainly conducted in surface waters considering phytoplankton in the euphotic zone as the main source. Already Lewitus and Broenkow (1985) speculated from their observations that the maximum of the deep red fluorescence is associated with the OMZ and that it might be a widespread feature. Combining the observation of the deep fluorescence with that of the 410 nm-absorption shoulder, this specific chromophor/fluorophor can be found in tropical region of all major oceans, as well as in subtropical and temperate regions of

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the Pacific Ocean. Concentrations in the temperate to subtropical Atlantic were relatively low, but still detectable. The “third” fluorescence maximum (sensu Broenkow et al., 1983) was found in depths of the OMZ, and the extent of the 410 nm-absorption shoulder was high in the OMZ, however, the absorption shoulder was also high in depths of the DCM below the vertical mixing zone in the tropical Atlantic. Any red fluorescence by this specific fluorophor might be covered by chlorophyll fluorescence from phytoplankton in the DCM. Broenkow et al., (1983, 1985, 1992), however, observed a clear fluorescence minimum between the surface maximum and the “third” maximum, which could not be observed yet for the absorption shoulder due to the low vertical resolution. More information is needed to resolve the relationship between OMZ and this fluorophor, but from the absorption results it is clear that it does not exclusively occur in the OMZ, e.g. the absorption shoulder was found in about 100–200 m of the Santa Barbara Channel, a coastal environment. More work is needed to quantitatively determine the concentration of this chromophor in the global ocean and to unravel its molecular origin.

#### 4.4 Possible origin of the chromophor/fluorophor

The data presented here show that a specific absorption is observed in the dissolved and in the particulate fraction at around 410–415 nm. In most cases the particulate absorption was too low to be measured accurately, but the absorption in the dissolved fraction (CDOM) was always high enough and, hence, often higher than that in the particulate absorption. Only when the particulate absorption was highest in deeper waters the extent of this absorption was the same in the particulate as in the dissolved fraction. It can be stated that this specific chromophor was generally found in the dissolved phase. However, it probably originates from the particulate fraction, either from bacteria or from detrital particles sinking down from the euphotic zone. Earlier observations were contradicting: Broenkow et al. (1992) concluded from an often close correlation with attenuation (mainly scattering by particles) that the deep fluorescence

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originated from the particulate fraction, whereas Breves et al. (2003) found it in the dissolved fraction. Here it is shown clearly that the specific absorption is detectable in the particles (Fig. 4), but that the main signal is from the dissolved fraction. No samples were taken to test whether the particulate absorption at 410 nm is giving a similar fluorescence signal than that in the CDOM, but due to the spectral similarity it is most likely that the particles serve as the source of the dissolved chromophor. As the absorption takes place in the visible spectral region this chromophor by definition is part of a pigment, and, hence, this observation shows the occurrence of high concentration of a pigment degradation product in the dissolved phase that is accumulated in sub-surface waters by either a high production or a low remineralization rate. The particulate absorption found in sub-surface waters is similar to earlier observations of bio-detritus (e.g. Kishino et al., 1985; Bricaud and Stramski, 1990). The absorption maxima at ca. 410 nm can be explained by absorption of heterotrophs, like bacteria, ciliates, flagellates (e.g. Stramski and Kiefer, 1990; Morel and Ahn, 1990, 1991; Stramski and Kiefer, 1998), and is due to absorption of the respiration pigments (e.g. cytochrome *c*). The similarity to the 410 nm absorption shoulder in CDOM gives evidence that this chromophor originates from respiration pigments in the particles, hence, from heterotrophs.

Thus, the optical characteristics of the chromophor/fluorophor did not support that the molecule is a chlorophyll *a* degradation product, but fits in its absorption and fluorescence to that of a cytochrome *c*. The particulate absorption of heterotrophic bacterial cultures showed maxima at 409 nm (Stramski and Kiefer, 1998) or 412–415 nm (Morel and Ahn, 1990), and specific weak absorption maxima at 525 and 555 nm (Morel and Ahn, 1991). Methanolic extract of the heterotrophic pigment showed an absorption maximum around 415 nm. These absorption features correspond exactly to the maxima found in the DOM fluorescence excitation spectra from subsurface samples (Fig. 7, ca. 512 and 552 nm). However, other sources are possible for the dissolved chromophor, such as release from detrital particle settling down from the euphotic zone that can either be from heterotrophic bacteria or from phytoplankton. E.g. in tropical

regions, where *Prochlorococcus* sp. and *Synechococcus* sp. often dominate the phytoplankton communities, the high chromophor concentrations found in CDOM from the DCM suggest both organism classes to be a potential source.

## 5 Conclusions

5 The detected fluorescence and absorption characteristics of an unknown chromophor in sub-surface waters of the sub-tropical to tropical ocean links observations of an absorption maxima at ca. 410 nm with earlier observations of a “deep red fluorescence” in these waters. This chromophor is not a chlorophyll-degradation product as speculated earlier but might originate from bacterial respiration pigments. The specific fluorescence at 650 nm and absorption at 410 nm are features of a single, relatively high concentrated pigment molecule (or set a similar molecules) in DOM, which makes it unique among all other molecules in the DOM pool of sub-surface waters. If the biogeochemistry (rates of production, remineralization, degradation etc.) of this chromophor is known, the easy optical detection of this chromophor could make it suitable as a tracer for e.g. bacterial production, or POM and DOM remineralization. Its molecular identity, 10 origin, global occurrence, and concentration currently remain unknown.

*Acknowledgements.* We thank captains and crews of RV “Polarstern”, RV “L’Alis”, and RV “Kilo Moana” for their help and support. The DOM extracts were kindly taken by Walter Geibert, Oliver Lechtenfeld, and Ruth Flerus. The participation of R. Röttgers on the RV “L’ Alis” cruise 15 was supported by the “VALHYBIO/PNTS” project.

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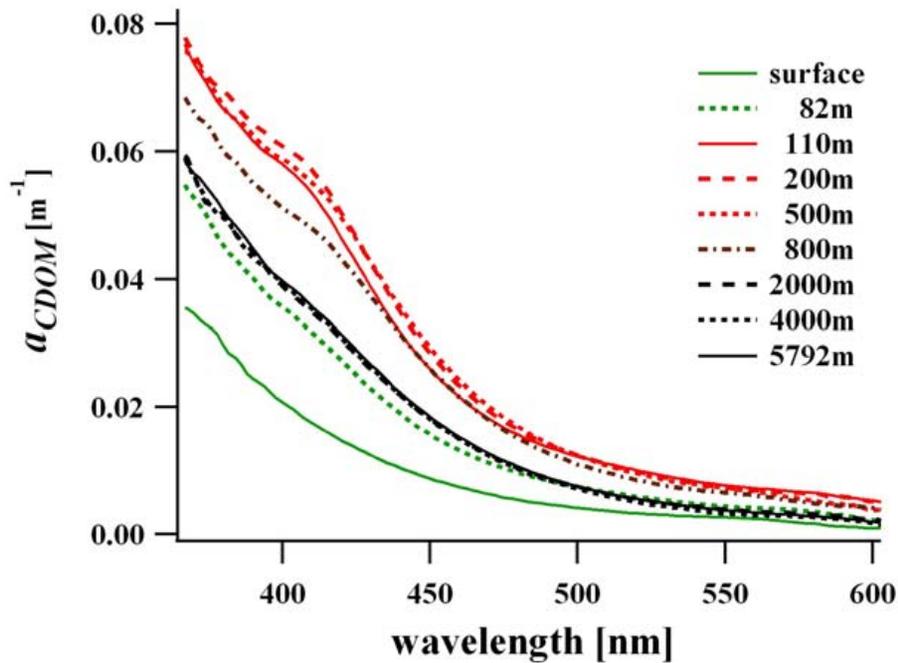
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**Fig. 1.** Depth-dependent light absorption of CDOM in the tropical Atlantic Ocean (S 12°, W 2°) in November 2005 (ANT XXIII/1) as a function of wavelength.

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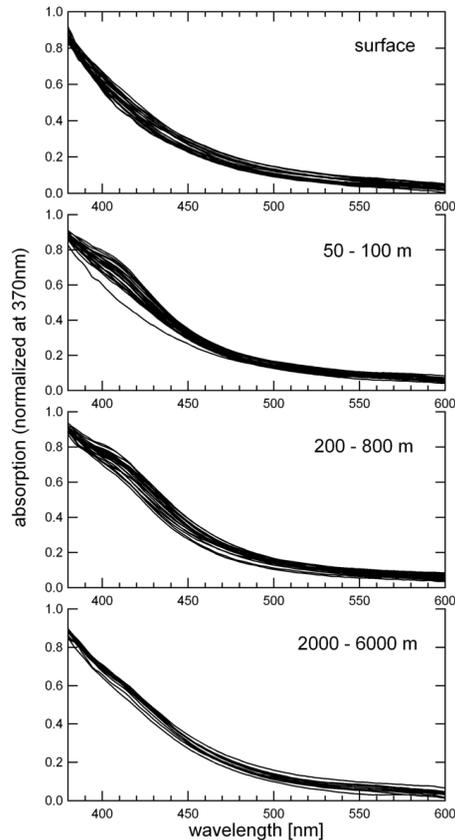
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**Fig. 2.** Normalized CDOM absorption spectra from a transect in the Western Atlantic Ocean (ANT XXIII/1), showing absorption for samples from **(a)** the surface and from depths of **(b)** 50–110 m, **(c)** 200–800 m, and **(d)** >2000 m. The absorption is normalized to the absolute absorption at 370 nm to adjust for differences in the absolute absorption.

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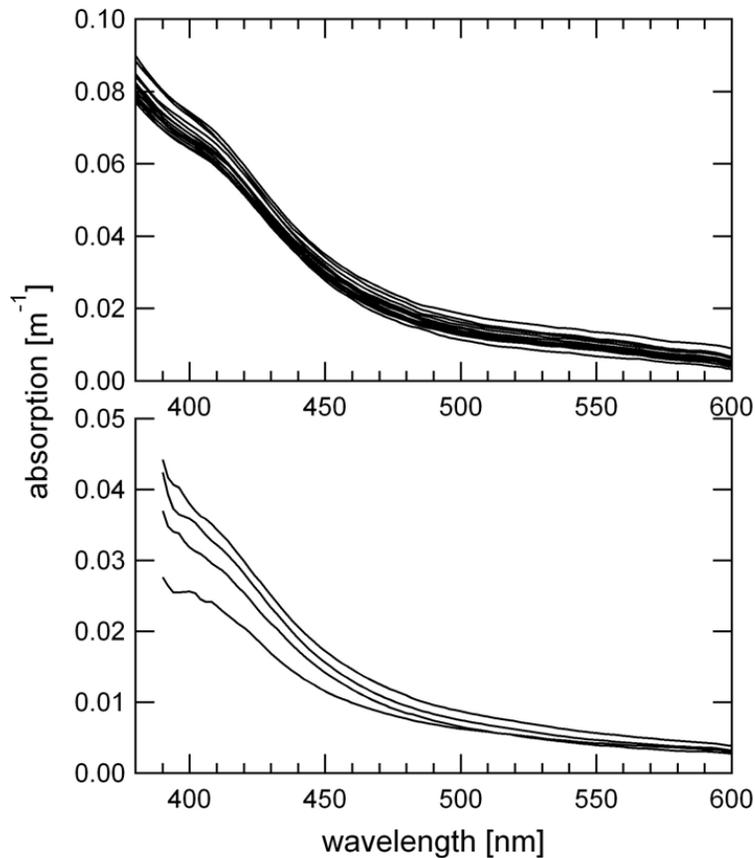
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**Fig. 3.** Light absorption of CDOM from sub-surface depth (80–200 m) of samples from **(a)** the Santa Barbara Channel (California, North-East Pacific) and **(b)** from offshore of New Caledonia (West Pacific).

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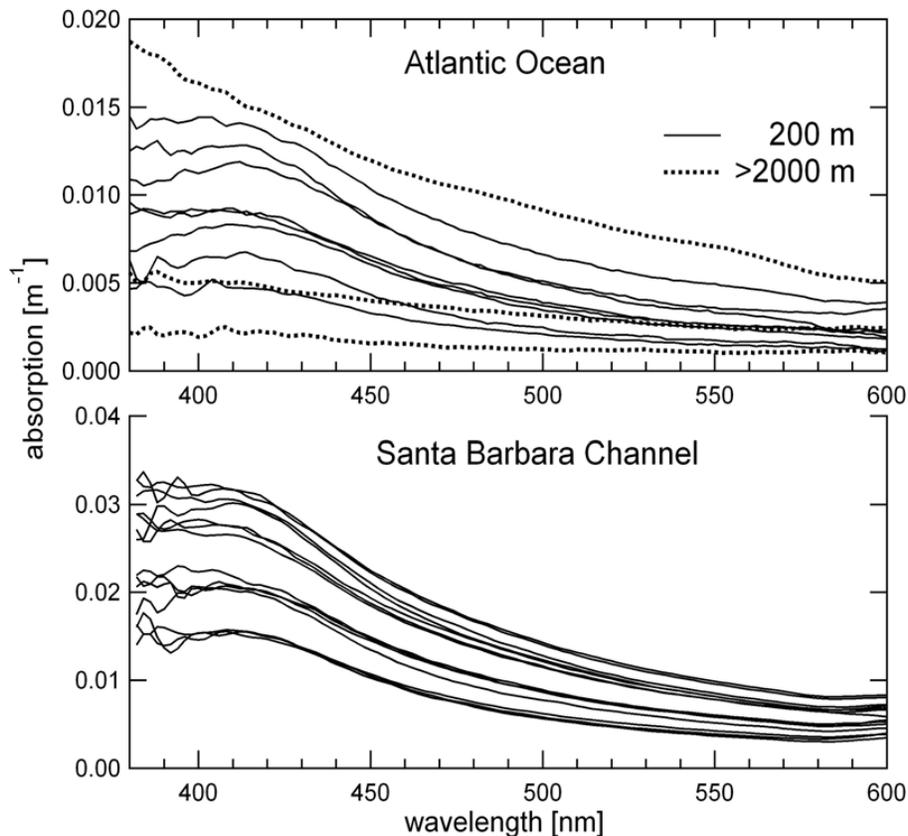
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**Fig. 4.** Light absorption by particles of samples from sub-surface depths taken in **(a)** the western Atlantic Ocean (200 m and >2000 m), and **(b)** the Santa Barbara Channel (California, North-East Pacific, 80–200 m).

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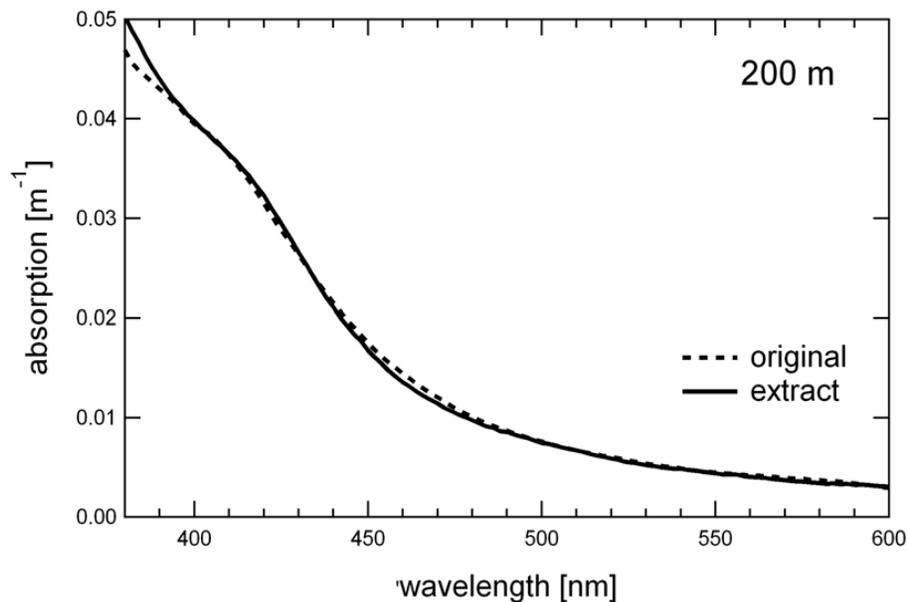
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**Fig. 5.** Normalized CDOM absorption spectra of a methanolic DOM extract from 200 m and the corresponding sample. The absorption is normalized to the absolute absorption at 380 nm to adjust for differences in the absolute absorption.

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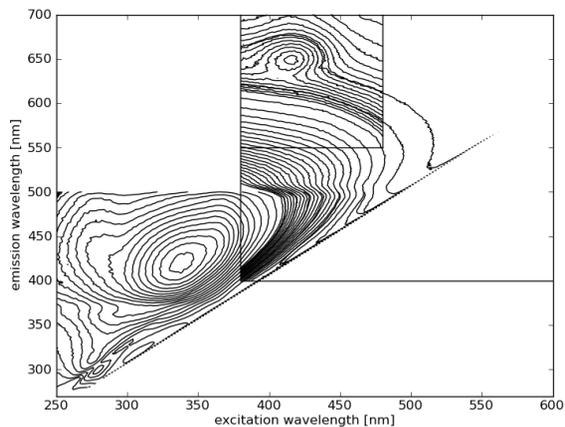
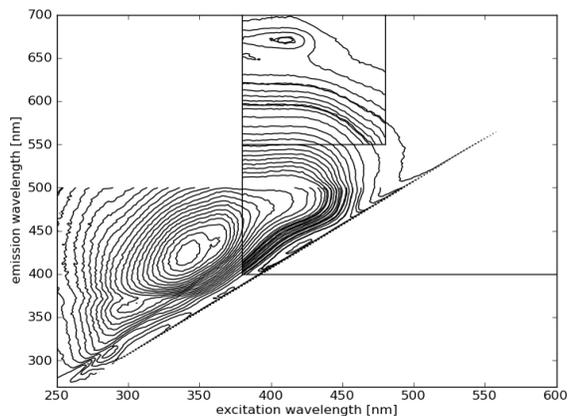
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**Fig. 6.** Fluorescence excitation-emission matrices of a sample taken at the surface (upper panel) and at 200 m depth (lower panel) in the Western Atlantic Ocean (ANT XXV/1). Each graph shows a composite of three matrices recorded from the same sample with different detector amplification to adjust for the low absolute fluorescence emission at longer wavelengths.

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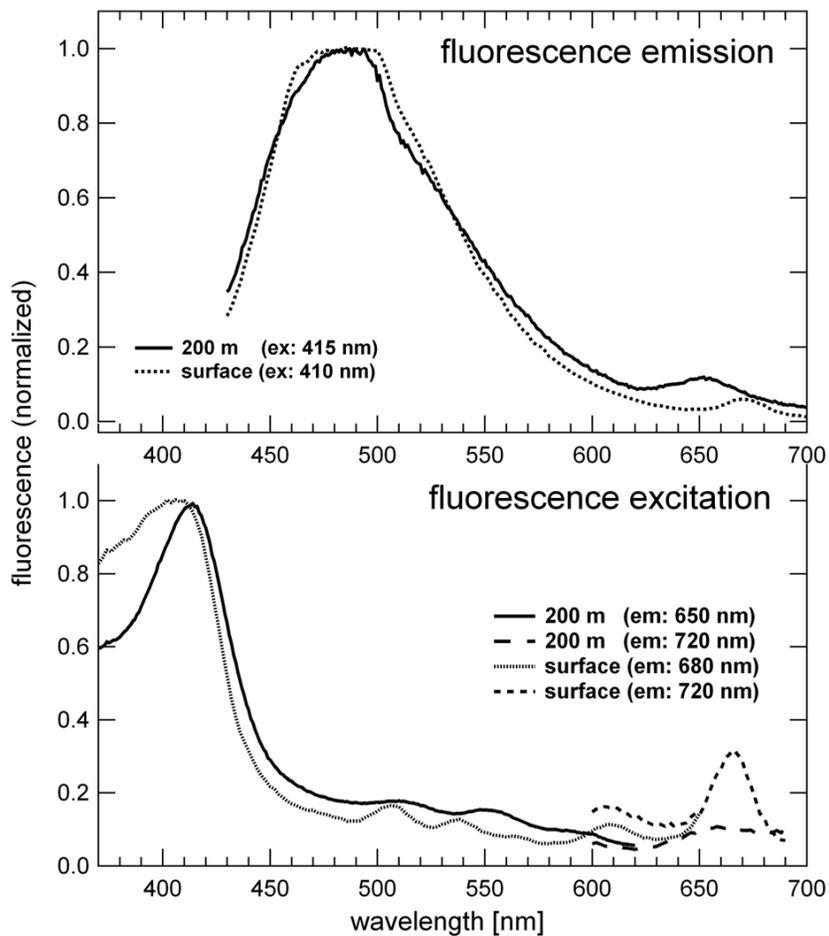
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**Fig. 7.** Single fluorescence emission (a) and excitation (b) spectra for the same two samples as in Fig. 6. Specific excitation and emission wavelengths are shown in the legend.

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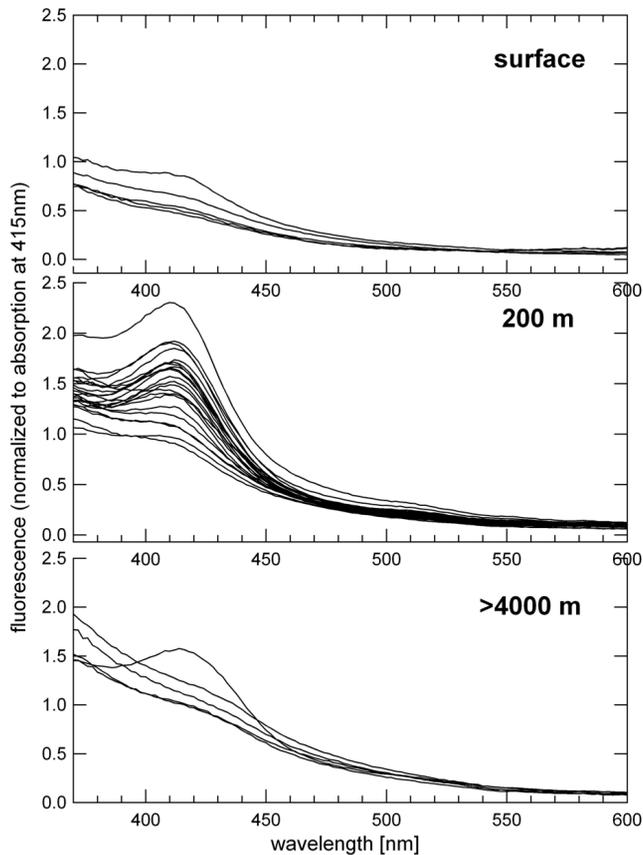
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**Fig. 8.** Excitation spectra for the 650 nm emission maxima for samples taken in the Western Atlantic Ocean (XXV/1) at **(a)** the surface, **(b)** a depth of 200 m **(c)** depths of >4000 m.

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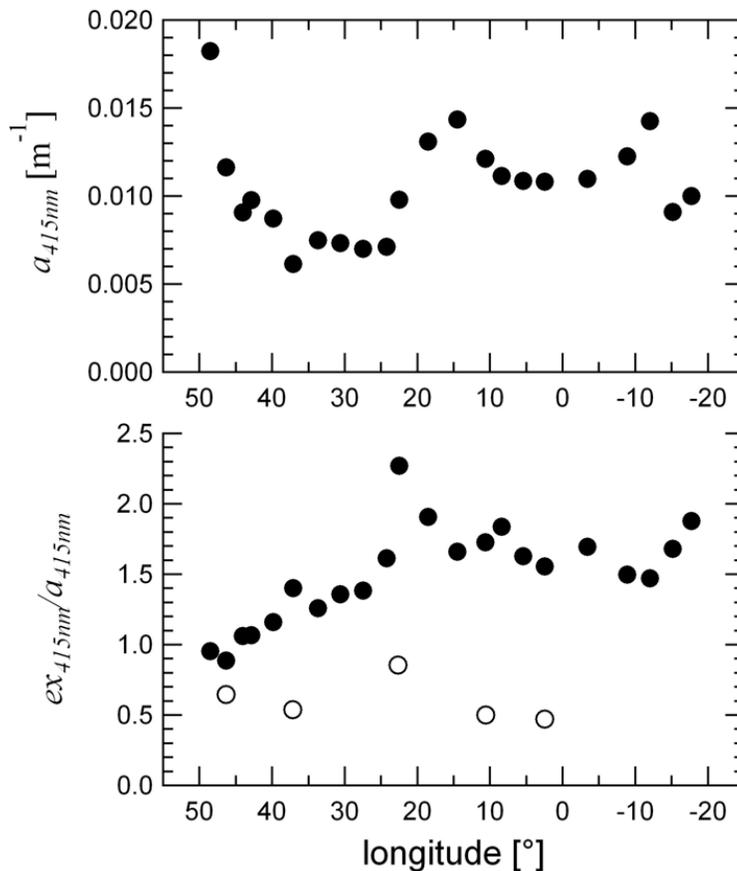
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**Fig. 9.** Latitudinal distribution of **(a)** the CDOM absorption at 415 nm, and **(b)** the 415 nm excitation maximum (em: 650 nm) normalized to the absorption at 415 nm for samples from 200 m (closed symbols) and the surface (open symbols). Samples were taken in the Western Atlantic Ocean during ANT XXV/1.

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