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Characterization of chromophoric dissolved organic matter (CDOM) in the Baltic Sea by excitation emission matrix fluorescence spectroscopy

Piotr Kowalczuk[a,*], Joanna Stoń-Egiert[a], William J. Cooper[b], Robert F. Whitehead[b], Michael J. Durako[b]

[a]*Institute of Oceanology of Polish Academy of Sciences, ul. Powstańców Warszawy 55, 81-712 Sopot, Poland*
[b]*Center for Marine Science, University of North Carolina at Wilmington, 5600 Marvin Moss Lane, Wilmington, NC 28409, USA*

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Abstract

Chromophoric dissolved organic matter (CDOM) is the major light absorber in the Baltic Sea. In this study, excitation emission matrix (EEM) fluorescence spectra and UV–visible absorption spectra of CDOM are reported as a function of salinity. Samples from different locations and over different seasons were collected during four cruises in 2002 and 2003 in the Baltic Sea in both Pomeranian Bay and the Gulf of Gdansk. Absorption by CDOM decreased with increased distance from the riverine source and reached a relatively stable absorption background in the open sea. Regression analysis showed that fluorescence intensity was linearly related to absorption by CDOM at 375 nm and $a_{CDOM}(375)$ absorption coefficients were inversely related to salinity. Analysis of CDOM-EEM spectra indicated that a change in composition of CDOM occurred along the salinity gradient in the Baltic Sea. Analysis of percent contribution of respective fluorophore groups to the total intensity of EEM spectra indicated that the fluorescence peaks associated with terrestrial humic components of the CDOM and total integrated fluorescence decreased with decreasing CDOM absorption. In contrast, the protein-like fraction of CDOM decreased to a lesser degree than the others. Analysis of the percent contribution of fluorescence peak intensities to the total fluorescence along the salinity gradient showed that the contribution of protein-like fluorophores increased from 2.6% to 5.1% in the high-salinity region of the transect. Fluorescence and absorption changes observed in the Baltic Sea were similar to those observed in similar transects that have been sampled elsewhere, e.g. in European estuaries, Gulf of Mexico, Mid-Atlantic Bight and the Cape Fear River plume in the South Atlantic Bight, although the changes in the Baltic Sea occurred over a much smaller salinity gradient.

Keywords: Chromophoric dissolved organic matter; Absorption; Fluorescence; Ocean optics; Estuary mixing processes; Baltic Sea

* Corresponding author. Tel.: +48 58 551 7281x218; fax: +48 58 551 2130.
E-mail address: piotr@iopan.gda.pl (P. Kowalczuk).

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1. Introduction

Dissolved organic matter, DOM, in natural waters is one of the largest pools of organic carbon in the biosphere. The fraction absorbing light from 300 to 800 nm, chromophoric dissolved organic matter (CDOM), historically referred to as Gelbstoff, yellow substances or humic material, is the primary absorber of sunlight. Thus, CDOM is a major determinant of the optical properties of natural waters and it directly affects both the availability and spectral quality of light. Through its effects on underwater solar radiation (Del Vecchio and Blough, 2005; Hargreaves, 2003), especially UV radiation, CDOM may stimulate or deter biological activity (e.g. Mopper and Kieber, 2002). CDOM also is an important component of remotely sensed ocean color (Siegel et al., 2002) and it plays a key role in marine photoreactions (Cooper et al., 1989; Whitehead and de Mora, 2000; Del Vecchio and Blough, 2005; Mopper and Kieber, 2002; Zepp, 2003; Kieber et al., 2003).

Light absorption by CDOM decreases exponentially toward longer wavelengths (Jerlov, 1976; Kirk, 1994), and it affects both the inherent and apparent optical properties of seawater. Physical, chemical and biological processes all influence the distribution, spatial and temporal variability and optical properties of CDOM (Whitehead et al., 2000; Siegel et al., 2002; Blough and Del Vecchio, 2002; Osburn and Morris, 2003; Zepp, 2003). CDOM in coastal environments generally has a terrestrial origin and is transported to the ocean via rivers. The highest concentrations of CDOM are found in coastal margins of oceans and in semi-enclosed seas, where direct sources of terrestrial organic matter are found. The geographical extent of the terrestrially dominated regions varies seasonally, depending on the magnitude of freshwater inputs (Blough et al., 1993; Nelson and Guarda, 1995; Vodacek et al., 1997; Rochelle-Newall and Fisher, 2002), and its dilution by physical mixing processes in the coastal areas. On local scales, in situ production from phytoplankton decomposition and extraction from benthic sediments may be an important source of CDOM (e.g. Kowalczuk, 1999; Kahru and Mitchell, 2001; Twardowski and Donaghay, 2001; Boss et al., 2001). In the central, oligotrophic regions of the oceans, CDOM is presumably created in situ by processes that remain poorly understood (Siegel et al., 2002).

CDOM is removed from water in three ways: coagulation and precipitation of the high molecular weight fraction of terrestrial DOM (Zamardi-Lamardo et al., 2004; Stabenau and Zika, 2004), photochemical reactions and/or microbial uptake (Osburn and Morris, 2003). Recently, field and laboratory studies have shown that photobleaching alone is a large sink of CDOM with half-lives ranging from hundreds to thousands of hours (e.g. Vähätalo and Wetzel, 2004). Photobleaching of CDOM may not change the dissolved organic carbon concentration but it may result in an increase in the spectral slope coefficient, mainly due to relatively faster photobleaching in the UV-A (Vodacek et al., 1997; Nelson et al., 1998; Grzybowski, 2000; Whitehead et al., 2000; Twardowski and Donaghay, 2001; Twardowski and Donaghay, 2002).

The fluorescent properties of CDOM have been known for a long time (Duursma, 1965) and the fluorescence signal has been used to estimate CDOM in marine waters (e.g. Højerslev, 1989). Numerous investigators have observed a linear relationship between fluorescence and absorption (Ferrari and Tassan, 1991; Hoge et al., 1993; Vodacek et al., 1997; Ferrari and Dowell, 1998; Ferrari, 2000). Fluorescence excitation emission matrices (EEM spectra) are obtained by acquiring emission spectra at a series of successively longer excitation wavelengths. The emission spectra are concatenated to generate a plot in which the fluorescence is displayed as a function of excitation and emission wavelengths. Although slower to collect, EEM spectra provide a more complete picture of CDOM emission properties and can often be used to discriminate among different classes of fluorophores based on their excitation emission maxima. It is also possible to use EEM spectra to follow changes in CDOM resulting from biological or physical processing of the material, or trace CDOM from different sources. Coble (1996) was the first to successfully apply this technique to field data analysis with descriptions of CDOM in the Caribbean, Arabian Sea and Gulf of Mexico (e.g. Coble et al., 1998; Del Castillo et al., 1999; Del Castillo et al., 2000).

The Baltic Sea has unique optical properties because of a very high input of freshwater from the large surrounding drainage area and limited water exchange with the North Sea through the Danish Straits. Earlier studies provided examples of CDOM absorption coefficients in the Baltic Sea and for a number of sites in the seas around Europe. The impact of CDOM absorption on apparent optical properties showed that characteristic optical properties of Baltic Sea waters are determined to a large extent by light absorption by CDOM (Højerslev, 1974, 1988, 1989; Lundgren, 1976). Nyquist (1979) suggested that a significant fraction of CDOM in the Baltic Sea consisted of lignin sulfonates. The Baltic Sea, which is located in the temperate climatic zone, except its northern part, the Sea of Bothnia, which is located in the boreal zone, undergoes a well-established seasonal hydrological cycle. The hydrological regime shapes inherent and apparent optical properties in the relatively low saline water above the permanent pycnocline (Sagan, 1991; Olszewski et al., 1992; Kowalczuk, 1999). Changes in inherent optical properties have an impact on apparent optical properties, including changes of the spectral signature (Darecki et al., 1995; Darecki et al., 2003; Kowalczuk et al., 1999; Kowalczuk et al., 2005).

The annual, well-defined hydrologic cycle controls terrestrial CDOM input and impacts the DOM dynamics in the Baltic Sea. Kowalczuk (1999) and Kowalczuk et al. (2005) found that annual CDOM cycling was determined by the annual maximum riverine discharge into the southern Baltic. The riverine source of CDOM, although most important, is not the only factor to affect DOM dynamics. Local production of CDOM is significant in remote areas not directly influenced by riverine plumes (Kowalczuk, 1999, 2001). Therefore, there is a need to better discriminate CDOM from various sources that will provide a new approach in modeling CDOM dynamics, e.g. by multivariable statistical modeling. From previous studies, it appears that the Baltic Sea offers a unique opportunity to study CDOM dynamics using the EEM spectroscopic technique.

In this study, we examined the variation in EEM spectra to characterize CDOM collected in the surface layer of the southern Baltic Sea in different locations and different seasons, to discriminate different sources of CDOM and to answer the following questions.

1. Are there significant qualitative and quantitative changes in CDOM EEM spectra collected in the Baltic Sea along salinity gradients from terrestrial sources to open seawaters?
2. What are the similarities and differences in cycling of specific fluorophores along the salinity gradient in the Baltic Sea and to those observed in similar transects that have been made elsewhere: e.g. in European estuaries, Gulf of Mexico, Mid-Atlantic Bight and the Cape Fear River plume in the South Atlantic Bight?

2. Materials and methods

This study was conducted from May 2002 to April 2003. Samples were collected during four cruises in the Baltic Sea, organized by the Institute of Oceanology Polish Academy of Science within the long-term bio-optical observation program. A majority of the sampling stations were located in the Pomeranian Bay and Gulf of Gdansk (Fig. 1). These regions are of particular interest because of the influence of two major rivers that drain most of Poland. The Vistula River flows into the Gulf of Gdansk and the estuary of the Odra River is connected to the Pomeranian Bay through three inlets: Peene, Dźwina and Świna Rivers. Nearly-eighty percent of the water exchanged between the Szczecin Lagoon (Odra River estuary) and Pomeranian Bay goes through the Świna River (Majewski, 1980). Reference samples were obtained in adjacent coastal zones with minimal river input and in the open sea.

The temporal distribution of the samples covered the important periods of the annual hydrologic cycle. The cruises in May 2002 and April 2003 corresponded to periods of maximum terrestrial input and the annual phytoplankton bloom. The cruise in October 2002 corresponded to a period of intense vertical mixing and the cruise in February 2003 corresponded to a period of minimal terrestrial input. The spatial and temporal distribution of samples was planned to provide high variability in CDOM. A CTD cast was performed at each station to determine salinity.

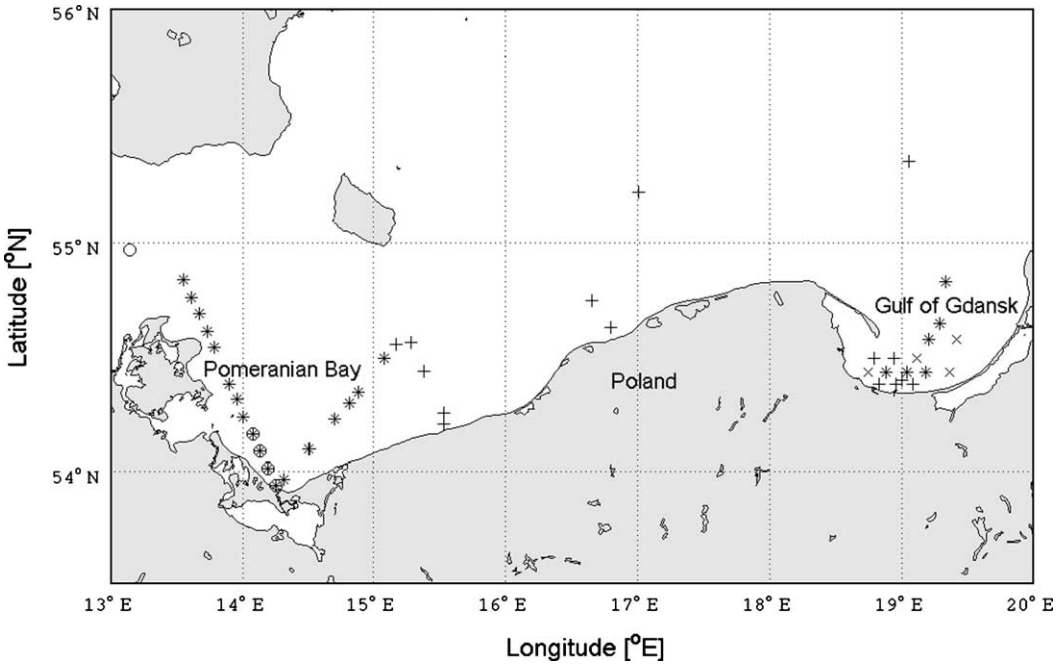

Fig. 1. Location of sampling stations in the Baltic Sea, May 2002 (*), October 2002 (○), February 2003 (+), April 2003 (×).

Water samples for the determination of CDOM absorption were collected at fixed depths at the surface layer (0 and 5 m) with Niskin bottles and processed according to the procedure of Reuter et al. (1986). Directly after collection, samples were filtered (on board) using sample pre-rinsed Whatman glass fibre filters (GF/F, nominal pore size 7 μm) to remove most suspended solids and plankton. The water was then filtered through acid-washed Sartorius 0.2 μm pore cellulose membrane filters to remove fine-sized particles. The first two portions of 250 mL filtered water were discarded and the third filtered sample stored in the dark at ~ 4 °C in 250 mL amber glass bottles with the Teflon lined caps for spectrophotometric scans (usually within a couple of hours). Absorbance of the samples was measured on the ship using a double-beam UNICAM UV/VIS spectrophotometer with 5 cm quartz cell in the spectral range 200–700 nm. A quartz cell with pre-filtered (0.2 μm) Milli-Q water was used as the reference for all samples. CDOM absorption coefficients, $a_{\mathrm{CDOM}}(\lambda)$, at each wavelength ($\lambda$) were calculated using the equation:

$$a_{\mathrm{CDOM}} = \frac{2.303A(\lambda)}{l} \quad (1)$$

in m^{-1}, where A is absorbance and l is length of the cell in meters. From measurements where both cells were filled with Milli-Q water, no significant differences were found in observed spectra for absorbance below 0.001, which corresponded to a detection level of 0.046 m^{-1} using 5 cm cells.

Following the recommendation of recent studies by Stedmon et al. (2000), the spectral slope coefficient S was calculated by applying the non-linear simple exponential model to fit the raw absorption spectra without offset correction. S was computed for two spectral ranges, $350 \leq \lambda \leq 550$ nm and $300 \leq \lambda \leq 650$, using the following equation:

$$a_{\mathrm{CDOM}}(\lambda) = a_{\mathrm{CDOM}}(\lambda_0)\mathrm{e}^{-S(\lambda_0-\lambda)} + K \quad (2)$$

where $a_{\mathrm{CDOM}}(\lambda)$ is the absorption coefficient at wavelength λ, λ_0 is a reference wavelength (375 nm), K is a background constant that allow any baseline shift caused by residual scattering by fine size particle fractions, micro-air bubbles or colloidal material present in the sample, refractive index differences between sample and the reference, or attenuation not due to organic matter. The parameters $a_{\mathrm{CDOM}}(375)$, S and K were estimated simultaneously via non-linear regression of Eq. (2) using a secant

iterative method implemented in SAS/STAT software package (SAS Institute Inc., 1994). This method, which preferentially weights regions at higher CDOM absorption rather than the areas of low absorption, has been found to give better fit of the model (significantly reduces the sum of residuals) to the observed spectrum than the linear regression of log transformed data. It also permits the use of a fixed spectral range for estimating the spectral slope coefficient for CDOM from different environments, which can then be compared more reliably, due to a significant reduction in the variability caused by using variable spectral ranges of log transformed data. The short wavelength limit for the first spectral range was applied to reduce the possible effect of the absorption due to the presence of lignin sulfonates (Nyquist, 1979), which was observed in some samples as a small bump in the spectral range 260–310 nm. Calculation of the spectral slope coefficient in second spectral range, 300–650 nm, was performed to extend modeling of the absorption spectrum into the ultraviolet, which is important for excitation of specific fluorophores and for photochemical decomposition of CDOM.

Samples for fluorescence measurements were treated in the same manner as those for absorbance measurements. After collection and filtration, samples were stored at 4 °C in the dark and shipped to the University of North Carolina at Wilmington, NC, for analysis. Excitation emission matrix (EEM) fluorescence spectra were obtained using a Jobin Yvon SPEX FluoroMax-3 scanning fluorometer equipped with a 150 W Xe arc lamp and a R928P detector. Highly absorbing samples were diluted with Milli-Q water to the point where A_{350} (1 cm path length) was ≤ 0.02 to minimize inner-filtering effects. The assignment of peaks was that of Coble (1996), where A was the terrestrial humic substances peak, C the terrestrial fulvic substances peak, M the marine fulvic substances peak and T the proteinaceous peak.

EEM spectra were constructed using excitation wavelengths from 250 to 500 nm (5 nm intervals) and scanning emission wavelengths from 280 to 600 nm (5 nm intervals). The instrument was configured to collect the signal in ratio mode with dark offset using 5 nm bandpass on both the excitation and emission monochromators. Scans were corrected for instrument configuration using factory-supplied correction factors, which were determined as described in Coble et al. (1993). Post-processing of scans was performed using FLToolbox 1.91 developed by Wade Sheldon (University of Georgia) for MATLAB® (Release 11) (Zepp et al., 2004). This software eliminates Rayleigh and Raman scattering peaks by excising portions (± 10–15 nm FW) of each scan centered on the respective scatter peak. The excised data were replaced using three-dimensional interpolation of the remaining data according to the Delaunay triangulation method and constraining the interpolation such that all non-excised data were retained. Following removal of scatter peaks, data were normalized to a daily-determined water Raman intensity (275 ex/303 em, 5 nm band pass) and converted to Raman-normalized quinine sulfate equivalents (QSE) in ppb (Coble et al., 1998). For samples that required dilution, the scatter-corrected fluorescence of the diluent Milli-Q was subtracted and the resulting fluorescence values were multiplied by the dilution factor to obtain the intensity for the original, undiluted sample. Replicate scans were generally within 5% agreement, in terms of intensity, and within-bandpass resolution, in terms of peak location. Sample post-processing and sample quality analysis and control is described in detail in Kowalczuk et al. (2003).

The EEM spectra were quantified using the integration option in FLToolbox (Zepp et al., 2004). Integration areas are shown in Figs. 3 and 4 as circles or ellipses centered in the respective peak's maxima. Integral of the whole EEM spectra was also calculated in the following spectral ranges: 250–500 nm for excitation and 280–600 nm for emission. Percentage contributions of individual peaks to the whole spectrum intensity were then calculated (Kowalczuk et al., 2003), by determining the ratio of the respective peak integrals of A, C, M and T to that of the whole EEM integral. These parameters were used to calculate relationships between absorption, fluorescence and salinity. Regression analysis and the significance level of the correlation coefficient were tested using the statistical package Statistica v. 6 (Computer program manual, Tulsa, StatSoft Inc., web: http://www.statsoft.com).

All samples were checked for possible bacterial contamination during storage and shipment. Bacteria have very strong fluorescence properties due to the abundance of aromatic amino acids, tryptophan and

tyrosine in the cells. Their fluorescence characteristics are very close to the tyrosine or tryptophan-like fluorophores (*T* peak) in natural waters (Determann et al., 1998). Published CDOM-EEM spectra (Coble, 1996; Del Castillo et al., 1999; Kowalczuk et al., 2003) show that the *T* peak intensity is always lower than *C* or *M* peaks; therefore, all samples with an unusually high intensity of the *T* peak were considered to contain bacterial contamination and eliminated from analysis.

3. Results

3.1. Quantitative description of CDOM changes in the study area

We chose the CDOM absorption coefficient at 375 nm, $a_{CDOM}(375)$, for describing changes in CDOM quantity and the CDOM spectral slope coefficient *S*, to differentiate CDOM pools. EEM spectra were used to detect qualitative changes in CDOM composition. Over the time period of this project, we collected 67 samples for CDOM absorption and 67 for EEM spectral analysis. Examples of CDOM absorption spectra for respective sampling areas are shown in Fig. 2. To compare data used in this study to previous data on CDOM dynamics in the Baltic Sea, we split this data set according to the criteria used by Kowalczuk (1999).

Temporal and spatial changes of CDOM optical properties generally followed the well-established seasonal pattern described in previous work (e.g. Kowalczuk, 1999, Kowalczuk et al., 2005), although small deviations exist. The lowest values of $a_{CDOM}(375)$ were recorded in open seawaters, both in autumn–winter (average $a_{CDOM}(375)=0.81\ m^{-1}$) and spring–winter (average $a_{CDOM}(375)=0.84\ m^{-1}$).

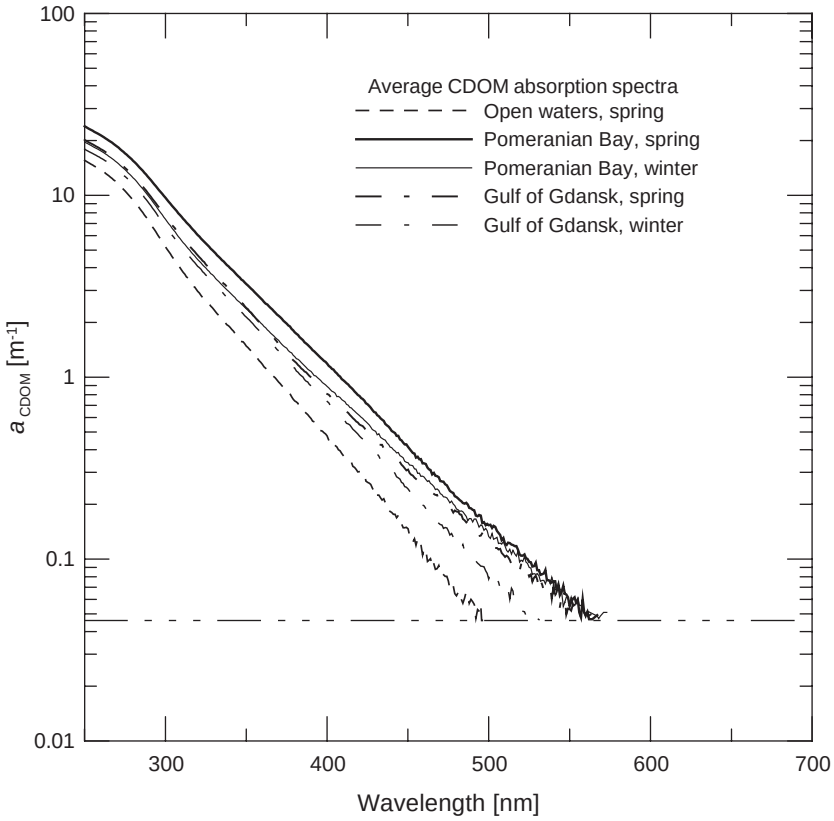

Fig. 2. Average CDOM absorption spectra in the study area for specific season and regions. Detection limit for the absorption coefficient measurement is marked as the dashed horizontal line.

Highest values were observed in Pomeranian Bay and the Gulf of Gdansk. The averaged value of absorption coefficient $a_{CDOM}(375)$ in autumn–winter (average $a_{CDOM}(375)=1.40$ m^{-1}, Pomeranian Bay; average $a_{CDOM}(375)=1.22$ m^{-1}, Gulf of Gdansk) was lower than in spring (average $a_{CDOM}(375)=1.95$ m^{-1}, Pomeranian Bay; average $a_{CDOM}(375)=1.33$ m^{-1}, Gulf of Gdansk). Some deviation in general trends were also observed in Pomeranian Bay and the Gulf of Gdansk. CDOM absorption in Pomeranian Bay was higher than in the Gulf of Gdansk, although long-term observational data suggest the reverse pattern. This observation may be explained by a small shift in sampling time in the regions. Observations in the Gulf of Gdansk were at the beginning of April 2003, just before peak Vistula River discharge. Observations in Pomeranian Bay were in mid May 2002 at the peak Świna River discharge.

During the course of this study, spatial and temporal distribution of the CDOM absorption spectral slope coefficient, S, in both spectral ranges, followed general trends observed in the Baltic Sea over the last 10 years of observations. Highest values of the slope coefficients were recorded in the open sea (average $S_{300–650}=0.0246$ nm^{-1} in autumn–winter and spring) and their values gradually decreased towards the embayments. Lowest values of S were recorded in Pomeranian Bay in autumn–winter (average $S_{300–650}=0.0197$ nm^{-1}). A general trend of increasing S with decreasing absorption was also observed in this data subset. In most cases, values of S in the autumn–winter samples were higher than in the spring in each region. When spectral and methodological corrections were applied to the present data set, CDOM absorption and S were usually within the seasonal variability range described by Kowalczuk (1999). Values of the slope coefficient, S, calculated over the extended spectral range (300–650 nm) were generally higher than values of S, calculated in the shorter spectral range. The differences in S values calculated using the two spectral ranges result from more weight being given to the shorter wavelengths, where CDOM absorption dominates the absorbance signal.

Two examples of CDOM fluorescence are shown in Figs. 3 and 4. These figures show EEM spectra of the two sites that characterize end members in our data set. The low-salinity (salinity=2.21) end member is represented by the sample collected in Pomeranian Bay in the vicinity of the Świna River mouth, 12 May 2002 ($a_{CDOM}(375)=3.31$ m^{-1}, $S=0.0199$ nm^{-1}), and the open sea high-salinity (relative to Baltic Sea hydrological conditions) end member (salinity=7.36) was a sample collected in the southwestern part of Bornholm Basin on February 9, 2003 ($a_{CDOM}(375)=0.83$ m^{-1}, $S=0.0212$ nm^{-1}). There was about a three-fold difference in fluorescence intensity of the major peak, A, which represents terrestrial humic substances, between these two stations. In the low-salinity environment, this peak is distinct and its shoulders extend into the T peak area. The C peak, which is attributed to terrestrial fulvic substances, was also clearly distinguishable on the Pomeranian Bay spectrum. In addition to the A and C peaks, the T peak, typically associated with the proteinaceous matter (Coble, 1996), was clearly visible in the open seawater EEM spectrum. The T peak intensity was proportionally higher than in the low-salinity end member (Fig. 3). All of the open seawater EEM spectrum peaks were reduced in their intensities, compared to those of the low-salinity end member.

Visual inspection of all EEM spectra from low-salinity water to the open seawater end members showed that, with decreasing absorption coefficients and fluorescence intensities, the relative T peak contribution to a whole EEM spectrum increased. Integration of EEM spectra enabled us to calculate a percent contribution of respective peaks to the total fluorescence intensity. The calculated ratio was a simple descriptor of the EEM spectrum, similar to the fluorescence index proposed by McKnight et al. (2001), and it may be used to quantify and characterize CDOM samples taken from different locations. For example, peak intensity to total EEM intensity ratios calculated for a sample with the terrestrially dominated CDOM were: A/TOT=18.1%, C/TOT=6.3%, M/TOT=7.8% and T/TOT=2.6%. In contrast, ratios calculated for an open seawater sample with less terrestrial influence were as follows: A/TOT=17.7%, C/TOT=5.3%, M/TOT=7.6% and T/TOT=5.1%. In the open sea, percent contribution of the A and M peaks varied little compared to the low-salinity end member, the C peak contribution dropped by 20% and T peak contribution nearly doubled. The numerical parameters used to characterize EEM

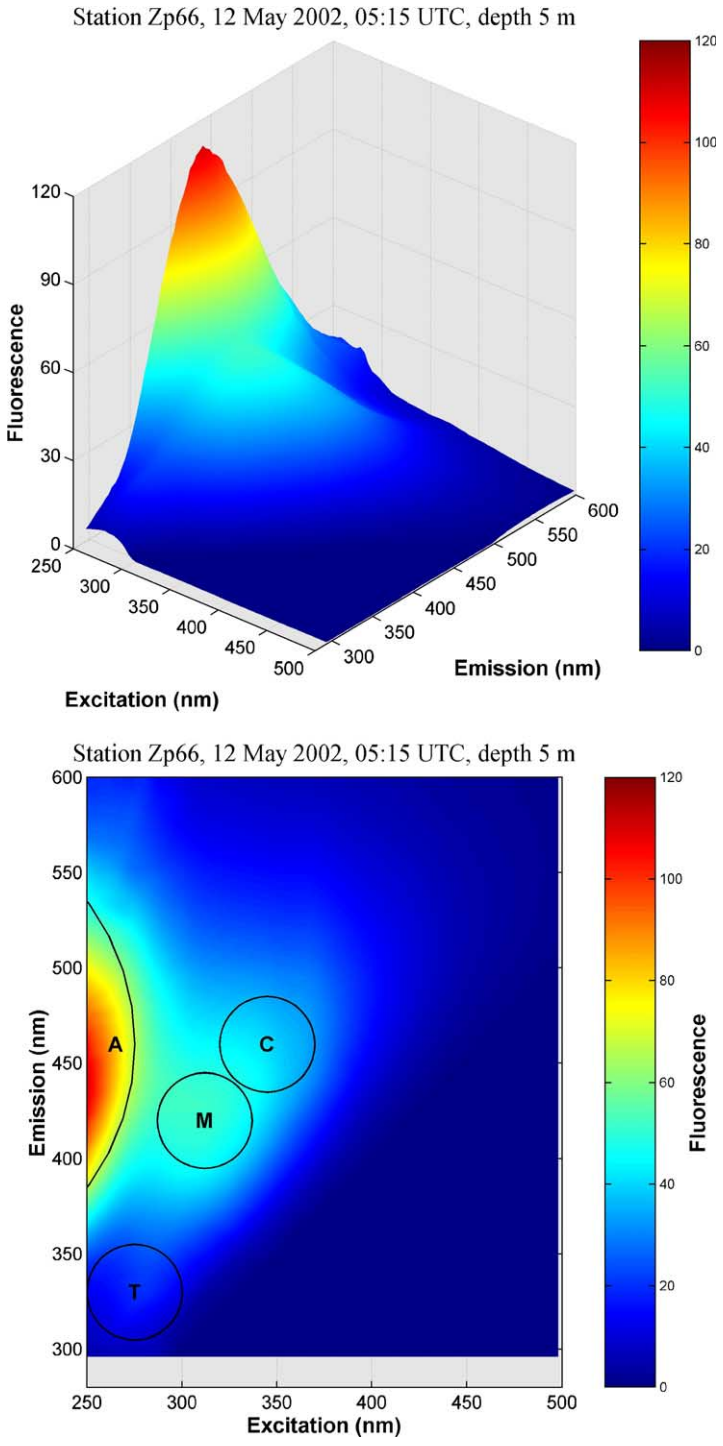

Fig. 3. The EEM spectra of a sample representative of the low-salinity end member in the data set. Sample taken at the vicinity of Świna River outlet, Pomeranian Bay. The *A*, *C*, *M* and *T* show locations of the respective fluorescence peaks according to Coble, 1986. The circles centered at peak maxima show the integration cross-section area.

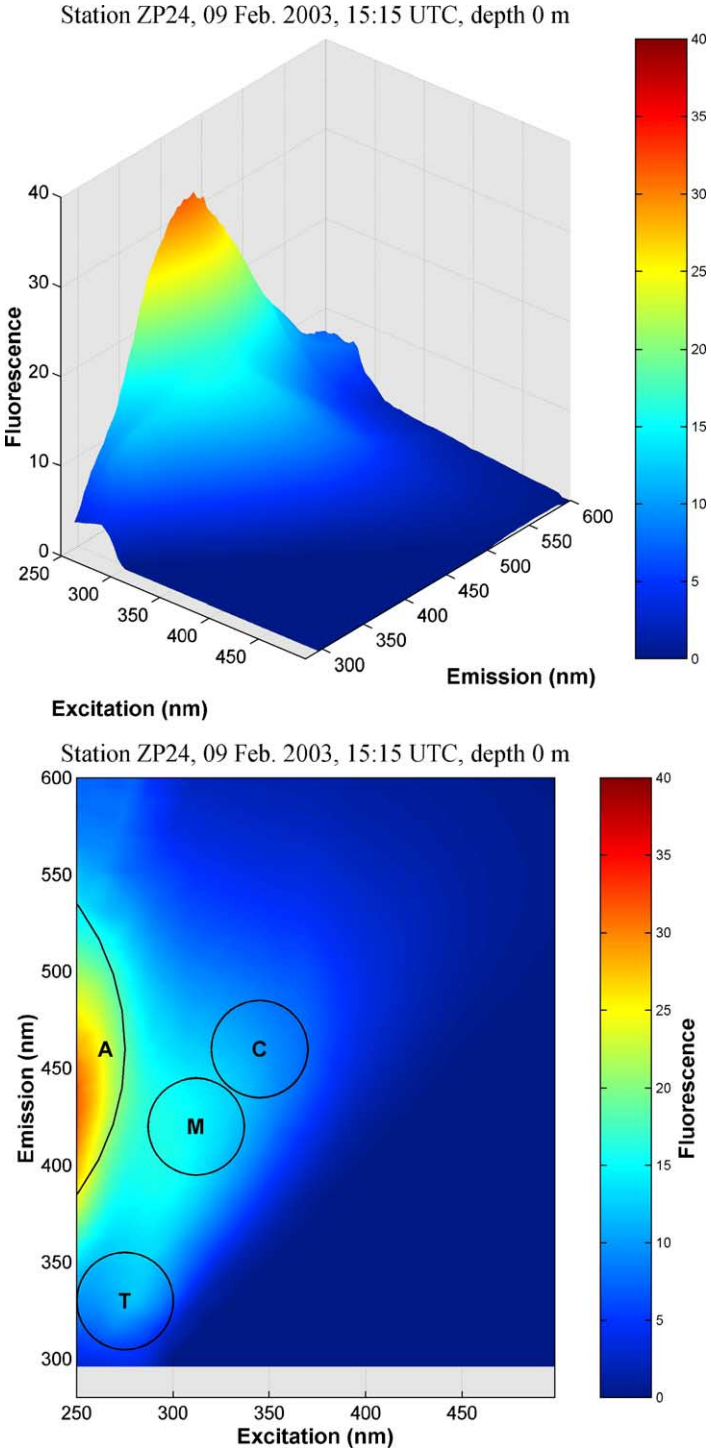

Fig. 4. The EEM spectra of a sample representative of the marine water end member. Sample of water from the open Baltic Sea (location, date and time on the graphs). The *A*, *C*, *M* and *T* show locations of the respective fluorescence peaks according to Coble, 1986. The circles centered at peak maxima show the integration cross-section area.

spectra may be applied in simple statistical regression analysis to observe changes in fluorescence ratios along the salinity gradient from the terrestrial source to the marine environment. The values of the *T*/TOT ratio may be a useful index to differentiate between fresh, terrestrially derived CDOM from one which is present in the marine environment for a longer time.

3.2. Empirical relationships between absorption, fluorescence and salinity

Dilution by mixing with the salt-water end member is the principal process leading to a decrease of CDOM absorption in the estuary. The overall negative relationship between CDOM absorption and salinity is well known; for this data set, the regression equation was $a_{CDOM}(375) = 5.056 - 0.57 * \text{salinity}$, with $r = -0.94$ and $n = 61$. Because CDOM absorption was negatively correlated with salinity, we expected similar results relating the respective peak intensities and the total EEM spectral intensity and salinity, because fluorescence can be regarded as a proxy for CDOM absorption. In other analyses, we intended to use CDOM absorption as an indicator of salinity; consequently, high CDOM absorption level would represent low-salinity freshwater influenced water and low CDOM absorption level would represent high-salinity (relative to local Baltic Sea conditions) marine waters.

Regression analyses verified the relationship between CDOM absorption and CDOM fluorescence (Fig. 5). We used the respective peak integrals as a measure of fluorescence intensity and plotted them against the absorption coefficient $a_{CDOM}(375)$ (Fig. 5). The regression equation and correlation coefficients are given in Table 1. The $\log_{10}$ of fluorescence intensities vs. $\log_{10}$ of absorption coefficients were, in most cases, linearly related with correlation coefficients with $r > 0.9$. All correlation coefficients were significant at $p < 0.05$. The best fit was obtained between $a_{CDOM}(375)$ and *A* and *C* peak integrals and total EEM integral. The *T* peak was the least correlated with absorption because its excitation and emission are located at wavelengths shorter than 375 nm, but it was still statistically significant.

One of the advantages of the EEM technique is the ability to determine qualitative changes in CDOM composition. Relative changes in intensities of specific peaks may reflect processes that lead to changes

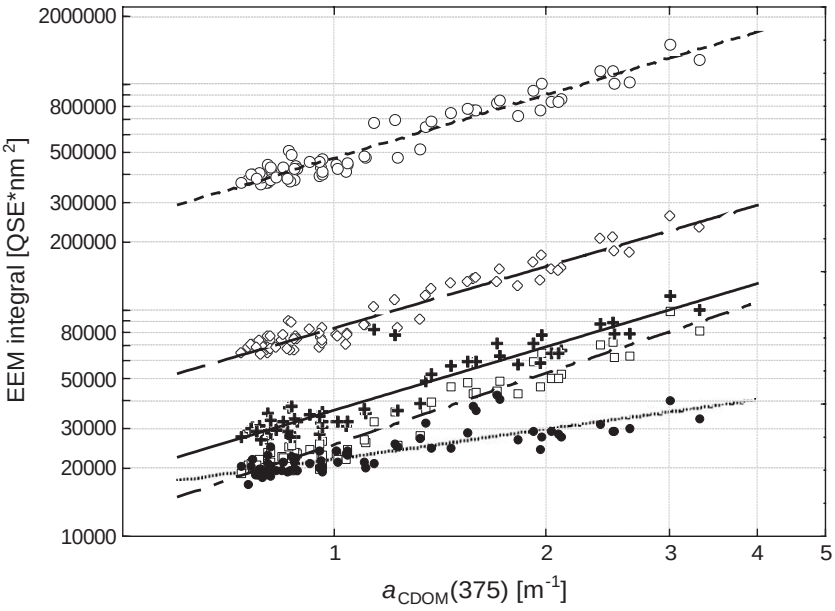

Fig. 5. Relationships (linear regression in log–log scale) between respective peak fluorescence intensities (expressed as the peaks integrals) and CDOM absorption coefficient at $\lambda = 375$ nm, $a_{CDOM}(375)$: total EEM scan integral (circles, dashed line), *A* peak integral (diamonds, long dashed line), *M* peak (crosses, solid line), *C* peak (squares, dash-dotted line) and *T* peak (dots, dotted line).

Table 1
Results of regression analysis between CDOM absorption and respective fluorescence peak intensities

Variables	Equation	Correlation coefficient	Sample size
$a_{CDOM}(375)$ vs. EEM total intensity	$EEM_{TOT} = 10^{(5.67+0.903(\log_{10}(x)))}$	$r=0.97$	$n=67$
$a_{CDOM}(375)$ vs. A peak intensity	$A_{peak} = 10^{(4.492+0.90(\log_{10}(x)))}$	$r=0.97$	$n=67$
$a_{CDOM}(375)$ vs. C peak intensity	$C_{peak} = 10^{(4.408+1.044(\log_{10}(x)))}$	$r=0.97$	$n=67$
$a_{CDOM}(375)$ vs. M peak intensity	$M_{peak} = 10^{(4.559+0.93(\log_{10}(x)))}$	$r=0.93$	$n=67$
$a_{CDOM}(375)$ vs. T peak intensity	$T_{peak} = 10^{(4.347+0.428(\log_{10}(x)))}$	$r=0.81$	$n=67$

All correlation coefficients are statistically significant at the significance level $p<0.05$.
$x=a_{CDOM}(375)$.

in composition of CDOM. Calculated as described above, the percent contribution of the respective peak intensities to the total EEM intensity was related to the CDOM absorption coefficient $a_{CDOM}(375)$ (Fig. 6). Two of the four peaks, A and M, did not show any significant change along the a_{CDOM} gradient. The T peak decreased, while the C peak increased with increasing a_{CDOM}. This graph clearly shows a relative increase of T peak contribution to the total fluorescence in open seawater. The correlation coefficients and regression equations are given in Table 2. The statistical approximation of these relationships between the respective peaks (in percent) to the total EEM spectral intensity vs. CDOM absorption worked reasonably well for the C and T peaks. Regression curves calculated for A and M peaks did not show any

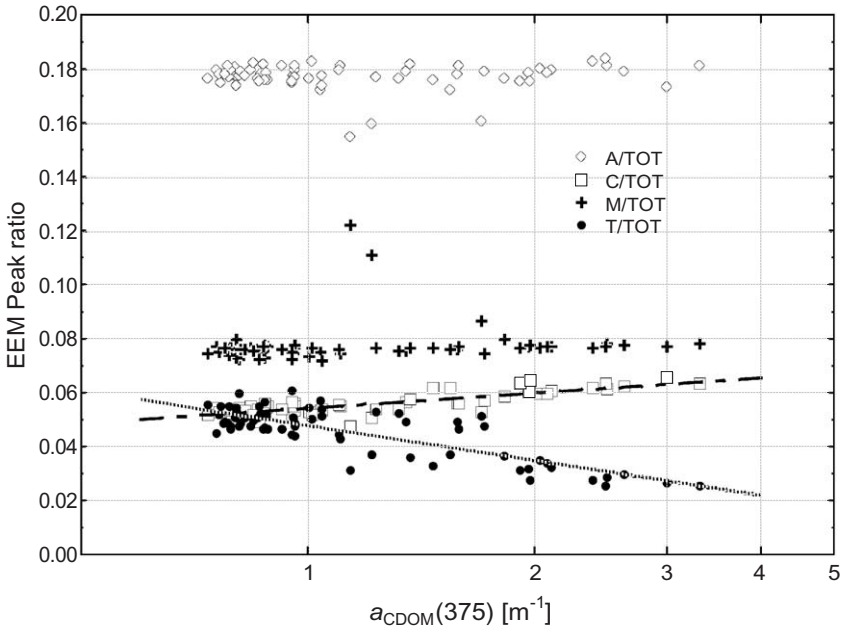

Fig. 6. Distribution of ratios of respective peak intensities to total EEM intensity in the function of CDOM absorption coefficient $a_{CDOM}(375)$: A/TOT (diamonds), M/TOT (crosses), C/TOT (squares, dash-dotted line) and T/TOT (dots, dotted line). Relationship (linear regression in semi-log scale) between C/TOT vs. $a_{CDOM}(375)$ is shown as dashed line and T/TOT vs. $a_{CDOM}(375)$ is shown as dotted line.

Table 2
Results of regression analysis between CDOM absorption and percent contribution of respective fluorescence peak intensities to the total EEM spectral intensity

Variables	Equation	Correlation coefficient	Sample size
$a_{CDOM}(375)$ vs. A/TOT	$A/\mathrm{TOT} = 0.171 + 0.0003(\log_{10}(x))$	$r=0.01$	$n=67$
$a_{CDOM}(375)$ vs. C/TOT	$C/\mathrm{TOT} = 0.0542 + 0.0187(\log_{10}(x))$	$\mathbf{r=0.83}$	$n=67$
$a_{CDOM}(375)$ vs. M/TOT	$M/\mathrm{TOT} = 0.0769 + 0.05(\log_{10}(x))$	$r=0.12$	$n=67$
$a_{CDOM}(375)$ vs. T/TOT	$T/\mathrm{TOT} = 0.478 - 0.0428(\log_{10}(x))$	$\mathbf{r=-0.81}$	$n=67$

Correlation coefficients statistically significant at the significance level $p<0.05$ given in bold face.
$x=a_{CDOM}(375)$; A, C, M, T—respective fluorescence peak intensity; TOT—the total EEM intensity.

statistically significant correlation. It was noted that the CDOM fractions with excitation wavelengths in the UV-B region, below 300 nm, did not change along the absorption (salinity) gradient. The relative decrease in fluorophores with excitation bands in the UV-A region (C peak) suggests that lower molecular weight terrestrial humic substances may be a source of the protein-like fraction of CDOM.

To examine possible sources for the increased presence of the protein-like fluorophores (T) in open seawaters we related the respective peak intensities and respective peak ratios to total EEM spectral intensity. The absolute values of respective peak intensities were linearly related with each other and correlation coefficients were always greater than 0.98 (data not shown). The relationship for each peak-percent contribution to the total EEM spectra was established. However, we have only shown the T peak contribution to total EEM intensity, relative to the three other peak ratios (Fig. 7). The scatter plot and

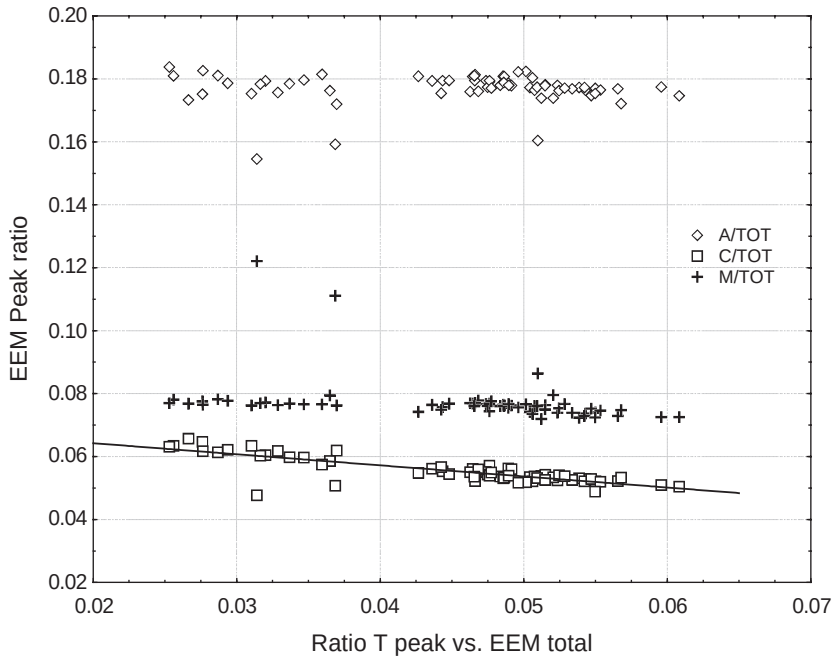

Fig. 7. Distribution of ratios of respective CDOM peak intensities to total EEM intensity vs. ratio of T peak intensity to total EEM intensity. Solid line represents the linear regression between C/TOT and T/TOT.

the fitted linear relationships described above show a trend of increased *T* peak contribution with a decrease in *C* peak contribution. The regression equation is as follows: $T/\mathrm{TOT} = 0.0713 - 0.352 * \mathrm{C/TOT}$, and the correlation coefficient calculated for this relationship was $r = -0.82$ and $n = 67$. The *T* peak contribution was much better correlated with peaks associated with terrestrial fulvic substances (*C* peak) than with other CDOM constituents. There was no correlation between *T* peak percent contribution and either the *A* or *M* peak. These data suggest that the *T* peak may arise from processes that modify the terrestrial fulvic fraction and not the high molecular weight terrestrial humic fraction of CDOM.

Twardowski et al. (2004) stated that a high proportion of the variability in values of the spectral slope coefficient is caused by inaccuracy of estimation of this parameter. Changes in values of the spectral slope coefficient may be regarded as an indicator of compositional changes in CDOM, if this source of error is reduced and effects associated with conservative mixing filter-out. Variability in the spectral slope coefficients reflects CDOM changes resulting from production, removal and mixing of different water masses characterized by contrasting optical properties of CDOM. Identification of a theoretical mixing line would make it possible to recognize non-conservative processes acting on the color fraction of the dissolved organic matter during mixing (Stedmon and Markager, 2003). A plot of the spectral slope coefficient in relation to $a_{\mathrm{CDOM}}(375)$ suggests a change in CDOM properties from terrestrial sources to more saline waters. The spectral slope coefficient calculated in two spectral ranges, the short (350–550 nm) and extended (300–650 nm), were plotted as a function of absorption coefficient (Fig. 8). In both cases, the distribution of data points showed a clear trend of increased slope coefficient with a decrease in absorption level. However, there is considerable scatter in the distribution of $S_{350-550}$, caused mostly by a lesser degree of accuracy in spectral slope estimation. Calculated correlation coefficients for the regression between $S_{350-550}$ and $a_{\mathrm{CDOM}}(375)$ were low, although statistically significant, and the regression equation could not be applied as a functional relationship (data not shown). $S_{300-650}$ data points are quite evenly distributed over the modeled regression line. There is a significant non-linear relationship, between $S_{300-650}$

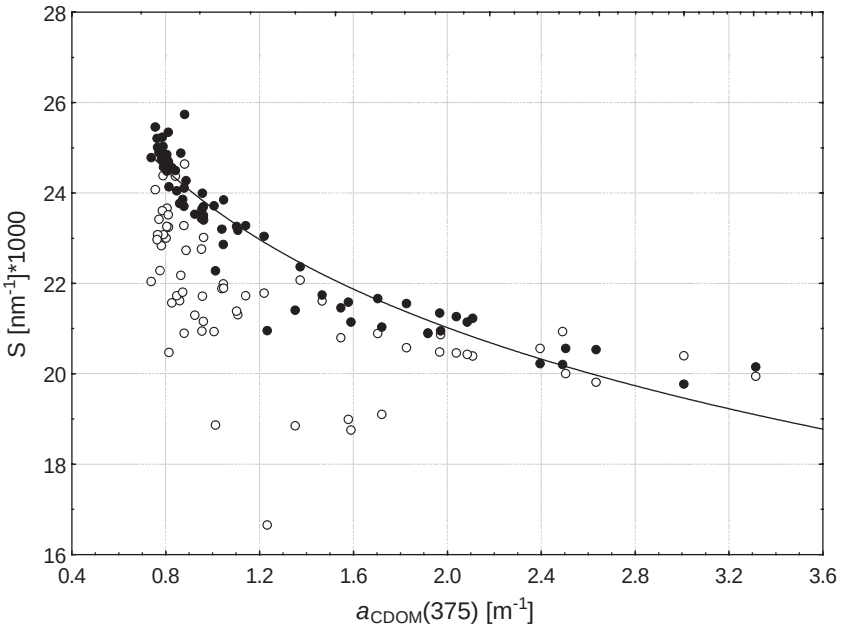

Fig. 8. Distribution of the spectral slope coefficient *S* calculated in the 350–550 nm spectral range (open circles) and the spectral slope coefficient *S* calculated in the 300–650 nm spectral range (dots) in the function of $a_{\mathrm{CDOM}}(375)$. Solid line represents non-linear regression (logarithmic fit) between $S_{300-650}$ and $a_{\mathrm{CDOM}}(375)$. Values of the spectral slope coefficient multiplied by 1000.

and $a_{CDOM}(375)$ (Fig. 8). The non-linear regression equation was $S_{300-650} = 23.667 - 8.79 * \log_{10}(x)$, where x is the $a_{CDOM}(375)$. The correlation coefficient for this relationship was $r = -0.95$, $n = 65$, $p < 0.05$. There was no significant seasonal dependency in the regression between spectral slope and the absorption coefficient; therefore, this relationship may be applied for modeling of optical properties of CDOM in the Baltic Sea.

Application of the relationship between spectral slope coefficient and absorption coefficient to differentiate between terrestrially derived CDOM from marine source derived CDOM was not possible in this study. The data subset used was too small to derive a reliable mixing model, which could be superimposed on the empirical distribution of $S_{300-650}$ and $a_{CDOM}(375)$ values.

4. Discussion

The Baltic Sea is a unique region in which to study CDOM dynamics. A well-recognized hydrological regime, significant seasonal changes typical for marine basins in the temperate zone and a long period of systematic hydrological, meteorological and oceanographic observations enable better interpretation of field data. Data collected in the Baltic cover a high proportion of the variability in $a_{CDOM}(\lambda)$ found in the world's ocean (e.g. Højerslev, 1988; Blough and Del Vecchio, 2002 and references therein). Although the observations presented in this study add little new information about seasonal cycling of CDOM optical properties in the Baltic Sea, when compared to the much larger data set analyzed by Kowalczuk (1999) and Kowalczuk et al. (2005), we have included a statistical description of CDOM optical properties, categorized and analyzed in the same way as in earlier studies. This analysis showed that this relatively small data set is representative of local, regional and seasonal conditions, and may be used as a good approximation of trend for the relationship between fluorescence intensities and salinity.

Calculation of the spectral slope coefficient over the extended spectral shape was much more accurate and enabled us to establish a very good statistical relationship between $S_{300-650}$ and $a_{CDOM}(375)$. This relationship had a high correlation coefficient and does not show any significant seasonal dependency. A relationship between spectral slope and the CDOM absorption coefficient in the Baltic Sea was published by Kowalczuk et al. (2005); however, the regression between those two variables was characterized by significantly lower correlation coefficient (0.61) and had a seasonal effect. The relationships presented in this study, although calculated for a limited sample size, enable significantly better formulae for modeling spectral properties of CDOM. The shape of a_{CDOM} vs. S presented in this study is similar to the curve presented by Stedmon and Markager (2001) in the Greenland Sea and the modeled mixing line presented by Stedmon and Markager (2003), and this curve may potentially be useful for differentiation of different CDOM pools. However, it is necessary to discriminate changes in the spectral slope coefficient that result from conservative mixing of two water masses with distinctly different optical properties of CDOM from those change that result from non-conservative production and degradation of CDOM, which influence optical properties of CDOM. This task may be achieved by superimposing modeled variation of S as a function of a_{CDOM} (conservative mixing model) on the empirical distribution of data points. Data points that significantly deviate from a conservative mixing line may have optical properties shaped by non-conservative processes. A further study of this relationship is needed, because the accuracy of a conservative mixing model depends on good characterization of optical properties of CDOM from contrasting water masses.

The percent contribution of fluorescence peaks to the total EEM intensity is a simple quantitative descriptor of the fluorescence properties of CDOM samples collected in different locations. This procedure also enables a statistical comparison of different fluorescence spectra. These quantitative indexes were applied by De Souza-Sierra et al. (1994) to observe compositional changes or as descriptors of the source of the organic matter (McKnight et al., 2001). The EEM spectra for samples representative of terrestrially derived CDOM were characterized by following peak ratios: A/TOT ≈ 18%, C/TOT ≈ 6%, M/TOT ≈ 7.8% and T/TOT ≈ 2.6%. The open seawater CDOM sample had the following EEM characteristics: A/TOT and M/TOT peak ratios values remain unchanged, compared to the riverine plume sample,

but *C*/TOT dropped to ca. 5% and *T*/TOT increased to over 5%. Percent contribution of fluorescence peaks to the total fluorescence intensity calculated for coastal waters at the continental shelf of southeastern United States have similar estuarine-to-oceanic tendencies of increased contribution of fluorophores associated with proteinaceous matter. The *T* peak contribution increased by factor of 4 from 2.5% to more than 10% from estuarine to oceanic waters (Kowalczuk et al., 2003). Therefore, the *T*/TOT ratio may be used as simple indicator to discriminate from fresh, terrestrially derived CDOM from one present in coastal and oceanic waters. An increase in the contribution of fluorophores excited at lower wavelengths in other low-to-high-salinity transition regions has been reported, in a transect from estuaries to the coastal Atlantic Ocean in Europe (De Souza-Sierra et al., 1994; De Souza-Sierra et al., 1997), the Orinoco River Plume (Del Castillo et al., 1999) and in the West Florida shelf (Del Castillo et al., 2000). This increasing importance of fluorophores excited at lower wavelength results in a significant blue shift of the emission maximum of CDOM excited at around 310 and 350 nm. Previously, this low-to-high-salinity relationship was established between CDOM absorption at the excitation wavelength and fluorescence intensity peak (Ferrari and Tassan, 1991; Hoge et al., 1993; Green and Blough, 1994; Vodacek et al., 1995; Vodacek et al., 1997; Ferrari et al., 1996; Ferrari and Dowell, 1998; Ferrari, 2000; Chen et al., 2002). The high correlation between these two parameters enables the use of fluorescence as a proxy for CDOM absorption. This approach is especially useful in clear waters with low CDOM concentrations because fluorescence is much more sensitive than absorption, making it possible to measure CDOM with enhanced accuracy. The studies cited above presented relationships between absorption coefficient and fluorescence intensity of a single fluorescence peak excited at a chosen wavelength. In our approach, it is possible to link the absorption coefficient with a global fluorescence intensity as well as with the fluorescence intensity of selected CDOM fractions. This approach may also be used over a very broad range of CDOM absorption coefficients, as shown by Kowalczuk et al. (2003) in the South Atlantic Bight. Within a fairly small area at the vicinity of the Cape Fear River mouth in Long Bay and in Onslow Bay, it is possible to measure the global variability range of CDOM absorption coefficients. Fig. 9 presents the relationships between total EEM

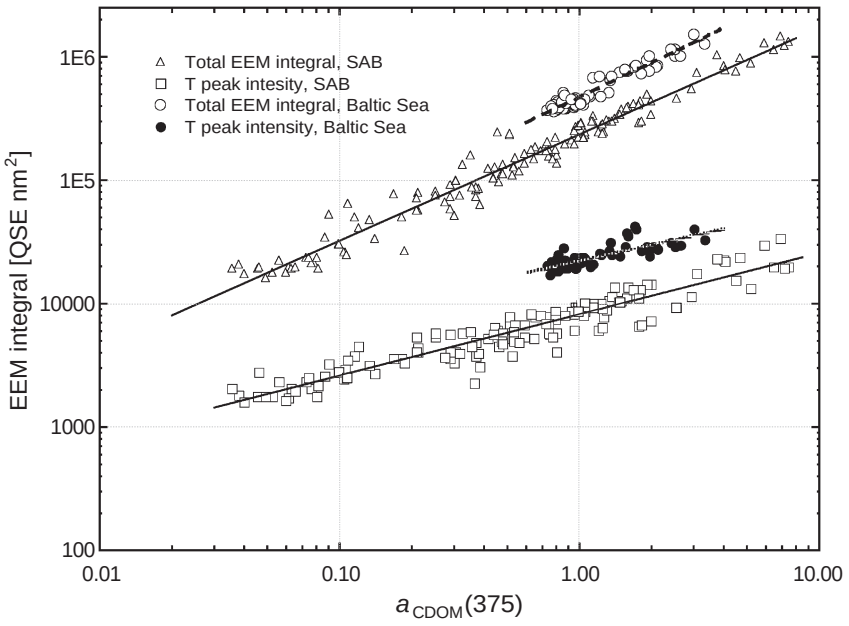

Fig. 9. Comparison between relationships (linear regression in log–log scale) of total integrated EEM intensity and *T* peak intensity vs. $a_{CDOM}(375)$ in the Cape Fear River plume area, South Atlantic Bight and Baltic Sea.

intensity, *T* peak intensity and CDOM absorption coefficients in South Atlantic Bight and Baltic Sea. Total fluorescence intensity or *T* peak intensity correlates well with the absorption coefficients (375 nm) in both geographic regions. The main difference between these two locations is associated with fluorescence efficiency. Chromophoric dissolved organic matter characterized by the same value of absorption coefficient produces higher fluorescence in the Baltic Sea than in South Atlantic Bight. However, this feature must be verified by studies on apparent florescence efficiency changes in both locations.

There are few reports that explain changes in EEM spectra. The systematic decrease of the three principal peaks, *A*, *M* and *C*, with decreasing absorption may reflect changes in CDOM concentration with relatively minor changes in composition during transport from the estuarine environment to oceanic waters. However, the relative increase of the *T* peak, which appears to relate to the fluorophores in proteins, with increased salinity and decreased absorption indicates that this fraction of CDOM may cycle differently than other CDOM constituents. Kowalczuk et al. (2003) recently reported a similar result in the South Atlantic Bight. Also, Stedmon et al. (2003) observed a relative increase in the proportion of fluorophores that could be attributed to fluorescent proteins in a fjord on the east coast of Jutland, Denmark.

New evidence of non-conservative behavior of the "protein-like" fluorophores has been reported by Vignudelli et al. (2004) in the Tyrrhenian Sea in the Mediterranean. Indirect corroboration of our findings also comes from the study by Schwarz et al. (2002) conducted in the Baltic Sea. Their report presented the application of a Gaussian fitting routine to deconvolute CDOM absorption spectra into a series of Gaussian curves. In the Bay of Gdansk, Baltic Sea, one of those curves, centered around 260 nm, exhibited a significant positive trend with salinity. Thus, the fraction of CDOM absorption at that wavelength becomes more prominent. Coincidentally, 260 nm is the excitation band of fluorescent proteins. Although the possible pathways of CDOM compositional changes are still little understood, there are several non-mutually exclusive explanations for the patterns in our data. (1) Protein-like fluorophores in our samples are a recalcitrant fraction of CDOM. (2) Protein-like fluorophores in our samples are a breakdown product of terrestrial CDOM. (3) Protein-like fluorophores are formed in marine environments.

The first possibility may be explained by the fact that under natural conditions there is no light available to directly bleach protein-like fluorophores, which absorbs light below 300 nm. This explanation is supported by the findings of Del Castillo et al. (1999) who reported relative bleaching resistance of fluorophores that absorb light below 300 nm. Therefore, only indirect bleaching processes are involved in their decomposition. Indirect bleaching processes that may involve reactions with reactive-oxygen species or other radicals may be much less efficient than direct bleaching (e.g. Cooper et al., 1989; Del Vecchio and Blough, 2002; Holder-Sandvik et al., 2000). Recent studies by Lepane et al. (2003) showed that, during a laboratory photobleaching experiment, using UV-B radiation, CDOM decomposed into lower molecular weight compounds, with a significant loss of absorption and fluorescence properties. This experiment also showed that extracted and concentrated fulvic and humic acids were more resistant towards UV-B radiation than samples of natural waters collected in the Baltic proper. Although more specifically designed laboratory experiments are needed to explore the possibility of protein-like fluorophore formation during decomposition of terrestrial CDOM, the second possibility does not seem likely since we did not observe an increase in absolute values of *T* but rather a relative increase. Likewise, the same reasoning seems to rule out, or at least limit, the third possibility as well. Therefore, the significant changes observed in our optical data may reflect dilution of the freshwater mass into the open seawaters, where *T* fluorophores are the dominant moieties. The importance of mixing alone has been examined by De Souza-Sierra et al. (1997) and Stedmon and Markager (2003). The exact mechanisms by which *T* peak percent contribution increases are yet to be resolved.

5. Conclusions

The Baltic Sea is characterized by strong seasonality in hydrological and physical processes, which strongly influence CDOM dynamics. Our data showed that there was a significant statistical relationship

between CDOM absorption and CDOM fluorescence intensity in log–log scale. The empirical relationship between those two parameters may be used for modeling CDOM absorption using fluorescence and also to predict the global intensity of the fluorescence as well as the intensity of selected CDOM fractions in the Baltic Sea. Based on our field data and experience in using EEM fluorescent analytical techniques in other marine basins as well as the results of other studies, we can address some preliminary conclusions. Application of the EEM technique allows us to trace different fractions of the CDOM pool in aquatic environments. Analyses of EEM spectra indicated that three of four principal fluorescence peaks and total integrated fluorescence decreased in a similar fashion with decreasing absorption. In contrast, the protein-like fraction of CDOM (*T* peak) decreases to a lesser degree than the other three fractions. Analysis of the percent contribution of fluorescence peak intensities to the total fluorescence along a salinity gradient showed that the contribution of the protein-like fluorophores fraction of the CDOM increases significantly in marine environment. The proportion of fluorophores with excitation bands below 300 nm does not change along the salinity gradient. The statistical significance of the empirical relationships between percent contribution of respective EEM peaks to the total EEM intensity suggests that those relationships may be used for modeling of CDOM dynamics in the absorption/salinity gradient in the Baltic Sea. The *T*/TOT ratio may be used as simple indicator to discriminate from fresh, terrestrially derived CDOM from one present in coastal and oceanic waters.

Further study of the behavior and distribution of each fluorescent component is needed to gain a better understanding of CDOM dynamics and processes leading to changes in CDOM composition. Additional studies, at sampling stations where depth profiles are obtained and EEM spectral data determined, should provide insight into the processes that are influencing CDOM as it is transported from the riverine sources to open seawater.

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