

TOOLS FOR ASSESSING CONTENT, SPECIATION AND ORIGIN OF DOM IN AQUATIC SYSTEMS

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ABSTRACT

Dissolved organic matter (DOM) is a major component of the carbon-pool in aquatic systems and thus represents an important pathway on the carbon cycle, especially in marine environments. For instance, studies have used DOM to assess drinking water quality, its importance on biogeochemical cycles, its usefulness as an environmental tracer, etc. This article discusses some of the current methods used to assess the amount and composition of DOM, and applications of DOM as an environmental tracer in aquatic systems. Different techniques varying from molecular, optical, and chemical analyses to satellite remote sensing have been employed to identify, characterize and quantify DOM and to assess its distribution, composition and dynamics in distinct aquatic environments. Those approaches however, focus only on specific fractions of the total DOM-pool. Hence, recent studies have attempted to link the results provided by such complimentary methods to reach a more comprehensive understanding on the total DOM-pool in aquatic systems. Additionally, DOM spectroscopic measurements (e.g. absorbance and fluorescence) are cost-effective tools and can be sampled with high resolution by autonomous devices such as fluorimeters. Furthermore, the optical properties of DOM have been shown to be reliable proxies for monitoring water quality and for tracing fresh water along the Arctic Ocean. With the climate change pressure on Arctic environments and the expected increase in fresh water and ancient carbon export from the continent to the ocean, optical analyses of DOM can be an easy-to-measure and affordable parameters for assessing and monitoring these effects in the Arctic environment.

KEYWORDS: DISSOLVED ORGANIC MATTER, CDOM, FDOM, OPTICAL INDICES, ENVIRONMENTAL ASSESSMENT

1. Introduction

With the climate change pressure on the environment, the scientific community has sought a more comprehensive understanding on the carbon cycle, its reservoirs and the processes governing their dynamics. Aquatic systems play an important role for the carbon cycle, for instance, as provision of a large pool of carbon, important sink and turnover of organic carbon. As a consequence, increasing effort has been devoted to study aquatic environments and processes governing the dynamics of both, organic and inorganic carbon. With more studies on the characterization of stocks, sources, dynamics, and fate of

both organic and inorganic carbon, a more comprehensive understanding of the carbon cycle would be reached, which is of great importance for improving forecasts of future climate scenarios.

Non-living organic matter is present in aquatic systems as particles, colloids, and dissolved molecules. The dissolved fraction of organic matter (DOM) is operationally defined by filtration with specific pore size, with 0.45 μm being the most accepted limit [Steinberg, 2003]. The DOM fraction encompasses a wide range of organic compounds with variable molecular complexity

and is one of the largest carbon-pools on Earth [Hedges, 1992]. The most frequently observed compounds in the DOM-pool are amino acids, carbohydrates, lipids, pigments, lignins, tannins, and proteins, whose relative contribution varies in different environments, in relation to its origin. For instance, terrestrially-derived humic acids derived from lignin, which is formed exclusively in vascular plants, contain large amounts of carbon in the form of aromatic carbons and phenols [Lebo et al., 2000]. Microbially-derived amino acids and proteins, on the other

hand, contain a low aromatic and phenolic content in relation to terrestrial sourced DOM [Geider and La Roche, 2002]. Given the huge variety in DOM composition and the wide panel of different approaches employed to analyze it, this article provides a brief review on some of the current methods applied to assess the amount and composition of DOM. Secondly, an overview on the applications of DOM properties as environmental tracers in aquatic systems is presented, with focus on the Arctic marine environment.

2. Quantitative and qualitative methods for DOM assessment

Different methods have been applied for performing chemical analyses of DOM. In general, those methods can be classified into two groups according to the preparation of the sample for analysis: the analyses involving purification and/or pre-concentration and the analyses performed in filtered original water (Figure 1). The purification and pre-concentration are employed to avoid interference of inorganic ions, which can affect highly to sensitive analyses. However, those methods can have analytical errors embedded in their analysis such as oxidation of only a part of DOM and increase in DOM concentration

during drying of samples [Bolan et al., 1996]. A wide range of methods have been developed using such techniques, for instance, solid phase extraction (SPE), ultrafiltration, nanofiltration, reverse osmosis, or electrodialysis. Although those methods have been widely applied in DOM studies, this article focuses on the approaches using filtered original water, which are here further divided into molecular and bulk analyses. The methods for DOM analysis using filtered original water can vary significantly with regards to the analytical procedure, including elemental analysis, isotopic analysis, chromatography, and mass spectrometry.

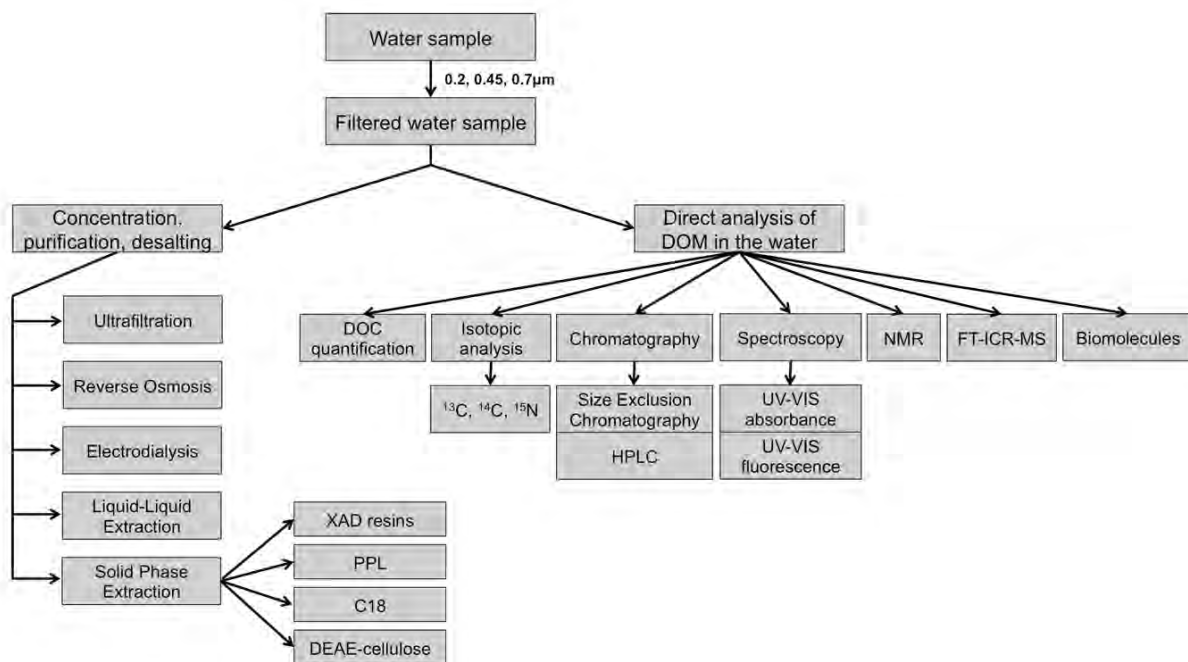


Figure 1. Strategies in the chemical analyses of DOM with respect to the pre-processing of samples [adapted from Dubinenkov, 2015].

2.1. Molecular analysis

By molecular analysis, this article refers to the quantification of an essentially pure type of organic compound or compound class. Two major groups of methods are applied in molecular analysis of DOM, the targeted and the non-targeted (Figure 2). Targeted methods are focused on analyzing specific organic compounds, which structure is well defined in the literature. In

DOM research, such molecules are usually referred to as biomarkers. Non-targeted methods are used to detect the analytical signal (or superposition of signals) from multiple molecular components with the DOM mixture. In addition, non-targeted methods can also be employed for the characterization of bulk DOM, since they can provide information on the carbon content in a water sample.

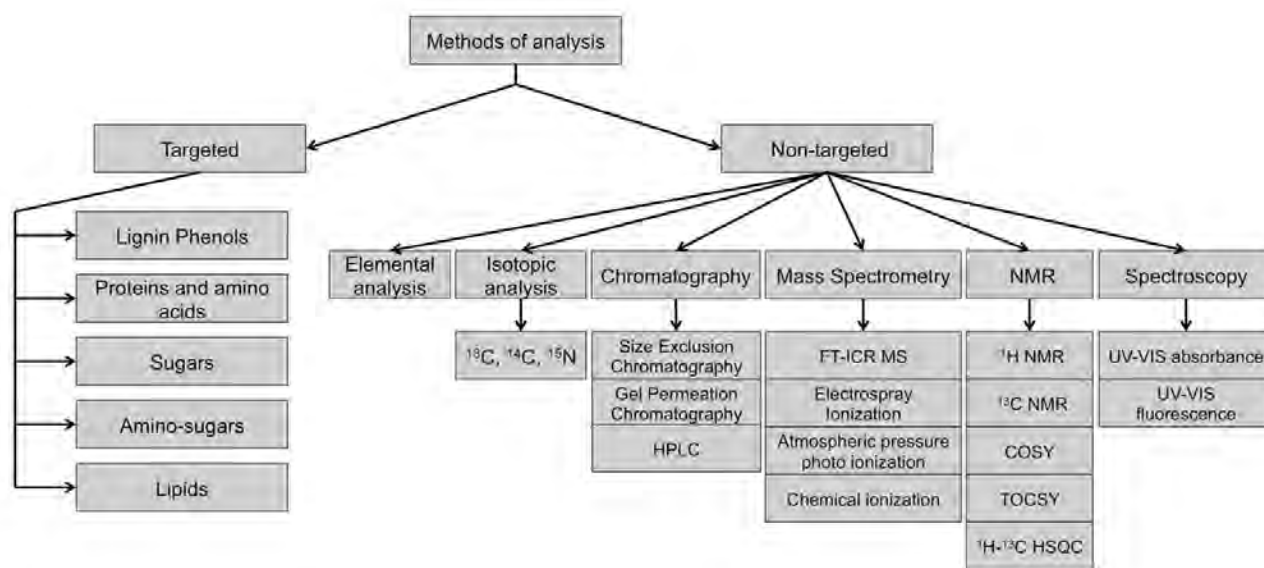


Figure 2. Strategies in the chemical analyses of DOM regarding the methods of analysis [adapted from Dubinenkov, 2015].

2.1.1. Targeted molecules

The analysis of targeted molecules consists of quantifying a specific compound based on its extraction from the water samples. Examples for targeted biomolecules are lignin phenols, proteins and amino acids, sugars, amino-sugars, and lipids. Such molecules can be analyzed by applying different techniques. For example, most proteinogenic

amino acids can be retained and separated by high-performance liquid chromatography (HPLC; Mopper and Lindroth, 1982) whereas some phenols can be separated using both gas chromatography [Benner and Opsahl, 2001] and HPLC.

2.1.2. Non-targeted molecules

Non-targeted approaches focus on the simultaneous detection of multiple molecular components. However, such methods can also provide bulk estimates of DOM. Among the several non-targeted methods for analysis of DOM, the nuclear magnetic resonance (NMR) and the Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) have been applied in many studies focusing on molecular characterization of DOM in the last decades.

Despite of the wide application of both methods, this subsection focuses on the FT-ICR MS method, given the significant increase in the number of studies using that method to chemically characterize DOM.

FT-ICR MS provides a detailed characterization of the diversity of molecular formulas contained in the analyzed DOM samples [Koch and Dittmar, 2006]. Three distinct ways are used to analyze DOM with mass spectrometry,

nevertheless all involving preliminary purification and concentration of samples: hyphenation with HPLC [Dittmar et al., 2007], direct injection of samples extracts [Kim et al., 2003] and chromatographic fractions [Koch et al., 2008]. The FT-ICR MS determines the mass-to-charge ratio (m/z) of ions based on their cyclotron frequency in a fixed magnetic field [Marshall and Hendrickson, 2002]. Given the high accuracy and sensitivity of the method, thousands of different mass peaks of DOM can be resolved

2.2. Bulk analyses

Bulk analyses to quantify and characterize DOM samples can be subdivided into elemental, isotopic, and spectroscopic methods. The most common quantitative representation of DOM in natural waters is dissolved organic carbon (DOC) concentration. It is usually quantified via high temperature catalytic oxidation to CO₂ [Sugimura and Suzuki, 1988]. Carbon isotopic measurements (e.g. ¹³C and ¹⁴C) can also provide information on bulk DOM in aquatic systems. Furthermore, such measurements can provide information on both mass and age of DOM [Williams and Druffel, 1987; Druffel and Bauer, 2000]. Studies have shown that DOC in deep waters presented q14C values

2.2.1. Chromophoric dissolved organic matter

Spectral analyses of DOM have been applied to assess the optically active fraction of DOM, the colored (or chromophoric) and fluorescent DOM (CDOM and FDOM, respectively). CDOM is the DOM fraction that absorbs light in the ultraviolet (UV) and visible wavelength ranges [Siegel et al., 2002], whereas a fraction of CDOM is able to fluoresce, characterizing the FDOM fraction. From the absorbance spectra obtained with spectrophotometers, the Napierian absorption coefficient of CDOM (a) at each wavelength (λ) is obtained from the given equation: $a(\lambda) = (2.303 \times A\lambda) / L$, where Aλ is the absorbance at specific wavelength and L is the cuvette path length in meters. That coefficient is adopted as an index of CDOM amount and different wavelengths have been chosen to determine a. Studies focusing on ocean color remote sensing previously

2.2.2. Fluorescent dissolved organic matter

FDOM has also been used as an index of DOM amount [Benner et al., 2005; Cooper et al., 2005]. Furthermore, it can provide information on the origin, mixing, and removal of different fractions of DOM [Yamashita and

and their respective molecular formulas can be assigned [Koch et al., 2005]. Several different approaches have been applied for visualizing the molecular information provided by the FT-ICR MS. Among them, the van Krevelen diagram is the most popular method applied [van Krevelen, 1950; Schmidt et al., 2009]. The diagram is constructed based on the atomic ratios of carbon compounds and is obtained from the hydrogen index (hydrogen:carbon) as a function of the oxygen index (oxygen:carbon).

reaching -502‰ (i.e., 5600 years) in the Southern Ocean [Druffel and Bauer, 2000] and -540‰ (i.e., 6240 years) in the central North Pacific Ocean [Williams and Druffel, 1987]. However, this article focuses on the spectroscopic methods, such as absorbance (section 2.2.1) and fluorescence (section 2.2.2) spectra. These parameters can be monitored with in situ autonomous platforms and ocean color remote sensing [Cooper et al., 2005; Siegel et al., 2005; Heim et al., 2014], and the derived optical indices (section 2.2.3) used to characterize and evaluate the transformation and reactivity of DOM.

presented results on absorption in the visible wavelength range, a₄₄₀ or a₄₄₃ [Siegel et al., 2002, 2005; Heim et al., 2014]. Other studies, on the other hand, used the absorption in the UV range (e.g. a₃₂₅ and a₃₅₀) because of its correlations with DOC and lignin concentration [Spencer et al., 2009; Stedmon et al., 2011], and also because CDOM is the most important optically active constituent of water in the open ocean with regards to absorption in the UV wavelength range [Nelson and Siegel, 2013]. Moreover, DOC has been shown to be strongly correlated with both CDOM and FDOM in the Arctic Ocean [Walker et al., 2013; Gonçalves-Araujo et al., 2015]. Therefore optically active fractions of DOM can be a proxy for the total DOM-pool (based on DOC measurements) in the Arctic environment.

Tanoue, 2003; Chari et al., 2013; Fukuzaki et al., 2014; Gonçalves-Araujo et al., 2015]. By acquiring the excitation-emission-matrices (EEMs), a qualitative evaluation of the different compounds of bulk DOM from spectroscopy

can be performed [Coble, 1996]. With the adaptation of the Parallel Factor Analysis (PARAFAC) for the analysis of DOM, a more holistic differentiation of underlying independent DOM components was possible [Stedmon et al., 2003; Stedmon and Bro, 2008]. The PARAFAC is a multi-way analysis that can be applied to decompose trilinear data arrays such as EEMs. Furthermore, EEMs must be corrected for inner-filter effects and for the Raman and Rayleigh scattering prior to PARAFAC modeling [Murphy et al., 2013]. Recent studies attempted to associate molecular groups and PARAFAC-derived DOM components [Stubbins et al., 2014; Kellerman et al., 2015; Wagner et al., 2015]. They found significant correlations between the humic-like fluorescent peak A [e.g., Coble, 2007] and high molecular weight compounds with little nitrogen, between the protein-like fluorescent peak T and low molecular weight aromatic compounds (such as amino acids) and between the humic-like fluorescent peak C and lignin-derived phenols. Moreover, a recent study pointed

2.2.3. Optical indices for DOM modification

The information contained in the spectral analysis of both CDOM and FDOM cannot only determine the amount and composition of DOM components, but it can also give insights into DOM origin and transformation. For that purpose, several optical indices have been developed. The spectral slope of absorption spectra (S) is obtained by applying an exponential function to the UV-VIS spectral range. It has been shown to be inversely correlated with the molecular weight of DOM and it can also be related to photobleaching [Helms et al., 2008]. The choice of each spectral range for assessment of the spectral slope varies among different studies and sampling regions. For instance, S values acquired in the UV region (e.g., 275–295 nm) can differ from results expressed by means of the VIS region (e.g., 350–400 nm) reflecting differences regarding the origin of DOM, as from terrestrial or marine character [Helms et al., 2008]. Other studies obtained S values considering the full UV-VIS regions, deriving S from the range between 300–650 nm [Stedmon and Markager, 2001]. A recent study showed that nitrate and cytochrome C exert strong influence on CDOM absorption spectra, given their absorbance peaks at 302 and 405 nm, respectively [Catalá et al., 2016]. Furthermore, that same study showed that those two chromophores can lead $S_{275-295}$ and $S_{350-400}$ to an overestimation by $13.3 \pm 6.0\%$ and $14.8 \pm 10.6\%$, respectively. The slope ratio (SR) is

obtained from the ratio between UV and VIS absorption spectral slope (275–295 and 350–400 nm, respectively) and provides strong differentiation between open ocean waters from those of near-shore coastal or estuarine origin [Helms et al., 2008]. The specific UV absorbance (SUVA) index is obtained as a function of the UV absorbance (at 254 nm) and DOC concentration, and it is used to trace the degree of aromaticity in CDOM samples [Weishaar et al., 2003], which is in turn correlated to the molecular weight [Helms et al., 2008].

Fluorescence is widely used to assess the degree of humification of bulk DOM, and thus to provide insights into the origin of DOM. The fluorescence index (FI) can be applied to distinguish sources of isolated aquatic fulvic acids. It is determined based on the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm, obtained with an excitation of 370 nm [Mcknight et al., 2001]. The humification index (HIX) estimates the degree of maturation of DOM [Zsolnay et al., 1999; Zsolnay, 2003], considering that humification is associated with an increase in the C/H ratio [Stevenson, 1994] and is thus reflected in emissions at longer wavelengths [Senesi et al., 1991]. This index is obtained from the ratio of the areas of two spectral wavelength regions (435–480 nm versus 300–345 nm) in the emission spectra for an excitation at 254 nm [Zsolnay et al., 1999]. An increase in the degree

of aromaticity (humification) leads to a red shift in the emission spectrum, which results in higher HIX values. The biological/autochthonous index (BIX) is used to assess the biological modification of DOM based on UV fluorescence. The BIX index is obtained by calculating the ratio of the emission at 380 and 430 nm, excited at 310 nm [Huguet et al., 2009]. High BIX values correspond to autochthonous origin of DOM, i.e., freshly released DOM, whereas low BIX values indicate allochthonous DOM [Huguet et al., 2009].

3. DOM as an environmental tracer

DOM has been shown to be a useful tool in a wide range of applications, from scientific to management interests. Studies have shown that FDOM measurements provide a fast and sensitive way to monitor the qualitative and quantitative variation of DOM in drinking water, and during the sewage treatment and also recycling water processes [Guo et al., 2010; Hambly et al., 2010; Murphy et al., 2011]. PARAFAC-derived protein-like components (and their relative contribution compared to humic-like components) were suggested to be a reliable tracer to monitor the relative amount of raw or treated sewage in China [Guo et al., 2010]. Another study, conducted with samples from municipal water systems, highlighted the dominance of the terrestrial humic-like PARAFAC-derived component, which has also been identified in other studies performed on engineered, wastewater impact environments [Murphy et al., 2011].

Besides its potential use to monitor water quality and sewage and wastewater treatment, DOM has been shown to be a water mass tracer, especially the fresh water fractions [Stedmon and Markager, 2001; Stedmon et al., 2015]. Strong correlations between CDOM and the fraction of meteoric water, which is a tracer of continental fresh water input [Dodd et al., 2012], can be used as a proxy to monitor the fresh water export from the Arctic to the Atlantic basins, given the high DOM concentrations in those waters. Furthermore, other optical parameters of DOM can be applied to trace the fraction of meteoric water in the Arctic Ocean. Studies have used the correlation between S and a_{375} to detect the fractions of meteoric water [Granskog et al., 2012; Stedmon et al., 2015] by applying the model proposed by Stedmon and Markager [2001]. It has also been demonstrated the potential of the visible wavelength fluorescence of DOM (VIS-FDOM) to trace the fresh

A recent study investigated the correlations between optical indices and molecular families derived from FT-ICR-MS measurements [Wagner et al., 2015]. The authors found that SUVA and HIX are effective in tracking terrestrially-derived groups of highly aromatic compounds with low N, P and S content, which have been previously pointed out by other studies to be photo-labile [Gonsior et al., 2009; Stubbins et al., 2010]. FI and BIX indices have been shown, on the other hand, to be associated to bio-labile aliphatic formulae [Wagner et al., 2015].

water content of the Arctic outflow [Gonçalves-Araujo et al., submitted]. Hence, FDOM can be used as a proxy to trace the origin of the waters occupying the Arctic surface layer, based on VIS-FDOM end-members proposed by that study, as being generated either in the Eurasian or Canadian basins, which cannot be detected by traditional analysis of hydrographical data (e.g. T-S diagrams and thermohaline intervals for water masses). Those authors concluded that their results provide an indication of which wavelength regions for DOM fluorescence carry information on DOM source and mixing. Such information has potential for supporting the design of in situ DOM fluorometers as a low-cost mechanism to provide high spatial and temporal resolution data for tracing the freshwater origins and decipher water mass mixing dynamics in the region, given the concern regarding the effects of climate change over the Arctic Ocean.

Given that CDOM absorbs light in both the UV and visible wavelength ranges, it can play an important role in the biogeochemical cycles in coastal and inner-shelf waters, being one of the dominant components interacting with the underwater light field in those environments [Siegel et al., 2002; Nelson and Siegel, 2013]. Furthermore, it can act as a shield for the aquatic biota from harmful UV radiation [Arrigo and Brown, 1996]. As a result of its UV absorbing properties, CDOM is susceptible to photo-degradation, which either induces direct mineralization or produces microbiologically labile low molecular weight compounds, which are subsequently utilized by bacteria [Mopper and Kieber, 2002]. CDOM does not only absorb UV-radiation, it also absorbs heat, thus influencing the light and heat penetration in surface waters [Granskog et al., 2015], especially in coastal and inner-shelf regions.

Finally, given the increased coastal erosion and permafrost thawing rates in the Arctic, with consequent release of ancient organic carbon [Aiken et al., 2014; Dubinenkov et al., 2015], many studies have attempted to trace and elucidate the fate of such compounds in the aquatic environment. A recent study showed that coastal erosion in the Yukon coast releases significant amounts of

DOC to the coastal Beaufort Sea [Tanski et al., 2016]. Based on the results of stable carbon isotope analysis it was also shown that, especially during summer and autumn, ancient DOC is released and will likely contribute to older DOC in the Yukon River and its tributaries in the coming decades [Aiken et al., 2014].

4. Conclusions

DOM has been shown to play an important role on the carbon cycle, acting as a link between terrestrial and aquatic systems. Furthermore, it is an easy-to-measure and affordable tool for monitoring water quality and sewage treatment. DOM is subject to several processes that affect its composition, amount and reactivity. However, the effects of such processes on different compounds, as well as the fate of DOM in aquatic systems are still under debate. With the ongoing climate change over the environment, more effort has been devoted to understand the role and fate of DOM in aquatic systems. A variety of new techniques have emerged in the last decades and better qualitative and quantitative assessment of the DOM is possible. However, there are still unresolved questions and unmet capabilities that need attention in the coming years. For instance, a more comprehensive understanding on the processes governing DOM dynamics, such as photo-oxidation, microbial turnover, adsorption/flocculation, etc. is needed

for a better estimation of DOM production rates as well as the rates of DOM transformation. A recent special issue gathered several papers linking the chemical and optical properties of DOM (“Linking optical and chemical properties of dissolved organic matter in natural waters”, *Frontiers in Journal, Section Marine Biogeochemistry*). Such studies can provide, for instance, a more consistent interpretation of optical indices of DOM modification and PARAFAC-derived fluorescent components. Finally, it is clear that implementation of new observing systems including new ocean color sensors and ocean observing systems, as well as the deployment of autonomous platforms with DOM-fluorometers, will be responsible for much of the future collection of data. Therefore, advances on the analysis and interpretation of the optical properties of DOM are required to improve the sensitivity and specificity of sensors deployable on these platforms.

5. Acknowledgements

The author thanks to Prof. Dr. Astrid Bracher and Lumi Haraguchi for their valuable comments and discussions. Rafael Gonçalves-Araujo is supported by a PhD fellowship from the Coordination for the Improvement of Higher

Level Personnel (CAPES-Brazil, Grant 12362/12-3) in collaboration with the German Academic Exchange Service (DAAD).

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