Limnologica 48 (2014) 57–64

Contents lists available at ScienceDirect

Limnologica

journal homepage: www.elsevier.com/locate/limno

Does filter type and pore size influence spectroscopic analysis of freshwater chromophoric DOM composition?

Jorge Nimptsch[a,*], Stefan Woelfl[a], Brian Kronvang[b], Ricardo Giesecke[a], Humberto E. González[a], Luciano Caputo[a], Jörg Gelbrecht[c], Wolf von Tuempling[d], Daniel Graeber[b]

[a] Instituto de Ciencias Marinas y Limnológicas, Facultad de Ciencias, Universidad Austral de Chile, Edificio Emilio Pugin, Campus Isla Teja, Valdivia, Chile
[b] Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark
[c] Department of Chemical Analytics and Biogeochemistry, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany
[d] Helmholtz Centre for Environmental Research – UFZ, Central Water Analytic & Chemometrics, Brückstraße 3a, 39114 Magdeburg, Germany

ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form 17 June 2014
Accepted 20 June 2014
Available online 9 July 2014

Keywords:
DOM
PARAFAC
EEM
Freshwater
Chromophoric

ABSTRACT

Dissolved organic matter (DOM) in freshwater ecosystems has its origin in a multitude of terrestrial and aquatic sources which determine amount, composition and thereby its functions. Spectroscopic methods are used to characterize chromophoric DOM. Among these methods, absorbance and fluorescence spectroscopy can provide indications of sources, behavior, and biogeochemical cycling of DOM. Since DOM is defined as the part of the organic matter pool which passes filters from 0.22 to 0.7 μm, sample filtration is required before spectroscopic measurements can be done. However, the use of different filter types might be influencing the results of DOM composition measurements. In order to assess the effect of different filters, we used three filter types with nominal pore sizes of 0.7, 0.45 or 0.22 μm, measured difference spectra, calculated spectroscopic indexes ($S_{275-295}$, $S_{350-400}$, S_r, $SUVA_{254}$, E2:E3, fluorescence index, β:α, humification index) and fluorescence components (parallel factor analysis, PARAFAC), obtained via spectroscopic measurements from water with different characteristics (e.g. river water, wetland, sewage effluent). Our results show that the filter types had significant but small effects on the chromophoric DOM composition and that meta-analyses of DOM composition based on studies with different pore size can be done, when keeping potential minor filtration effects in mind. This is important for further meta-analysis which might inter-compare data sets on spectroscopic characterization of DOM.

Introduction

Dissolved organic matter (DOM) in freshwater ecosystems has its origin in a multitude of terrestrial and aquatic sources which determine amount, composition and thereby its functions (Fellman et al., 2010). Besides physico-chemical properties such as pH buffering of aquatic matrixes (Roila et al., 1994), light absorption (Roulet and Moore, 2006), interaction with metals (Yamashita and Jaffé, 2008) and organic contaminants (e.g. pesticides, Beale et al., 2013) together with absorption to surfaces, DOM is the most important vector of carbon loss from terrestrial to aquatic ecosystems and, in general, plays a fundamental role within terrestrial–aquatic connectivity (Thacker et al., 2005). Furthermore, amount and composition of DOM are affected by changes in land-use and effects of climate change (Worrell et al., 2003; Graeber et al., 2012).

For describing the main properties of DOM, different analytical methods are developed and available. In routine measurements, usually the bulk dissolved organic carbon (DOC) concentration is determined, which does not give information on DOM composition. Furthermore, detailed information can be obtained if special but very expensive analytical methods like Fourier transform ion cyclotron resonance mass spectrometry (FT-ICRMS) are used (Flerus et al., 2012). Spectroscopic methods pose a trade-off between the aforementioned methods, as they are relatively fast and easy to measure but still give information on DOM composition with reasonable detail. These can be used to characterize DOM, since in all natural waters a part of the dissolved organic matter is chromophoric (Green and Blough, 1994), which means that it absorbs light, where fluorescing material is a subset of the

* Corresponding author. Tel.: +56 63 293954.
E-mail address: jorge.nimptsch@uach.cl (J. Nimptsch).

http://dx.doi.org/10.1016/j.limno.2014.06.003
0075-9511/

chromophoric material which fluoresces in the UV and visible regions of the spectrum (Chen and Bada, 1992). For example, based on spectral UV absorption at 254 nm and DOC concentration, specific ultraviolet absorption at 254 nm (SUVA254) can be calculated, which is a good indicator of DOM aromaticity (Weishaar et al., 2003). In addition, indexes of absorbance slope steepness (Helms et al., 2008) or ratios of absorbance at different wavelengths have been developed (e.g. E2:E3, De Haan and De Boer, 1987), which can indicate DOM molecular size and bioavailability. Fluorescence of DOM is an even more detailed method for assessing DOM composition, as it can be used for studying changes in composition and amount of DOM in aquatic systems for large amounts of samples with a high resolution (Fellman et al., 2010). It is measured across a range of excitation and emission wavelengths to generate Excitation-Emission Matrices (EEMs), generating a three-dimensional fluorescence – intensity landscape in which the presence of distinctive peaks can provide indications of sources, behavior, and biogeochemical cycling of DOM (Coble et al., 1990). The advantage of applying fluorescence spectroscopic measurements relies on the fact that it is a highly sensitive semi quantitative and qualitative method. However, it has been shown that the fluorescence signal might be instrument dependent and has to be inter-calibrated by removal of the instrument specific spectral bias, correction of inner filter effect; and fluorescence intensity calibration (Lawaetz and Stedmon, 2009; Murphy et al., 2010).

Before DOM can be measured it needs to be separated from particles contained in the matrix of the sample. Filtration is the usual procedure in which DOM is the part of the organic matter pool which passes filters with pore sizes of 0.7, 0.45 or 0.2 μm (Ortega-Retuerta et al., 2009; Spencer et al., 2009; Zhang et al., 2009). It has been shown by Karanfil et al. (2003) that different filter types ranging from glass fiber, nylon, polyethersulfon, between others, with different pore sizes in the range of 0.2–1.5 μm may interfere in spectroscopic measurements needing to be washed and pre-conditioned in order to give reliable results in UV_{254} and DOC measurements. The filter type and pore size thereby influences (i) the inner-filter effect of the separated DOM, (ii) potential adsorption and release of DOM, (iii) generation of particulate organic matter (POM) from DOM by cavitation due to filtration pressure and finally (iv) the inclusion of bacteria and organic matter aggregates (particulate organic matter, POM) in the DOM sample. The inner filter effect is the portion of light that is absorbed by a sample during fluorescence measurements. It can be absorbed either as exciting light going into the sample from the light source or as emitted light going out from the sample to the detector (see Kothawala et al., 2013; Ohno, 2002 for details). If a sample is filtered with a large pore size, the inner-filter effect may be higher due to remaining particles. This is corrected for by a mathematical inner-filter correction (Ohno, 2002; Lakowicz, 2006). With this correction, the filter effect should be accounted for but still small effects of the inner-filter effect may remain (Ohno, 2002). Furthermore, typically used filters are composed of organic material, exceptions being the rarely used expensive silver filters and glass filters. Therefore, filters can release or adsorb DOM which may produce measurement artifacts (Zsolnay, 2003; Karanfil et al., 2003). Moreover, pressure changes during filtration can result in cavitation resulting in the formation of small gas bubbles. Surface active DOM can then adsorb on the bubble surfaces, and when the bubbles collapse this adsorbed DOM unites as POM (Zsolnay, 2003). Finally, bacteria can also affect fluorescence because these can either be a source of DOM or can take up bioavailable DOM from the analyzed matrix. This can be a problem, when a sample is filtered with pore sizes >0.2 μm, since only with this pore size all bacteria can be removed from a sample. Consequently, the alteration of DOM molecular composition by bacteria can confound DOM spectroscopic measurements. On the other hand, different filter pore sizes can also result in the exclusion or inclusion of POM of different sizes. Fluorescence of POM is rarely investigated, however a study of riverine organic matter fractions found that the POM can be fluorescent after it is separated from DOM (Mounier et al., 1999). Moreover, it can bind or release chromophoric DOM which was shown to alter fluorescence of surface water DOM samples (Komada et al., 2002) and may affect fluorescence-based estimations of DOM concentration (Hoge et al., 1993). If filters are prepared and used correctly, these effects are only minor but potentially sum up to a filtration effect which may affect the outcome of studies in which samples were filtered with different filter types and pore sizes. Only very few studies have made approaches toward the effect of filtration on DOM fluorescence, such as Baker et al. (2007), who investigated the effect of pH and two filter types toward two components (i.e. tryptophan and humic acid), modeled by parallel factor analysis (PARAFAC). His results showed different fluorescence intensities of especially tryptophan like component between 0.2 and 1.2 μm nominal filter cutoff, whereas humic like component was not influenced significantly. However 1.2 μm are not commonly used in DOM fluorescence studies. Furthermore, Cohen et al. (2014) evaluated the effect of different waste water treatment steps on 0.45 μm filtrated PARAFAC modeled fluorescence intensities waste water samples in order to evidence waste water treatment efficiency.

The aim of the present study is focused to obtain evidence on influence of filter type and pore size during sample preparation on spectroscopic analysis of freshwater chromophoric DOM composition. Hence, to get information on the uncertainty of EEM analysis based on different filtration procedures, the present investigation compares spectroscopic indexes, fluorescence components using PARAFAC and difference spectra obtained via spectroscopic measurements using three different typically used types of filters (Millipore Millex® 0.2 and 0.45 μm, Whatman® GF/F) with different pore size and filter material for sample preparation of water samples with different characteristics (river water, wetland, sewage effluent). Our hypothesis was that there is an influence of sample preparation using different filter types and pore sizes upon freshwater DOM composition obtained via spectroscopic analysis due to the several potential mechanisms in which different filter types may affect the composition of a given DOM sample.

Materials and methods

Sampling and sample preparation

Two sampling campaigns were carried out within the North Patagonian Cruces River – Wetland system (Ramsar Carlos Anwandter Wetland, Valdivia – Chile) (Fig. 1), a sampling campaign in summer 2011 (hereafter referred to as dry season – summer campaign, March 2011) and one in winter 2012 (wet season – winter campaign, July 2011). Cruces River is about 125 km long and drains a catchment area of 3233 km^2. It flows through the towns of *Lanco* and *San Jose de Mariquina* before entering Carlos Anwandter wetland which was created in 1960 as a result of an earthquake and subsequent "Tsunami". Due to this natural force, large areas around the river Cruces (Valdivia) were transformed into a permanent wetland system. The main urban settlement (Valdivia city) is located downstream of the Wetland.

Fourteen sampling stations with different characteristics and different anthropogenic influence (e.g. river: R1–R3, wetland: W1–W8, urban area of Valdivia and sewage effluent of Mariquina and Valdivia: U1–U3) were used for sampling (Fig. 1). At ten of the 14 sampling stations, samples were taken in the top 10 cm surface layer and in the bottom layer 50 cm above sediment and at the four river stations only one sample was taken near the water surface, since maximum depth was below 2 m. Twenty-four samples