Using fluorescence to characterize dissolved organic matter in Antarctic sea ice brines

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[1] Sea ice plays a dynamic role in the air-sea exchange of CO_2. In addition to abiotic inorganic carbon fluxes, an active microbial community produces and remineralizes organic carbon, which can accumulate in sea ice brines as dissolved organic matter (DOM). In this study, the characteristics of DOM fluorescence in Antarctic sea ice brines from the western Weddell Sea were investigated. Two humic-like components were identified, which were identical to those previously found to accumulate in the deep ocean and represent refractory material. Three amino-acid-like signals were found, one of which was unique to the brines and another that was spectrally very similar to tryptophan and found both in seawater and in brine samples. The tryptophan-like fluorescence in the brines exhibited intensities higher than could be explained by conservative behavior during the freezing of seawater. Its fluorescence was correlated with the accumulation of nitrogen-rich DOM to concentrations up to 900 μmol L^{-1} as dissolved organic carbon (DOC) and, thus, potentially represented proteins released by ice organisms. A second, nitrogen-poor DOM fraction also accumulated in the brines to concentrations up to 200 μmol L^{-1} but was not correlated with any of the fluorescence signals identified. Because of the high C:N ratio and lack of fluorescence, this material is thought to represent extracellular polymeric substances, which consist primarily of polysaccharides. The clear grouping of the DOM pool into either proteinaceous or carbohydrate-dominated material indicates that the production and accumulation of these two subpools of DOM in sea ice brines is, to some extent, decoupled.

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1. Introduction

[2] Sea ice forms from super-cooled surface seawater in oceans and inland seas of the Polar Regions. During sea ice formation and growth, the solutes present in the parent seawater mass are rejected from the ice crystal matrix into a residual hypersaline solution (brine, S > 35). A large part of this brine drains into the underlying water by gravity, while a fraction (10%–40%) remains entrained in channels within the sea ice, becoming saltier with decreasing ice temperatures, and vice versa [*Petrich and Eicken*, 2010]. Despite the extreme nature of the sea ice environment with its low temperatures and high brine salinities, there is a very productive biological community exploiting this niche [*Arrigo et al.*, 2010; *Deming*, 2010], which drives the internal cycling of carbon and nitrogen between the inorganic and organic reservoirs.

[3] Our understanding of the role of sea ice in the global carbon cycle is changing. Rather than representing an inert cover retarding atmosphere-ocean CO_2 exchange, it is now apparent that there are significant fluxes of carbon through the ice into the atmosphere and the ocean [*Thomas and Dieckmann*, 2010; *Miller et al.*, 2011; *Rysgaard et al.*, 2011]. In addition to the important role in abiotic inorganic carbon cycling, autotrophic, and heterotrophic activity, the latter fuelled by high concentrations of dissolved organic matter (DOM) within the ice, change the concentrations and dynamics of inorganic and organic carbon, and thereby the fluxes of CO_2.

[4] The carbon cycle in sea ice includes inorganic carbon fluxes to the underlying ocean associated with sea ice formation and melting [*Rysgaard et al.*, 2007], as well as considerable entrapment, production, and accumulation of

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organic carbon in particulate and dissolved forms [*Thomas et al.*, 2010]. The characteristics of this material, including its susceptibility to remineralization still remain poorly understood. The internal pool of DOM in sea ice is composed of the component that originates from the parent seawater from which the ice is formed [*Giannelli et al.*, 2001; *Stedmon et al.*, 2007a] and that produced internally from the sympagic biological assemblages [*Smith et al.*, 1997; *Riedel et al.*, 2007]. In turn, the DOM composition of the parent seawater is a combination of material produced by microbial activity within the water itself and material derived from nonmarine (i.e., terrestrial) sources. The latter source can be expected to be negligible in the waters of the polar Southern Ocean [*Wedborg et al.*, 2007], and hence sea ice formed from these waters should be devoid of terrestrial organic matter input.

[5] Previous studies have shown that sea ice from the Arctic and Southern oceans, as well as from the Baltic Sea, can contain high concentrations of both dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) [*Thomas et al.*, 1995; *Stedmon et al.*, 2007a; *Norman et al.*, 2011]. Absorption spectra of colored DOM in Antarctic sea ice have revealed that there are two general pools that can be differentiated. A fraction derived from in ice production and a fraction that resembles that present in seawater [*Norman et al.*, 2011]. There is, however, still a need to expand this further to provide a better characterization of the DOM that accumulates. In addition, before the role of DOM in carbon cycling in sea ice can be determined, it is essential to be able to differentiate between the conservative DOM fraction originating in surface seawater prior to the sea ice formation and those that are produced internally in the sea ice system by the productive sympagic communities. In addition to the direct role that these fractions may have in the turnover of carbon in sea ice, it is also likely that this material plays a role in shaping the environment that sea ice microbes inhabit. The chemical characterization of a heterogeneous DOM pool is complex; sea ice to date has largely been restricted to quantifying specialized components, such as carbohydrates [*Herborg et al.*, 2001; *Underwood et al.*, 2010], amino acids [*Amon et al.*, 2001; *Uusikivi et al.*, 2010], or extracellular polymeric substances [*Krembs et al.*, 2011]. Optical measurements of the absorption and fluorescence spectral properties of DOM can provide useful information because they may facilitate the separation between the autochthonous and allochthonous DOM fractions in sea ice [*Stedmon et al.*, 2007a].

[6] The aim of this study was to provide a first attempt at the fluorescence characterization of the DOM in Antarctic sea ice. Our objective was to investigate these characteristics directly in sea ice brines, as opposed to melted bulk sea ice, using fluorescence spectroscopy. Direct observation in brines means that artifacts resulting from melting ice core sections and the ensuing osmotic shock on the included sympagic organisms are absent. The analyzed brines were collected from ice floes in the northwest Weddell Sea, Antarctica, and had high DOC and DON concentrations [*Meiners et al.*, 2009; *Norman et al.*, 2011]. In the current study, the fluorescence intensity of the DOM produced within the sea ice is considered separately from that of the DOM present in the parent seawater and physically concentrated in the brines during sea ice formation and consolidation.

2. Material and Methods

[7] The study was conducted in the transition from winter to spring (September–October 2006) during the *Winter Weddell Outflow Study* (WWOS) on board RV *Polarstern*. The cruise track took an east to west transect between 60°S–61°S and 40°W–52°W, and a northwest to southwest transect between 60°S and 65°S, over a large expanse of the pack ice in the northwestern Weddell Sea [*Lemke*, 2009]. Seawater, brine, and ice core samples were obtained at 22 ice stations during a 38-day period. Several physical, chemical, and biological parameters from the sea ice at the WWOS ice stations have been reported by *Meiners et al.* [2009] and *Norman et al.* [2011].

[8] Under-ice seawater samples were taken using a Seabird 911+ CTD rosette equipped with 12L Niskin bottles. Depths sampled ranged from 19 to 2442 m. Brine samples were collected using the sackhole-sampling method by manually drilling partial boreholes into the ice surface after removing the snow cover and allowing the brine from the surrounding brine channels to percolate into the sackhole for a period of up to 1 h, depending on ice temperature [*Gleitz et al.*, 1995; *Papadimitriou et al.*, 2007]. Between three and six sackholes were drilled on each occasion using a 14 cm (ID) Kovacs Mark V ice corer within approximately a 2 m^2 ice surface to depths between 16 and 186 cm, but mostly in the 30 to 60 cm range, depending on the thickness of the ice. Ice shavings and slush were removed, and Styrofoam covers were placed over the top of the sackholes to ensure that samples were not compromised by snow, slush, or debris and to minimize air-brine interaction [*Papadimitriou et al.*, 2007]. The brine samples were collected into acid-cleaned 1 L plastic containers using an acid-cleaned 100 mL plastic syringe fitted with a 30 cm length of Teflon tubing. Deeper sackholes were sampled with a clean stainless steel ladle attached to a pole [*Norman et al.*, 2011].

[9] All salinity measurements were taken at laboratory temperature (17–22°C) using a SEMAT Cond 315i/SET salinometer with WTW Tetracon 325 probe. Samples with salinity higher than 70 were first diluted with ultrapure water. The seawater and brine samples were filtered through precombusted filters (Whatmann, GF/F, 500°C, 3 h). The filtrate provided samples for DOC, DON, and fluorescent DOM measurements. The DON samples were stored in acid-cleaned 20 mL scintillation vials, while the DOC samples were stored acidified with ~20 μL of 85% orthophosphoric acid in precombusted (500°C, 3 h) 4 mL borosilicate vials with Teflon-lined screw caps, both at −20°C, until analysis in the home laboratory. The samples for DOM absorption and fluorescence were stored refrigerated in the dark in acid-cleaned plastic bottles and were analyzed upon arrival in Denmark approximately 4 months after collection. DOC concentrations were determined by high-temperature combustion on an MQ 1001 TOC Analyzer [*Qian and Mopper*, 1996]. DON was determined by subtraction of NO_3 and NH_4 from the total dissolved nitrogen (TDN) analyzed by flow injection analysis on a LACHAT autoanalyzer using online peroxodisulfate oxidation coupled

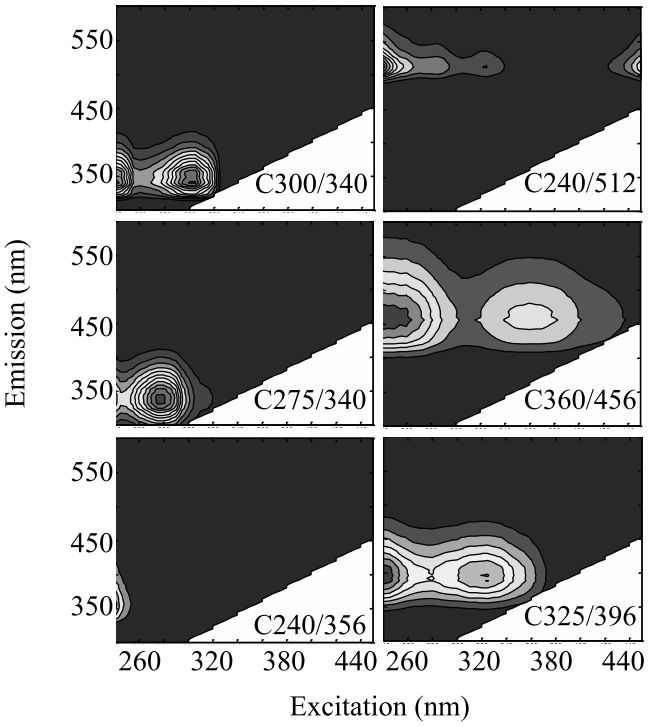

Figure 1. The spectral properties of the six components identified by the parallel factor analysis. The components are labeled according to the wavelengths of their excitation and emission maxima (CEx./Em).

with ultraviolet radiation at pH 9.0 and 100°C [*Kroon*, 1993].

[10] Fluorescence properties of DOM were measured on a Varian Eclipse fluorescence spectrophotometer, with both excitation and emission slit widths at 5 nm. Excitation-emission matrices (EEM) were obtained by repeatedly scanning over emission wavelengths between 300 and 600 nm for each excitation wavelength between 240 and 450 nm with 5 nm increments. The spectra were corrected by using the instruments correction factors and calibrated to Raman units using the approach described in *Lawaetz and Stedmon* [2009]. Before calibration, inner filter effects were removed using absorbance spectra of the same sample according to the method by *Lakowicz* [1999]. Absorbance spectra were measured on a Shimadzu UV-2401PC UV-Visible spectrophotometer with pure MilliQ water as the blank and using either a 5 or 10 cm quartz cuvette.

[11] A data set of 89 EEMs was compiled and characterized by parallel factor analysis with the use of the DOMFluor MATLAB toolbox. The data consisted of 62 brine samples, 8 seawater samples, and 19 ice melt samples. A PARAFAC model was derived following the recommendations presented in *Stedmon and Bro* [2008]. Six independent components were validated by split half and random initialization analysis and were found to characterize the fluorescence in these samples (Figure 1). In order to avoid confusion when comparing with earlier studies, the components in this study are named according to their excitation and emission maxima. All components had excitation maxima below 240 nm, so where a clear maximum at greater wavelengths was detected, this wavelength was used for naming rather than 240 nm.

3. Results

[12] The salinity of the sackhole brines ranged from 58 to 134 and their temperature from −3.6°C to −8.7°C (n = 126) [*Norman et al.*, 2011]. The temperature in the ice column either increased systematically with depth from the coldest uppermost layer to the warmest bottommost layer or was approximately constant and almost uniformly lowest in the upper quarter to half of the ice column, increasing thereafter to the bottom of the sea ice. In both cases, the measured temperature at the bottommost ice sections was always close to the freezing point of the underlying surface oceanic water (−2.1 to −1.9°C). In comparison, the temperature of the brines was lower than that of the bottom sections of the sea ice, indicating their origin in the coldest upper sea ice layers. The chlorophyll maximum was observed in the lowest part of the ice column close to the ice-seawater interface in all cases except on 2 occasions, when the chlorophyll maximum was located in the uppermost part of the ice column, and on one occasion, when the chlorophyll maximum was located internally [*Norman et al.*, 2011].

3.1. Fluorescence Characteristics

[13] Four of the identified components had signals very similar to those identified in earlier marine studies and could be grouped into two types according to their emission properties. Fluorescence with an emission less than 360 nm is often referred to as protein or amino-acid-like fluorescence, because this is the region where three amino acids found in proteins, tryptophan, tyrosine, and phenylalanine, exhibit fluorescence [*Coble* 1996; *Yamashita and Tanoue*, 2003]. Two components, C300/340 and C275/340, had this type of fluorescence signal. The C300/340 component has been found in several earlier studies, and its excitation and emission spectra are plotted in Figure 2a together with three components found in oceanic and Baltic Sea samples (C7 from *Murphy et al.* [2006, 2008] and C4 as given by *Stedmon et al.* [2007a, 2007b]). The emission spectrum of this component is similar to that of tryptophan, but its excitation maximum is at 300 nm rather than 275 nm. The second amino-acid-like component (C275/340) has spectral properties that were very similar to those of free dissolved tryptophan and has also been reported in surface marine waters [*Murphy et al.*, 2006]. Its spectral properties are plotted together with pure tryptophan and C6 from *Murphy et al.* [2006, 2008] in Figure 2b.

[14] Two of the identified components had fluorescent properties that are often referred to as humic-like [*Coble*, 1996], with broader excitation spectra and emission maxima above 380 nm (Figure 1). These also overlapped to a certain extent with the fluorescent signals found in several earlier studies. For example, the spectral properties of C360/456 and C325/396 are compared with those of C1 and C2 by *Yamashita et al.* [2010] in Figures 2c and 2d. These two peaks are often referred to as C and M peaks, respectively, and are present across a wide range of environments [*Coble*, 1996, 2007; *Stedmon et al.*, 2007a, 2007b; *Fellman et al.*, 2010; *Jørgensen et al.*, 2011].

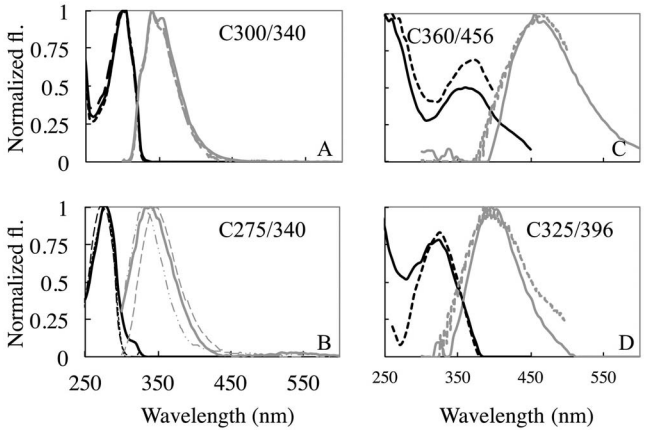

Figure 2. Comparison of the excitation (black) and emission (gray) spectra of four components with a selection of previously published spectra. (a) C300/340 with component 4 given by *Stedmon et al.* [2007a, 2007b]. (b) C275/240 with C6 given by *Murphy et al.* [2006] and tryptophan dissolved in MilliQ water. (c) C360/456 with component 1 given by *Yamashita et al.* [2010].(d) C325/396 with component 2 given by *Yamashita et al.* [2010].

[15] To our knowledge, the remaining two identified fluorescent signals have previously not been reported in aquatic DOM samples. C240/512 had a very characteristic fluorescent signal with a broad excitation spectrum covering the whole measured range and maxima below 240 nm and above 450 nm. In contrast, the emission spectrum was very narrow. These spectral properties are similar in shape to compounds, such as chlorophyll, phycobiliproteins (produced by blue-green and red algae), and riboflavin, but the position of the maxima is different. C240/356 had an excitation maximum below that of the range measured, but its emission spectrum overlapped considerably with that of C300/340. This component of our knowledge had not been identified in earlier published studies.

3.2. Preservation, Production, and Removal of DOM Fractions in Brines

[16] The median fluorescence intensities of each component identified by the PARAFAC analysis in the seawater and brine samples are shown in Figure 3a. The C300/340 component was absent in seawater. In the brines, the C300/340 fluorescence intensity was much higher and very variable, varying between 0 and 5.7 nm^{-1}. In comparison, the fluorescence intensity of the other components was much less variable in both the seawater and the brine samples. The relative distribution of these components in each sample type was, however, different (Figure 3a). The fluorescence spectra of the 19 ice melt samples were dominated by the three protein-like fluorescence components and very variable (data not shown) between samples. This was considered to be a result of the release of DOM by organisms experiencing osmotic shock during melting. As a result these data will not be considered further here, because it is questionable how representative they are of DOM present in the sampled ice.

[17] In order to differentiate between the two sources of DOM in sea ice, that which is present in the parent seawater and that which is produced by biological activity within the sea ice, the effect of physical concentration during brine formation through seawater freezing was removed using average values calculated from the seawater samples for a seawater salinity of 35 as follows:

$$F^{acc} = F^{B} - \left(\frac{F^{SW}}{35} S^{B}\right) \quad (1)$$

where F^{acc} is accumulated fluorescence, F^{B} is fluorescence intensity of brine, S^{B} is brine salinity, and F^{SW} is average fluorescence of the seawater. The term "accumulated" here and onward is used to describe the excess material present in the brine after subtraction from the total measured value of the amount resulting from the conservative behavior of the seawater component during the freezing of seawater. Figure 3b shows the fluorescence characteristics for the material that accumulated in the brine, calculated according to equation (1) for each fluorescent component. Because no replicate samples were measured, it is difficult to determine the precision of the fluorescence measurements, but a first-order estimate can be obtained by using the standard deviations of the mean of the seawater samples, where the fluorescence signal was comparatively invariable despite the range of depths sampled. These data are plotted as error bars in Figure 3b and provide an indication of how significant the average accumulated fluorescence values are. When error bars overlap with zero, it can be assumed that no significant accumulation of this material occurred in the brines. The results show that the variability in the fluorescence of C240/512 and C325/396 was, on average, largely due to the physical concentration of original seawater solutes. In contrast, the fluorescence of C300/340, C275/340, and C360/

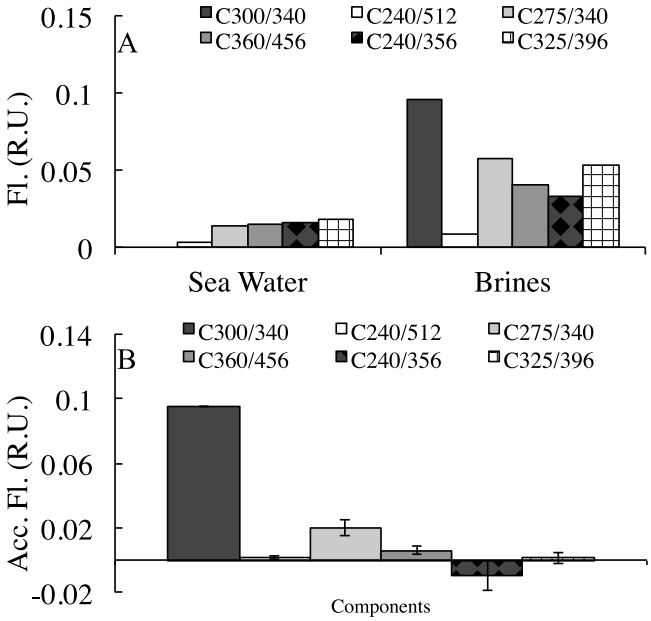

Figure 3. (a) Average fluorescence intensity of each of the six components in seawater and in the brines. (b) Average accumulated fluorescence calculated for all brine samples for each component. The fluorescence derived from the original seawater has been removed, using the average values shown in Figure 3a.

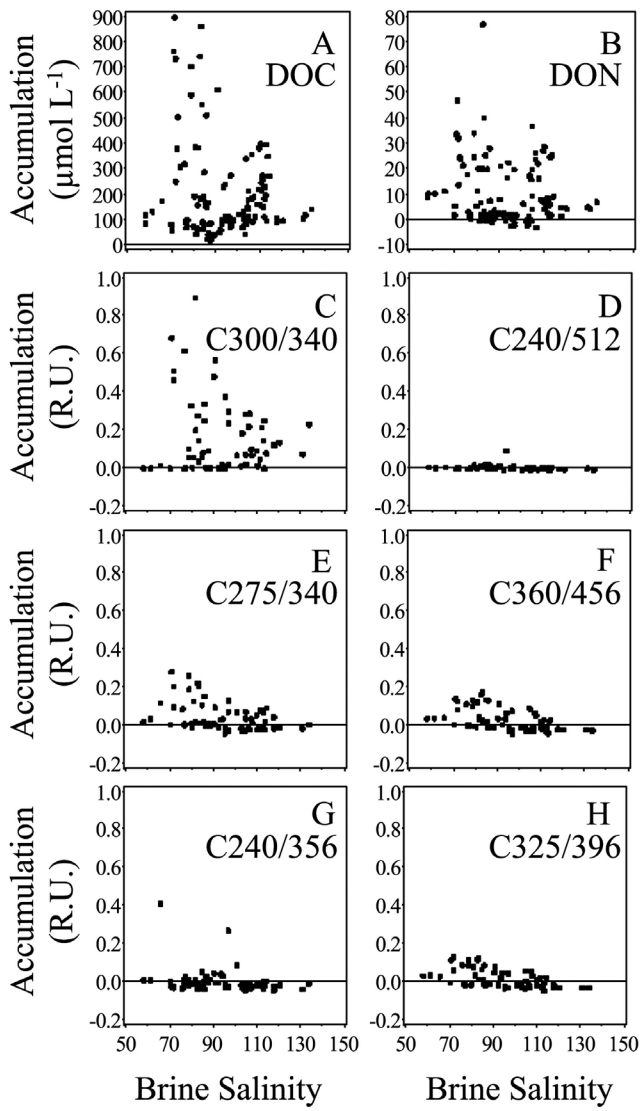

Figure 4. Accumulated dissolved organic carbon and nitrogen (DOC, DON) and six fluorescent components in each brine sample plotted against brine salinity. Reference line is shown at zero, and samples falling upon this line indicate that only concentrated seawater DOM was present.

456 appeared to accumulate in the brines via in situ production (Figure 3b). One fluorescent fraction, C240/356, exhibited on average net removal from the brines.

[18] The C and N concentrations of the accumulated brine DOM can be calculated in the same fashion as the accumulated fluorescence (equation (1)), and all calculated observations are plotted against brine salinity in Figure 4. According to this analysis, some of the sampled brines exhibited little or no DOM accumulation and others substantial accumulation up to 900 μmol DOC L^{-1} and 80 μmol DON L^{-1} (Figures 4a and 4b). No systematic trend with geographical location or sackhole depth in the ice was found. In addition, no clear trends in DOM accumulation with increasing brine salinity were apparent, although there was a tendency for the greatest accumulation at brine salinities between 60 and 90, corresponding to temperatures between −5.5°C and −3.5°C [*Norman et al.*, 2011]. The calculated accumulated DOC concentrations corresponded to 18%–92% of the total DOC concentration in the brine, with a mean of 61%. The calculated concentrations of accumulated DON in the brine were similarly variable, corresponding to 13%–92% of the total DON concentration in the brine, and with a mean of 50%. The C-to-N ratio of the accumulated DOM varied between 5 and 100, with a mean value of 26. For comparison, the C:N of the total measured DOM was between 6 and 35 with a mean of 19. Plotting the C:N of the accumulated DOM against the accumulated DOC revealed two trends indicating two general types of samples (Figure 5). The first group of observations represented a wide span of accumulated DOC concentrations (≈300–900 μmol L^{-1}) with a comparatively low C:N (<30). The second group of observations represented brines with moderate DOC accumulation (0–200 μmol L^{-1}) and elevated C:N (up to 100).

[19] There was a net accumulation of C300/340, C275/340, and C360/456 in the majority of the samples (Figures 4c, 4e, and 4f) and, to a slightly lesser extent, C325/396 (Figure 4h). Because C300/340 was absent from seawater, its presence in the sea ice appears to be solely attributed to a process occurring in the sea ice. However, its production and subsequent persistence is clearly not ubiquitous, because it was not identified as part of the DOM pool in several of the brines sampled (Figure 4c). Although the tryptophan-like fluorescence of C275/340 was present in seawater, the calculations indicate that there was additional production and accumulation for this material in the brines (Figure 4e), with, on average, 35% of the measured concentrations attributed to net production and retention in sea ice (Figure 3). The same was true for the humic-like fluorescence of C360/456, where, on average, about 15% of the total fluorescence was generated internally in sea ice compared with that expected from conservative behavior during brine formation. The fluorescence of the remaining components did not differ notably from that expected from conservative behavior during the freezing of seawater (Figures 3 and 4).

[20] The relationships between the accumulated DOC and DON concentrations and the fluorescent components were tested using linear regression analysis. Significant relationships were only found for C275/340 and C360/456 (r^2 0.5–0.7, $p < 0.01$). Although there was a reasonable and significant correlation, the residuals were skewed, with the high DOC and DON concentrations in particular being underestimated. This indicated that the linear relationships were inadequate for predicting DOC and DON, but were more representative of the fact that the accumulation of DOM fluorescence was associated with the accumulation of DOC and DON. This implies a common internal driving mechanism(s) in the sea ice biota. Further analysis revealed that the fluorescence of C275/340 was associated with the accumulation of nitrogen-rich DOM in the brines (Figure 5).

4. Discussion

[21] The characterization of the fluorescence properties of DOM in Antarctic sea ice brines has revealed that it is composed of six independent fractions differing in their spectral fluorescence properties and distribution. The fluorescence characterization allows us to differentiate between these fractions and trace material that either behaved con-

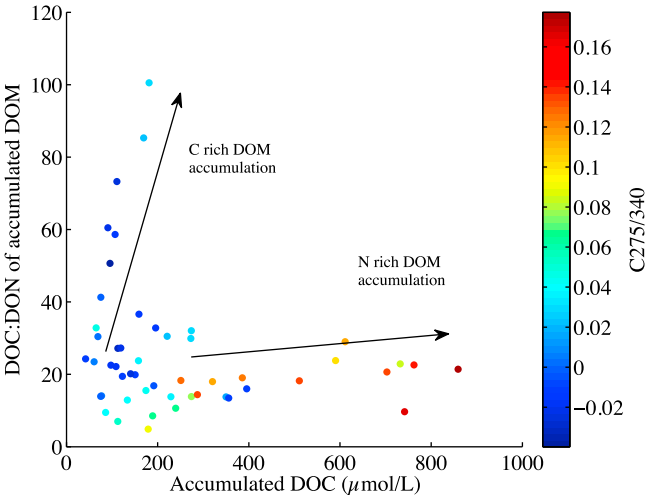

Figure 5. Comparison of the DOC:DON molar ratio of the accumulated DOM in the brines with the accumulated DOC. The symbols change with respect to the intensity of the fluorescence of C275/340. Two types of samples are apparent: accumulating a N-rich DOM and accumulating a C-rich DOM.

servatively (relative to salinity) during seawater freezing or was produced within sea ice. The effect of the physical process of sea ice formation on the characteristics of DOM is to date poorly resolved. The extent to which different DOM fractions are preferentially retained in brine channels during ice formation is unclear. The network of sea ice brine channels may preferentially retain larger high molecular weight material, which is operationally defined as dissolved but behaves more like particles [*Müller et al.*, 2011]. However, because of the brine-sampling strategy in this study, it is likely that this material is not detected because it would remain in the channels during brine drainage into the sackhole. The same behavior has been found to affect quantitative assessment of particle concentrations in sackhole brines [*Weissenberger*, 1992]. Furthermore, the physical concentration of seawater DOM in the residual brine retained in channels together with other solutes during sea ice formation is not the only process that affects the internal DOM pool in sea ice (Figure 4). Additional processes also act to alter DOM composition and concentration in sea ice brines.

[22] First, photochemical reactions, especially in the upper sea ice layers, where irradiance is higher, can lead to the direct remineralization of DOM to CO and CO_2, as well as formation of biolabile organic compounds, subsequently taken up by bacteria [*Moran and Zepp*, 1997; *Xie and Gosselin*, 2005]. *Norman et al.* [2011] presented results on the photoreactivity of DOM in the same brine samples and showed notable photobleaching but no significant change in bulk DOC and DON concentrations when exposed to natural surface light conditions. However, the light climate within the ice column, especially when covered by snow, can be expected to change dramatically, with the rapid attenuation of wavelengths driving photochemical reactions [*Belzile et al.*, 2000]. This would suggest that, although photochemical reactions can be important at the surface of sea ice, they are unlikely to be responsible for widespread alteration of the concentration and the optical character of DOM in internal brines.

[23] Second, sea ice hosts a large range of microbial organisms, at times present in high abundances because of the constrained space and high concentrations of nutrients [*Thomas and Dieckmann*, 2010]. Their presence and activity will influence brine DOM concentrations and characteristics via active release of compounds for osmoregulation, such as amino acids, carbohydrates [*Pomeroy et al.*, 1990; *Amon et al.*, 2001; *Herborg et al.*, 2001], and proline [*Krell et al.*, 2007], and for regulation of their physical environment, such as ice binding proteins [*Raymond et al.*, 2007] and extracellular polysaccharide substances (EPS) [*Underwood et al.*, 2010; *Krembs et al.*, 2011], as well as via excretion [*Thomas et al.*, 1998]. Such compounds are often found to accumulate in high concentrations in sea ice brines. The living biomass can also be expected to release DOM passively from their cells [*Bjørnsen*, 1988]. Additionally, there is a constant supply of a complex range of metabolites originating from the decaying microbial necro mass. However, with the high abundances of heterotrophic bacteria in sea ice [*Deming*, 2010], it is remarkable that compounds, such as amino acids and carbohydrates, which are considered highly biolabile, accumulate in sea ice brines. *Pomeroy and Wiebe* [2001] suggest that this can be due to a temperature-induced reduction in bacterial DOM affinity. While high levels of bacterial production (activity) can be detected in sea ice at very low temperatures, they require much higher ambient DOM concentrations to function optimally. In support of this, *Junge et al.* [2004] have showed that the majority of active sea ice bacteria are actually associated with particles, and it is likely they primarily utilize material directly released from the particles. The combination of these mechanisms, temperature limitation and dominance of particle-associated bacteria, can explain why high concentrations of otherwise biolabile DOM can accumulate in brines. Although abiotic "humification" transformations may also occur, converting relatively simple organic exudates into more complex structures [*Harvey et al.*, 1984], the low temperatures that characterize the sea ice would make this pathway very slow.

[24] From these findings, it is clear that the DOM found to accumulate via internal sea ice processes in the current brine samples most likely originated from autochthonous biological production rather than preferential retention during sea ice formation or photochemical reactions. The DOM fluorescence characteristics presented here offer several intriguing insights into the dynamics of DOM in sea ice, revealing different fractions and tracing how physical brine formation and biological processes alter its characteristics. Two tryptophan-like fluorescence components were shown to accumulate in the brine, and this most likely originates from protein material released by organisms. The correlation with the accumulation of N-rich DOM supports this assignment (Figure 5). Earlier work has shown that fluorescent material with the same spectral characteristics (Figure 2) can be produced in sea ice and surface marine waters [*Murphy et al.*, 2006, 2008; *Stedmon et al.*, 2007a] and is sensitive to photodegradation [*Stedmon et al.*, 2007b]. A recent study from Alaska also demonstrated that this type of fluorescence signature was prevalent in DOM in glacial meltwater and represented material that was labile to marine

microbes [*Hood et al.*, 2009]. Ice binding proteins, which are known to contain tryptophan [*Raymond et al.*, 2007, 2009], are a possible source for this fluorescent material.

[25] The narrow bands of salinity (S = 60 and 90) and corresponding temperature (−5.5°C to −3.5°C), where the greatest net DOM accumulation was observed, suggest a relationship between the observed net DOM accumulation, the biological activity responsible for it, and the physical characteristics of the brine environment as represented by its salinity and temperature. This putative link, however, may be incidental because the salinity and temperature of sea ice brines are transient, constantly evolving features through the seasons. The brines sampled here certainly had a complex thermal and compositional history, from their young stage in newly formed sea ice, potentially harboring an active autumnal biological community from the surface seawater, through their coldest hypersaline stage in winter to their status in early spring, possibly involving some degree of dilution by meltwater during inevitable warming as insolation increased. The net DOM accumulation, in other words, may not have been a recent event, which occurred at the observed brine salinity and temperature. Instead, it can be conceived equally realistically as a relic, or even evolving, in either case tightly coupled to thermal and compositional history of the brines, which the current data cannot disentangle further.

[26] The two humic-like fluorescent components (C360/456 and C325/396) correspond to what is termed the C and M peak regions, and fluorescence at these wavelengths is found across a wide range of systems [*Coble*, 1996; *Fellman et al.*, 2010]. Their spectral properties, however, are identical to two components found to be widespread in oceanic waters [*Yamashita et al.*, 2010; *Jørgensen et al.*, 2011]. The fluorescence of both these components is greatest in deep ocean waters and is correlated with oxygen utilization [*Chen and Bada*, 1992; *Hayase and Shinozuka* 1995; *Yamashita and Tanoue*, 2008; *Jørgensen et al.*, 2011]. In the surface ocean, their fluorescence is generally removed by photodegradation, but high levels can be maintained because of increased supply in productive waters, in oceanic regions influenced by terrestrial organic matter inputs, or in upwelling zones [*Nieto-Cid et al.*, 2005, 2006; *Jørgensen et al.*, 2011]. The majority of the humic fluorescence in the brines originated from the parent seawater, but there was evidence for a slight production and accumulation internally in sea ice (Figures 4f and 4h). This is most likely representative of microbially mediated humification similar to that observed in deep ocean waters [*Wedborg et al.*, 2007; *Yamashita and Tanoue*, 2008; *Jørgensen et al.*, 2011]. The fact that C360/456 is more prevalent than C325/396 also agrees with the observation by *Jørgensen et al.* [2011], which indicated that C360/456 fluorescence increases 1.3 times faster with oxygen utilization than C325/396.

[27] These results indicate that the accumulated DOM in sea ice brines results from the accumulation of two separate pools, an N-rich and a C-rich pool, differing in their C:N and fluorescence characteristics (Figure 5). The accumulation of the N-rich pool (C:N < 30) was correlated with the fluorescence signals at C275/340, which agrees with the fact that this fluorescence was nearly identical to that of the amino acid tryptophan. The net accumulated DOC concentration for this N-rich pool was up to approximately 900 μmol L^{-1}. The other fraction was comparatively C-rich (C:N > 30) and did not correlate with any of the fluorescence signals. With net accumulated DOC concentrations of up to 200 μmol L^{-1}, this C-rich pool is likely to represent EPS material. In addition, the accumulation of the humic-like fluorescence signal of C360/456 in the brines suggests the occurrence of microbial humification in sea ice. The average intensity of the fluorescence of C360/456 in the accumulated brine DOM was comparable to deep ocean levels, where this signal is produced over timescales from decades to centuries [*Yamashita and Tanoue*, 2008; *Jørgensen et al.*, 2011]. It is apparent that the production of this fluorescence signal in sea ice occurs at much faster timescales (months) than the deep ocean, reflecting the rapid turnover of DOM by the sympagic microbial loop.

[28] Fluorescence spectroscopy provides a useful tool for investigating different fractions of sea ice DOM. Combined with DOC and DON measurements, it offers a rapid approach suitable for assessing the development of the DOM character, and the effects of subsequent sympagic biological production on it during ice formation and growth in polar oceans. Three distinct pools were identified in the current samples: proteinaceous, carbohydrate, and humic-like. The greatest DOC concentrations were associated with the accumulation of fluorescing proteinaceous material. Accumulation of carbon-rich, nonfluorescent DOM material, consistent with the internal production of carbohydrates as EPS by sympagic organisms, was also identified and was apparently decoupled from both the amino acid and humic accumulation. The net accumulation of DOM in sea ice brines and the chemical characteristics of this material are shaped by the thermal, compositional, and biological history of the sea ice. At present, this is difficult to disentangle, but it is clear that fluorescence spectroscopy is a useful approach for tracing both the retention of seawater DOM in brines during ice formation and the effects of subsequent biological activity by sea ice organisms in the internal brine DOM pool.

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