FLUORESCENCE OF DISSOLVED ORGANIC MATTER IN SEAWATER AT LOW TEMPERATURES AND DURING ICE FORMATION

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ABSTRACT

Measuring the chromophoric fraction of dissolved organic matter (CDOM) in natural waters using its fluorescence signal is extremely useful in a variety of marine and freshwater applications. The aim of this research was to study the changes in CDOM optical properties at low temperatures and during ice formation from seawater. In a laboratory study it was found that CDOM fluorescence intensity reversibly decreases with increasing water temperature. The study of the temperature effect is important for future calibration of lidar data measured in regions with horizontal temperature gradients, and for correction of *in situ* measured CDOM fluorescence depth profiles.

Changes in CDOM fluorescence due to variations in concentration and possibly composition under controlled conditions of ice cover developing were studied during experiments using artificial ice sheets produced in large-scale tank experiments. Fluorescence emission, fluorescence excitation and absorption spectra of filtered samples from ice, ice brine and melted ice were measured in the laboratory at fixed room temperature. Sea ice brines showed high fluorescence signals due to the accumulation of CDOM in channels filled with brine during ice growth. CDOM fluorescence slightly increased in seawater underlying the ice sheet due to exclusion of DOM from ice. Ice samples separated from brine had low fluorescence signals. Humic-type fluorescence intensity (270, 308 or 355 nm excitation) was directly proportional to salinity as well as to dissolved organic carbon (DOC) concentrations in many samples of ice to highly concentrated brine.

Keywords: CDOM, seawater fluorescence, ice, brine

INTRODUCTION

Measuring the chromophoric fraction of dissolved organic matter (gelbstoff, yellow substance, CDOM) in natural waters using its fluorescence signal is extremely useful in a variety of marine and freshwater applications (see, for example, (1,2,3,4,5)). In remote sensing, measurements of CDOM distributions are of interest due to their effect on light propagation in surface waters but also due to their characteristics to represent natural water mass tracers. In oligotrophic and mesotrophic waters, dissolved organic carbon (DOC) is usually the largest pool of organic carbon and is an important constituent of the biogeochemical carbon cycle. Moreover, CDOM is photochemically active and can be photo-oxidised to lower molecular weight components that are then available to the microbial food web.

Because CDOM contributes significantly to the absorption of coastal waters, its presence substantially impacts the underwater light field. Thus an understanding of its sources and sinks, as well as the nature of the light-absorbing constituents is needed to predict its spatial and temporal variability in coastal (and open-ocean) waters.

Our interest to study the CDOM in water during ice formation is caused by the fact that sea ice at its maximum extent covers more than 13% of the earth's surface, and is one of the largest biomes on earth (6). Unlike freshwater ice, frozen seawater forms a semi-solid matrix permeated by a net-

work of channels and pores filled with concentrated brine. These channels are home to the microscopic organisms trapped in the sea-ice so not only do the organisms have to survive their incarceration into a semi-solid matrix, they also have to survive drastic chemical changes to their surroundings (7). Sea ice is dominated by strong gradients in temperature, salinity, space and light, although salinity is the dominant factor in external chemistry to influence the biological assemblages within the sea ice. Hyperoxic brines depleted in carbon dioxide with high DOM content, low concentrations of nutrients and elevated pH levels are all a consequence of biological activity within the confined system (8,9).

Temperature directly influences the fluorescence efficiency. The study of temperature effects is important for further calibration of fluorescence lidar data (see, for example, (10,11)) measured in regions with horizontal temperature gradients, and for the correction of *in situ* measured depth profiles of gelbstoff fluorescence. The objective of the investigations was to find answers for the following questions:

- do variations of the water temperature affect the measured fluorescence intensities?
- are there any changes in the fluorescence band-shape due to temperature variations?
- is the effect of temperature reversible?
- does freezing and melting of samples affect the fluorescence intensity and band-shape; can previously frozen samples be used to estimate the original CDOM content in water?

Changes in CDOM optical response due to alteration of its content and composition at low temperatures were studied during the ARCTECLAB experiment (Hamburg Ship Model Basin, May 2001) under controlled conditions of ice cover developing from seawater which was enriched with marine plankton cultures. The aim of the ARCTECLAB experiment was to determine the factors initiating and controlling the specific chemistry within sea ice that are evidently associated with sea ice diatom growth. This is a vital link for an understanding and interpretation of field measurements. It is well known that salts and inorganic nutrients are expelled from the sea ice crystal lattice during ice formation. The brine is enriched in salts and inorganic nutrients. Since DOM is also concentrated, one would expect that CDOM is also concentrated in brine. The questions we intended to answer by means of fluorescence measurements were the following

- how is CDOM portioning between seawater, ice and brine?
- what is a relationship of CDOM fluorescence to DOC in phases of ice and water?
- what is the relationship of CDOM fluorescence to other chemical parameters?
- are there any changes in chromophoric composition of DOM in water, ice and brine samples?

Fluorescence and absorption spectra were measured in filtered samples of seawater under ice, brine from the ice and melted ice in laboratory at fixed room temperature. The optical properties of the samples were compared with their chemical parameters.

METHODS

ARCTECLAB experiment

Controlled incubations of artificial seawater (Instant Ocean Salts) were set up concurrently in four 4 m^3 custom-made polyethylene tanks, which were placed in a large experimental basin at Hamburg Ship Model Basin (HSVA), Hamburg, Germany (http://www.hsva.de). The seawater was enriched with inorganic nutrients to concentrations of 680-770 µM dissolved inorganic nitrogen, 60-200 µM dissolved silicate and ~40 µM dissolved inorganic phosporus (12). Further details of the experimental facilities are described in (13). Three tanks (A, B and C) were inoculated with a dense, non-axenic Antarctic diatom culture (*Fragilariopsis cylindrus*), resulting in an initial chlorophyll *a* concentration of ~11 µg L^{-1} (13) and a particulate organic carbon (POC) concentration of 500 µg C L^{-1} (~40 µM; unpublished data). Tank D was free of diatoms. Tanks A and B were kept at an ambient

irradiance of 34 µmol photons m^{-2} s^{-1}, while the irradiance level over tank C was kept at approximately half that value. The cultures were allowed to establish in the seawater for 7 days at air temperature of 0°C. Then ice growth was initiated by lowering the air temperature to (-10 ± 2)°C, facilitated by fine water spraying over the seawater surface. During the subsequent 18 days these freezing conditions were maintained, and the thickness of the ice sheet increased steadily at a rate of 0.54 mm h^{-1} to 25-27 cm by the end of the experiment (13). The maximum temperature gradient through the ice sheet ranged from -6.7°C in its top 4 cm to -2.2°C near the ice-seawater interface. The seawater before freezing, and, thereafter, underneath the closed ice cover, was completely mixed at all times by using electric water pumps without filters.

Sampling and Analytical Methods

Sampling and data from the ice sheet and the under-ice seawater are reported with respect to the day of the onset of ice formation (*t*=0). The ice sheet was sampled by manually drilling ice cores with a 12 cm (external diameter) ice auger on *t* = 2, 4, 9, 15 days (tanks B and C) and 2, 4, 9, 16 days (tanks A and D). The ice cores from the first two time points were treated as single sections of ~5 cm and ~7 cm respectively, while the ice cores from the final two time points were sectioned in two (*t*=9) and three ~7 cm slices (*t*=15 and 16). The ice sections were centrifuged at 1500 rpm and -5°C for 15 min to separate the brine inclusions from the solid ice matrix (14). Additional sampling of brine inclusions was done on t = 8, 11 and 18 days in all tanks by drilling shallow (~7 cm) sackholes through the surface of the ice sheet and allowing brine from adjacent brine channels and pockets to seep into the sackhole for 30-60 min (hereafter, shallow sackhole brine), with the hole covered with a plastic lid (15). Brine was obtained also from a deeper (~15 cm) sackhole on the final day of the incubation (*t*=18). The salinity of the seawater and brine inclusions was measured with a WTW LF 191 conductivity meter (12).

Samples for dissolved organic carbon (DOC) and CDOM measurements were taken manually by immersing 20 mL plastic syringes below the surface of the seawater through openings in the ice sheet, into sackhole brine, or into the centrifuged brine immediately after centrifugation of ice core segments. The samples were immediately filtered through glass fibre syringe filters (Whatman GF/D, 0.45 µm). DOC samples were sealed in pre-combusted glass ampoules and preserved at -20°C until analysis by high temperature oxidation on an MQ 1001 total organic carbon analyser (16).

Spectral measurements

Spectral measurements were done on filtered samples of seawater, brine and melted ice in the laboratory at fixed room temperature. Emission and excitation fluorescence spectra were measured using a Perkin Elmer Model LS50 luminescence spectrometer (parameters are given in Table 1).

Table 1: Parameters of excitation and emission registration used for spectral measurements of CDOM fluorescence with its fluorescence bands attribution.

Type of scan	Excitation wavelength, nm	Emission wavelength, nm	Excitation /emission slit width, nm	Scanning speed, nm/min	Fluorescence band attribution
Emission	230	235…445	7/7	100	Protein-type and humic-type
Emission	270	280…525	7/7	100	Protein-type and humic-type
Emission	308	320…550	7/7	100	Marine humic-type
Emission	355	370…550	7/7	100	Humic-type
Excitation	220…285	300	7/7	50	Tyrosine-type
Excitation	220…335	350	7/7	50	Tryptophan-type
Excitation	200…365	380	7/7	100	Marine humic-type
Excitation	230…425	440	7/7	100	Humic-type

The temperature effect on fluorescence spectra was studied in the laboratory with a Perkin Elmer Model LS50 luminescence spectrometer. Water samples were filled in a 1-cm-pathlength quartz cuvette, while temperature was controlled by pumping water at a given temperature through the metallic cuvette-holder. The sample temperature was measured with an electronic temperature sensor with an accuracy of 0.05°C. The temperature was altered within a range of 6 to 70°C. During fluorescence measurements the temperature was stabilised within 0.1°C at 20°C, and 0.5°C at the highest and lowest temperature values. Original and filtered seawater samples were investigated. Filtration was done with glass-fibre filters (Schleicher & Schuell GF 50) which were pre-combusted at 450°C for 6 hours, or pre-washed cellulose acetate filters with 0.2 µm pore size (Sartorius).

Absorption spectra were measured with a Perkin Elmer Lambda18 spectrophotometer using a 1-cm optical path quartz cuvette. As a reference sample for absorption measurement, water purified with the SeraDest system was used showing no fluorescence emission signal at all used wavelengths of excitation.

RESULTS

Absorption spectra of DOM seawater, brine and ice samples

Absorption of samples increased at shorter wavelengths in the UV range, having a shoulder at 270 to 300 nm. Absorption for melted ice samples was marginal for wavelengths longer 240 nm. Absorbance values measured at certain wavelengths increased from seawater to brine, and within brine samples, from samples taken from the bottom ice section to the top ice section. Absorption of seawater samples did not change during the experiment, whereas that of brine increased throughout the experiment. In Figure 1 absorption spectra are shown for the samples taken at the end of the ARCTECLAB experiment in tank A.

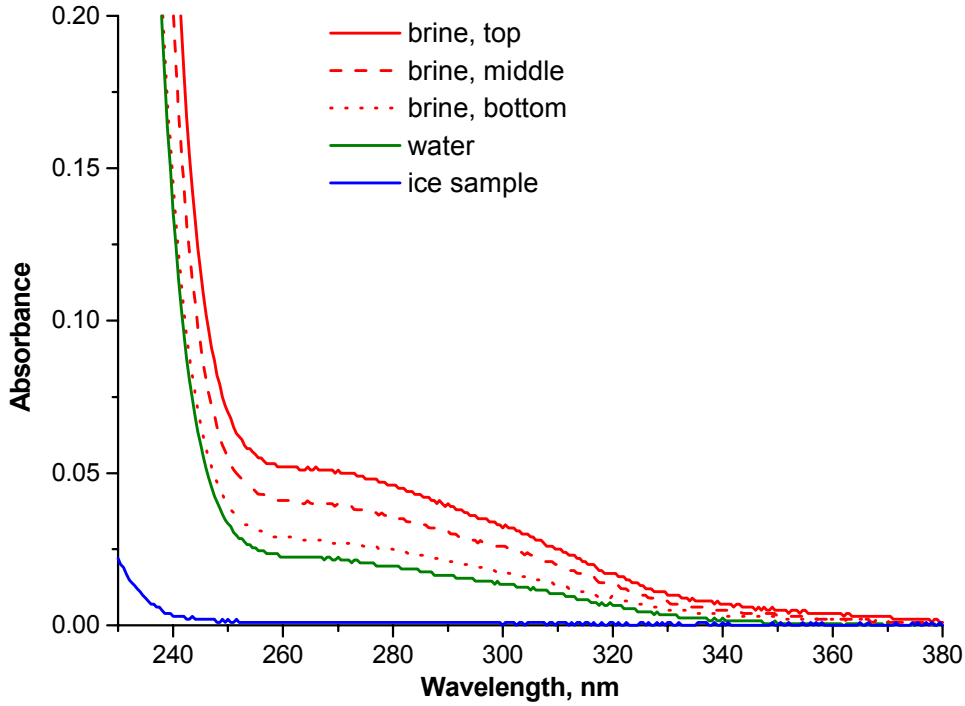

Figure 1: Absorbance spectra for samples taken at the end of the ARCTECLAB experiment. The optical path length is 1 cm.

Fluorescence of seawater, brine and ice samples excited at different wavelength

Typical fluorescence spectra of seawater, ice and brine samples excited at different wavelengths are shown in Figure 2. The position of the water Raman scattering peak was dependent on the excitation wavelength.

There are three main components in typical fluorescence spectra of natural water samples. The band with a maximum at 420 to 450 nm represents the emission of yellow substance, or gelbstoff (1,5). This band is also called as humic-type fluorescence (2). The tryptophan- and tyrosine-type fluorescence bands have maxima at 345 and 295 nm in relation to the emission maxima of the corresponding amino acids (3). These two emission bands can be excited at 270 nm or shorter wavelengths. Both tryptophan-type and tyrosine-type fluorescence bands can be denoted as protein-type fluorescence since they resemble well the fluorescence emission of proteins in organisms.

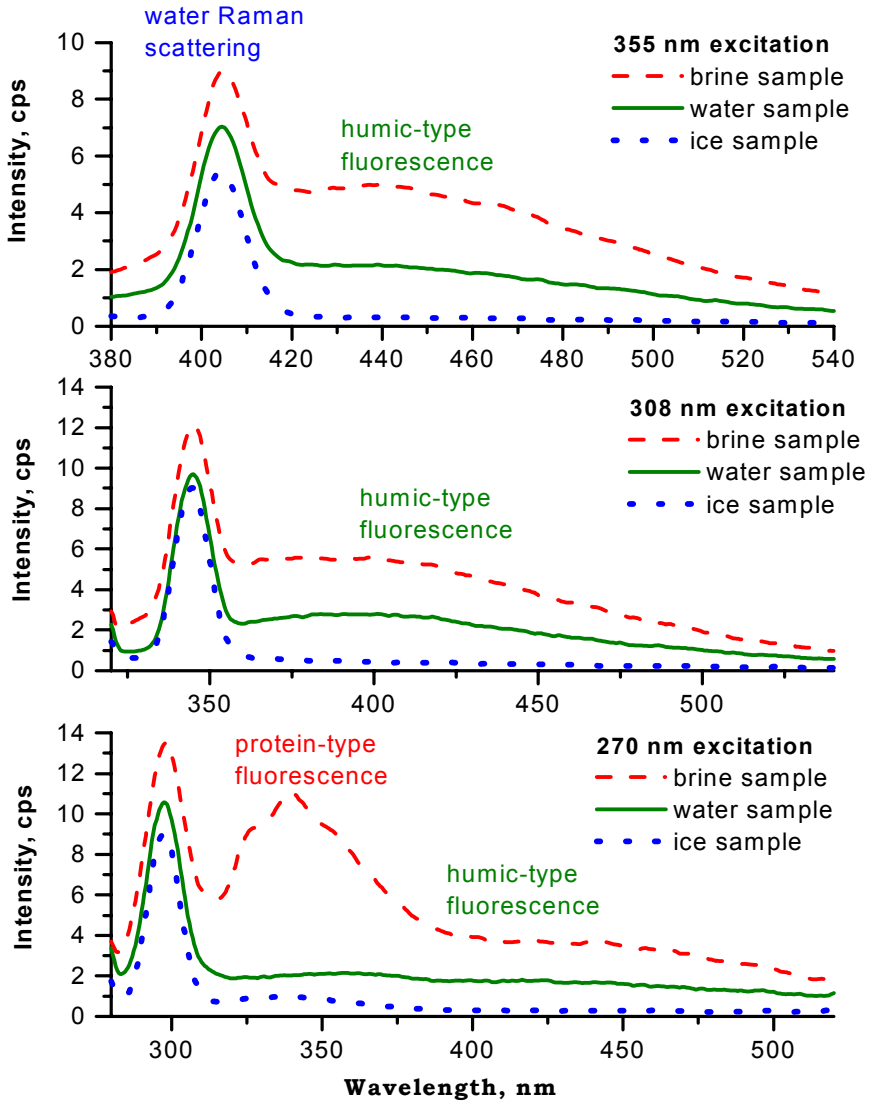

Figure 2: CDOM fluorescence spectra for samples taken at the end of the ARCTECLAB experiment.

Temperature dependence: humic-type fluorescence

The CDOM fluorescence intensity was found to decrease with rising temperature. A range of 6 to 70°C was used to find the temperature coefficient for humic-type fluorescence. The temperature dependence of fluorescence intensity normalised by the intensity of water Raman scattering is shown in Figure 3.

A linear fit of the normalised fluorescence intensity versus temperature yields a mean value of the temperature coefficient of -1.06 10^{-4} /°C. This corresponds to -0.45% /°C relative to the intensity at 20°C. In experiments at sea one typically finds variations in water temperature through the column of about 10…15°C, which, assuming an invariant CDOM concentration, causes relative changes of up to 7% in fluorescence intensity.

The estimated error of fluorescence readings was about 2%, if we take into consideration only the data measured during each step of the experiment, either heating or cooling of the sample. The duration of each step was about 3h. The full duration of the experiment was 9h, and at the end the CDOM fluorescence was the same within 10% as at the beginning, the slight changes possibly occurring from biological processes in the unfiltered sample. Thus, the temperature effect of CDOM fluorescence is reversible within 2% if measurements are done within short times where the DOM content remains invariant. Changes in the CDOM fluorescence band-shape were not observed due to temperature variation within the temperature range of 6 to 70°C.

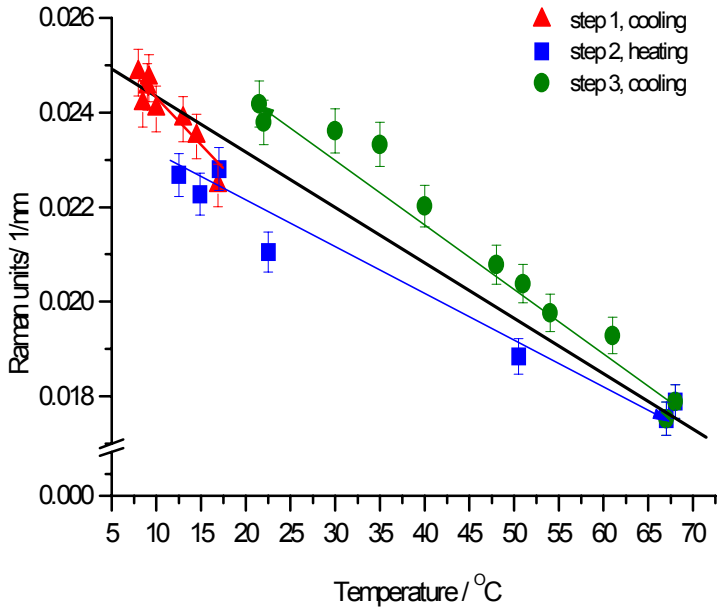

Figure 3: Temperature dependence of humic-type fluorescence intensity normalized by water Raman scattering. Different colours show steps of the experiment: cooling from room temperature to 6°C, heating up to 70°C, and then cooling back to room temperature.

Temperature dependence: protein-type fluorescence

Protein-type fluorescence at 300 to 350 nm was measured in unfiltered seawater samples at 230 or 270 nm excitation. The temperature coefficient of protein-type fluorescence varied from -0.5 to -1.0% /°C for different samples. The estimated error in these measurements is higher than for humic-type fluorescence because, in addition to temperature-dependent fluorescence quenching, other (physical-chemical, biological) processes in water can change the true fluorophore content.

As with the humic-type, the temperature coefficient of protein-type fluorescence is negative, but it differs from that of humic-type fluorescence. Assuming a constant fluorophore concentration, a temperature variation through the water column of 10 to 15°C would cause changes of up to 15% in protein-like fluorescence intensity. The difference in the temperature coefficient of humic-type and protein-type fluorescence affects their intensity ratio. These changes due to temperature variation are marginal from the point of view of absolute intensity measurements for DOM mapping used in remote sensing, but could be noticeable when fluorescence data are used for DOM type characterization.

Frozen and melted samples: fluorescence intensity and band-shape

To answer the question in which way freezing of water samples would affect the fluorescence of water samples we studied previously frozen and melted samples of seawater (filtered and unfiltered). Both the fluorescence band-shape and fluorescence intensity of CDOM were shown to remain constant after freezing, melting and re-adjustment of the temperature to its initial value (see Figure 4). Hence, freezing of samples does not affect the estimation of CDOM concentrations with fluorescence spectroscopy.

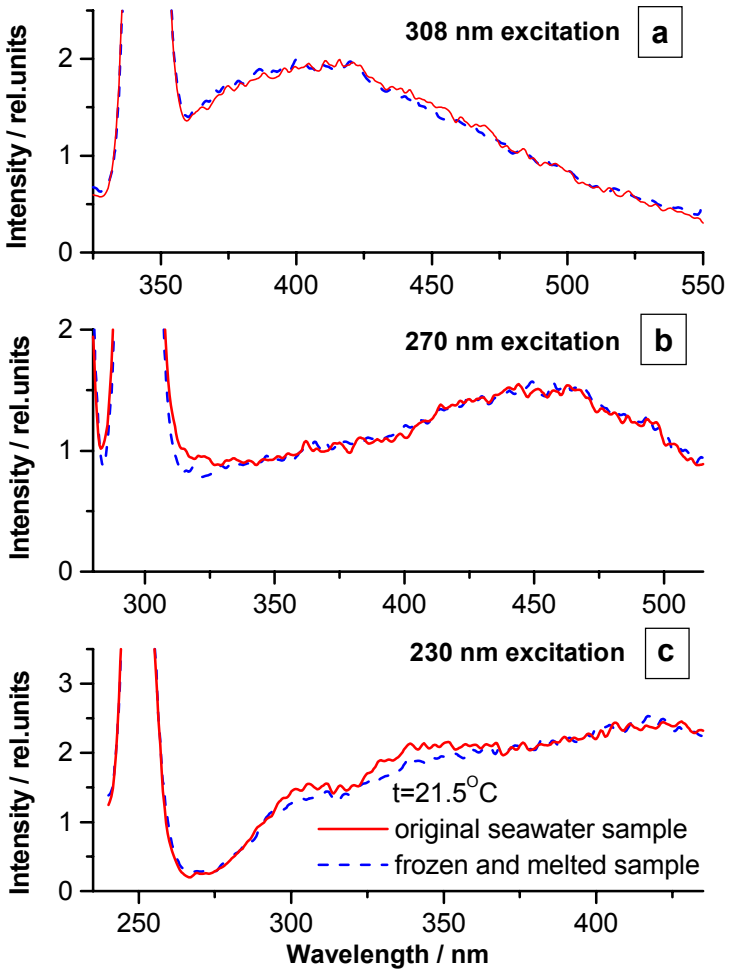

Figure 4. Fluorescence spectra of the original and previously frozen seawater samples.

ARCTECLAB experiment: CDOM fluorescence in seawater, brine and ice samples

Raman normalised fluorescence intensities were measured for humic-type fluorescence excited at 308 nm (emission at 410 nm) and 355 nm (emission at 440 nm) as suggested in (3). The results are shown in Figure 5.

In all four tanks the fluorescence of ice samples was very low and decreased during the experiment. The CDOM fluorescence in seawater samples did not change significantly during the sampling period. The fluorescence levels of brine samples were much higher than that for seawater, and increased rapidly with ice growth in all the tanks. The brine fluorescence levels show large variations, as it was observed with salinity and DOC concentrations. Higher levels of CDOM fluorescence as well as higher levels of salinity and DOC were found in brine samples from the top layer of the ice cover. Correspondingly, the lowest levels of CDOM fluorescence, salinity and DOC were observed for brine samples from bottom layers of ice.

CDOM correlation with DOC concentration and salinity

To check the relationship between salinity and DOC concentration and fluorescence intensity we analysed the data sets with different levels of salinity and DOC – from ice to top brine samples – for each tank. The large variety of samples from ice to highly concentrated brine showed a statistically positive relationship of fluorescence intensities (ex/em 308/410), salinity and DOC with an r^2 value of 0.91 and 0.87 correspondingly for tank A, 0.80 and 0.86 for tank B, 0.80 and 0.91 for tank C and 0.86 and 0.85 for tank D.

Also other excitation wavelengths (ex/em 270/440 and 355/440) gave a statistically significant linear relationship of humic-type fluorescence intensities with both salinity and DOC, with r^2 values of

about 0.8-0.9. Hence all three wavelengths excite the same type of chromophore responsible for the blue humic-like fluorescence.

In contrast to this, the correlation of fluorescence intensities (ex/em 270/440, 270/340, 308/380, 308/410, and 355/440) with other biochemical parameters (particulate organic carbon, particulate organic nitrogen, chlorophyll *a* and phaeophytin concentrations, pH, dissolved oxygen) was rather poor, with r^2 values less than 0.3. It is known from the literature (17) that changes in CDOM fluorescence properties do not reflect synchronously but with a time lag biological processes in water.

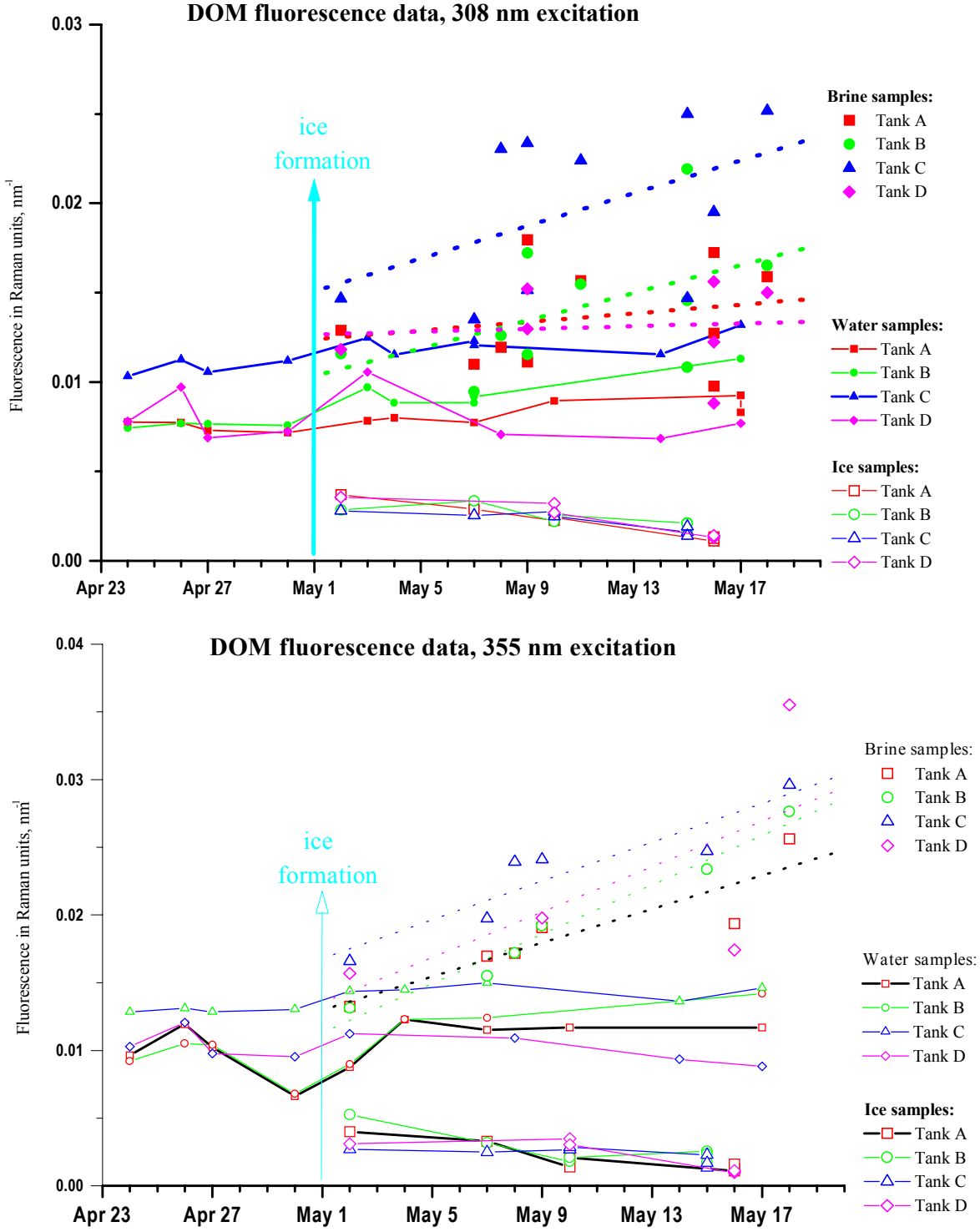

Figure 5. CDOM fluorescence intensities during the ARCTECLAB experiment. The date of ice formation is marked. 308 nm (top picture) and 355 nm excitation (bottom picture).

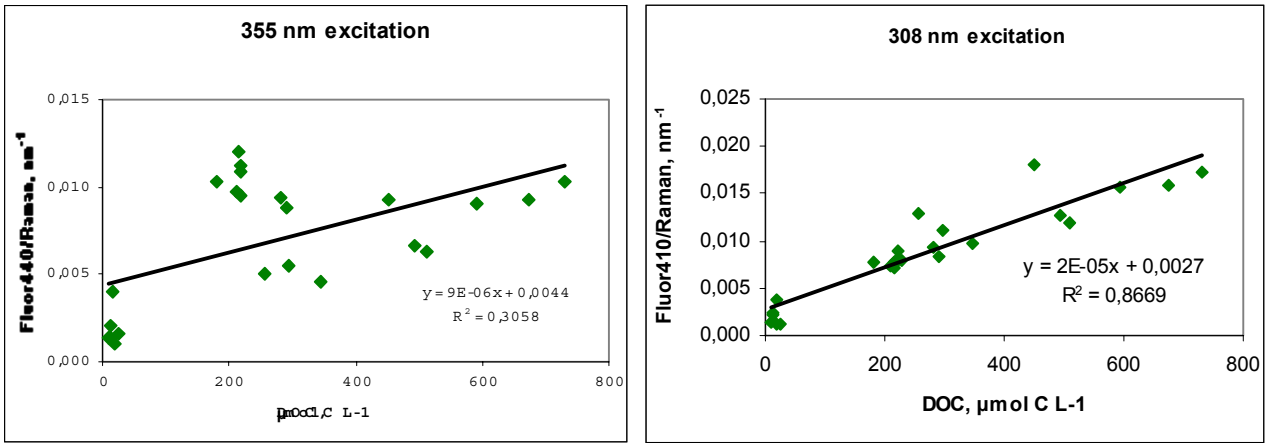

Figure 6. Fluorescence intensities versus DOC concentration for tank A, excitation at 355 and 308 nm.

DISCUSSION

Temperature effect on CDOM fluorescence

A linear fit of the normalised humic-type fluorescence versus temperature in the range of 6 to 70°C gives a mean value of -0.45% /°C of the temperature coefficient of intensity with respect to the intensity measured at 20°C.

A correction of the temperature dependence of fluorescence intensities can be accomplished using the following formula:

$$\frac{flu(\theta)}{flu(\theta_o)} = 1 + TC_{flu}(\theta - \theta_o) \ ,$$

where $flu(\theta)$ and $flu(\theta_o)$ represent the fluorescence signal measured at temperatures θ and θ_0 in °C, and TC_{flu} is the temperature coefficient. With θ_0 = 0°C it follows TC_{flu} = 0.0042. With θ_0 = 20°C one has TC_{flu} = 0.0045.

It has been found previously (18) that the temperature dependence of intensity for both emission and excitation spectra is well described by an exponential function $I=I_0 \exp(-a(\theta - \theta_0))$. At θ_0 = 20°C one finds a=0.0082 for emission spectra (337 nm excitation) and a=0.0078 for excitation spectra (425 nm excitation). Figure 7 presents a fitting of the results of two experiments, i.e. a linear fit for the data given here (experiment 1), and an exponential fit for the data reported earlier ((18), experiment 2). The slope of the curve for experiment 2 is higher than for the results given in this paper. This might be due to a different origin of the samples. In this paper an unfiltered seawater sample from 1000 m depth was used, while a Baltic Sea sample with increased gelbstoff content prepared using ultrafiltration had been investigated in experiment 2.

Generally, a temperature increase results in a decrease in the fluorescence quantum yield. This holds also for the fluorescence lifetime since non-radiative processes of excited states related to thermal agitation are more efficient at higher temperatures. Experiments are often in good agreement with an empirical linear variation such as ln [1 / (flu (T) -1)] versus 1/T, with the absolute temperature T in Kelvin (19). This is graphically shown in Figure 7, where the fluorescence intensity is set to 1 at 0°C. The data in the temperature range of 0 to 70°C can be well fitted by a linear function. This indicates that our experimental data can be well described by using a linear fit. However, the slope of the curve given by the Valeur relation (19) is much smaller than for the experimental data. Hence, in addition to a thermal quenching of fluorescence other mechanisms might influence the CDOM fluorescence as well. This could be aggregation of DOM in larger aggregates at low temperatures, or changes in its macromolecular conformation. An understanding of processes,

which take place in natural water and affect its fluorescence spectra, requires further experimental work.

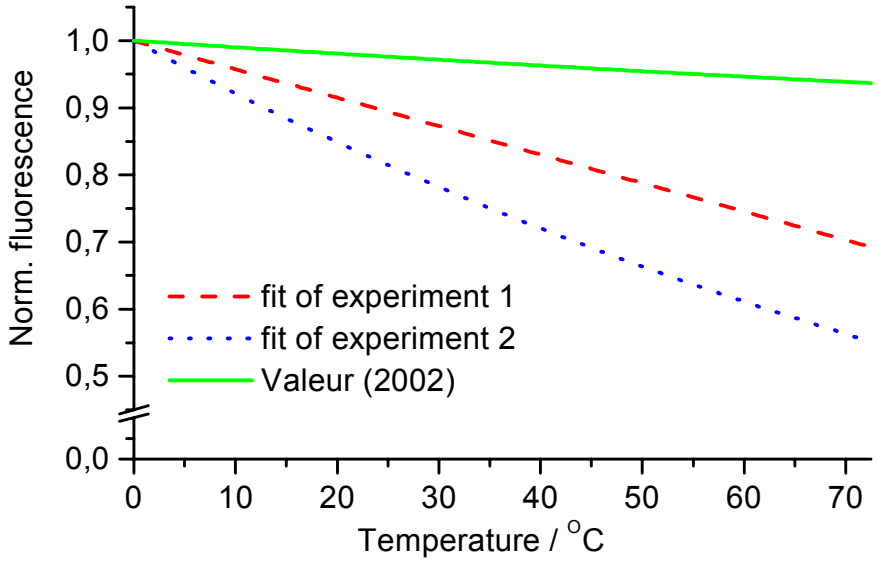

Figure 7: Fit of experimental data and theoretical values for intensity in relative units. Further explanations are given in the text.

Partitioning of CDOM between seawater, brine and ice during freeze-up experiment

CDOM fluorescence has been measured in water column samples from a variety of aquatic ecosystems. Much less is known, however, about the CDOM fluorescence in ice and brine, and the portioning and CDOM compositional transformations during ice growing from seawater.

It is known that gases, ions, and nutrients are extracted from ice during freezing and that this exclusion is more effective when ice growth is slow. Sea ice is often enriched in CDOM or DOC through algae production in ice and the release of algal exudates (20,21). In the experiment reported here we observed a continuous increase of humic-type fluorescence levels in brine during freezing, while the fluorescence in the liquid seawater remained quite stable. The brine samples from top ice layers showed higher levels of CDOM fluorescence signals than those from bottom ice layers, and fluorescence for the latter was higher than for the underlying seawater. All ice samples had low signals for the humic-type fluorescence, and showed a trend to decrease during ice growing. Typically, upper ice samples had smaller fluorescence levels than bottom ice samples.

The variety of samples with different levels of salt and DOC concentration – from ice to top brine samples demonstrates a statistically positive relationship of humic-type fluorescence intensities (ex/em 270/440, 308/410 and 355/440) versus both salinity and DOC. The correlation of CDOM fluorescence intensities with other biochemical parameters (particulate organic carbon, particulate organic nitrogen, chlorophyll a and phaeophytin concentrations, pH, dissolved oxygen) was rather low. We assume this fact as evidence that humic-type fluorescence in our experiment did not result from biological activity of microorganisms trapped in the ice.

CONCLUSIONS

The CDOM fluorescence intensity can be used in remote sensing with fluorescence lidar as a measure of CDOM concentrations with good accuracy, if the effect of fluorescence quenching by temperature is taken into consideration. The humic-type fluorescence is, on the average, decreasing by 0.5% /°C. Intensity changes are practically reversible. The fluorescence band-shape remains invariant.

CDOM fluorescence band-shape and intensity are constant after freezing and melting of the samples, and with the temperature adjusted to the initial temperature.

The changes in CDOM fluorescence response due to alteration of its concentration and possibly composition under controlled conditions of ice cover development were studied during the ARCTECLAB experiment. Fluorescence emission, fluorescence excitation and absorption of filtered seawater samples under ice, brine and melted ice were measured in the laboratory at fixed room temperature. Brine samples show high fluorescence signals due to the process of CDOM concentration increase in channels filled with brine during ice growing. CDOM fluorescence is slightly increased for seawater samples due to exclusion of DOM from ice into underlying water.

Ice samples separated from brine had low fluorescence signals. The humic-type fluorescence intensity (270, 308 or 355 nm excitation) is linearly proportional to salinity as well to DOC in a large variety of samples from ice to highly concentrated brine.

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