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Temperature dependence of fluorescence spectra of natural organic matter in seawater

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ABSTRACT: Water temperature is one of the environmental factors influencing fluorescence of chromophoric dissolved organic matter (CDOM) in natural waters. The study of the temperature effect on gelbstoff fluorescence is important for further calibration of lidar data measured in regions with horizontal temperature gradients, and for correction of *in situ* measured gelbstoff fluorescence depth profiles. Fluorescence spectra of naturally occurring organic matter in seawater were studied in the laboratory at temperatures from 6 to 70°C using a Perkin Elmer LS50 luminescence spectrometer.

Fluorescence intensity is decreasing with rising temperature; this temperature effect is practically reversible. For gelbstoff fluorescence excited at 230, 270 or 308 nm with maximum emission at 410…450 nm the temperature coefficient of fluorescence is (0.5 ± 0.1)% / °C. No changes in the gelbstoff fluorescence band-shape were observed due to temperature. Gelbstoff fluorescence band-shape and intensity keep also constant after freezing and melting of samples, and re-adjustment to the initial temperature. Hence, the intensity of gelbstoff fluorescence can be used in remote sensing using laser spectroscopy (fluorescence lidar) as a measure of the gelbstoff content with good accuracy, if the effect of fluorescence quenching by temperature is taken into consideration.

The temperature coefficient of protein-like fluorescence observed under 230 or 270 nm excitation with maximum emission at 300…350 nm is varying for different samples from 0.5 % to 1.0% / °C. The uncertainty of the data for protein-like fluorescence is higher than for gelbstoff fluorescence because other than temperature-dependent fluorescence quenching processes (physical-chemical, biological) can influence the fluorescence signal.

1 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, Kalle, 1966; Mopper and Schultz, 1993; Determann et al, 1994, Belin et al, 1994; De Souza Sierra, 1994). In remote sensing, measurements of gelbstoff distributions are of interest due to their effect on daylight in surface waters, and as a natural water mass tracer.

Water temperature is one of the environmental factors influencing fluorescence efficiency. The study of temperature effects on fluorescence is important for further calibration of fluorescence lidar data (see, for example, Measures, 1984; Reuter, 2001) measured in regions with horizontal temperature gradients, and for the correction of *in situ* measured gelbstoff fluorescence depth profiles.

The objective of the investigations was to find the 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? Can previously frozen samples be used to estimate the original CDOM concentration?

2 METHODS AND MATERIALS

Fluorescence spectra were studied in the laboratory with a Perkin Elmer Model LS50 luminescence spectrometer. The water samples were filled in a 1-cm 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 the range of 6…70°C. During fluorescence measurements the temperature was stabilised within 0.1 °C at 20 °C, and 0.5 °C at the minimal and maximal temperature values.

Original and filtered seawater samples were investigated. Filtration was done with glass-fibre filters (GF 50, Schleicher & Schuell) which were pre-combusted at 450 °C during 6 hours, or pre-washed cellulose acetate filters with pore size 0.2 µm (Sartorius).

A seawater sample taken in January 1996 in the North Atlantic Ocean from 1000 m depth and stored in refrigerator was used for fluorescence measurements, and as a medium to grow marine algae. A culture of *Amphidinium Carterer* was grown during 1 month. After storage in the refrigerator at 6 °C during 9 months it was filtered to prepare a sample of dissolved organic matter of marine type.

3 EXPERIMENTAL RESULTS

3.1 *Fluorescence of seawater samples*

The typical fluorescence response of seawater excited at different excitation wavelengths is shown in Fig. 1 for a sample of dissolved organic matter produced by algae. The position of water Raman scattering peak is dependent on the excitation wavelength.

There are three main components in typical fluorescence spectra of natural water samples. The fluorescence band with maximum at 420…440 nm represents the emission of yellow substance, or gelbstoff (Kalle, 1966; Belin at al, 1994). The tryptophan- and tyrosine-like fluorescence bands have maxima at 345 and 295 nm in relation to the emission maxima of the corresponding amino acids (Determann et al, 1994, Patsayeva and Reuter, 1995). These two emission bands can be excited at wavelengths of 270 nm or shorter. They are well distinguished in Fig. 1c as Gaussian components of the integral fluorescence spectrum of seawater DOM.

Both tryptophan-like and tyrosine-like fluorescence bands can be denoted as protein-like fluorescence since they resemble well fluorescence emission of proteins in organisms.

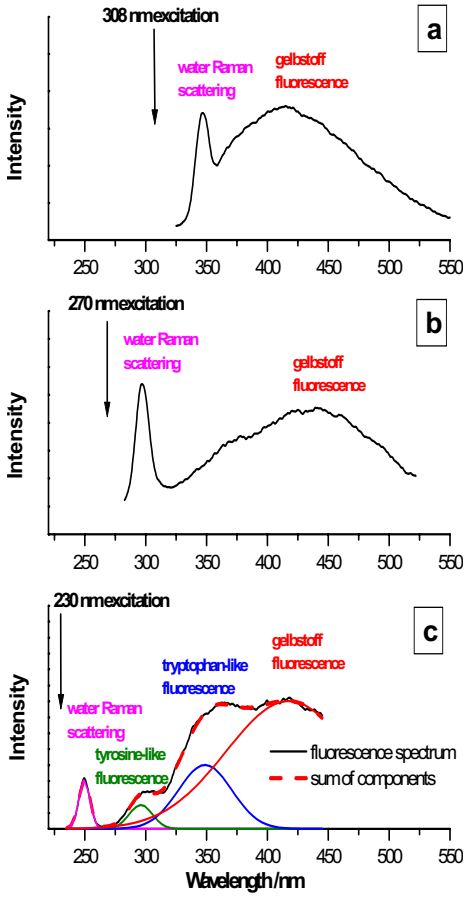

Figure 1. Fluorescence response of dissolved organic matter in filtered seawater excited at 308, 270 and 230 nm.

3.2 *Temperature dependence: gelbstoff fluorescence intensity*

In filtered and unfiltered samples of seawater, gelbstoff fluorescence was detected at 230 (fluorescence maximum at 420 nm), 270 (450) or 308 (410) nm excitation.

The fluorescence intensity of gelbstoff emission was found to decrease with rising temperature. Fig. 2 shows fluorescence spectra of seawater measured at three temperature values.

The full temperature range of 6…70 °C was used to find the temperature coefficient for gelbstoff fluorescence. The temperature dependence of fluorescence intensity normalised by the intensity of water Raman scattering is shown in Fig. 3.

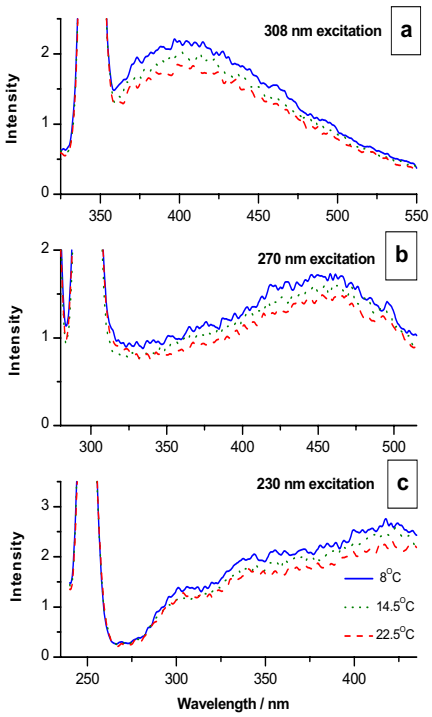

Figure 2. Fluorescence spectra of unfiltered seawater measured at different temperatures.

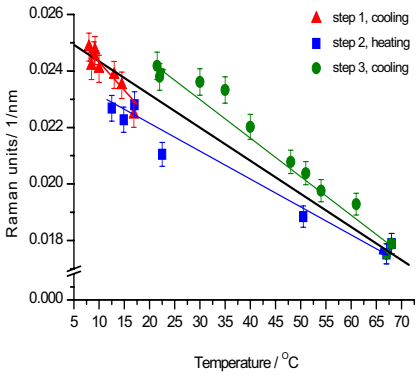

Figure 3. Temperature dependence of fluorescence intensity normalized by water Raman scattering. Different colours show steps of the experiment: cooling from room temperature to 6°C, heating up to 70°C, and then cooling to room temperature.

A linear fit of the normalised fluorescence intensity versus temperature gives the mean value of temperature coefficient of -1.06 10-4 /oC. This corresponds to -0.45% /oC 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 gelbstoff concentration, cause 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 experiment, either heating or cooling of the sample. The duration of each step was about 3h. The full duration of the experiment was 9hs, and at the end the gelbstoff fluorescence was the same as at the beginning within 10%, possibly due to biological processes in the unfiltered sample.

Thus, the temperature effect of gelbstoff fluorescence is reversible with the reproducibility of 2% if we perform measurements within one hour to avoid fast cells growing in natural water sample.

3.3 *Temperature dependence: gelbstoff fluorescence band-shape*

Changes in the gelbstoff fluorescence band-shape were not observed due to temperature variation. Fluorescence spectra measured at different temperatures within the range of 6…70 °C in Fig. 4a-c are normalised by fluorescence intensity at 400 nm to compare their band-shape.

To answer the question what happens with fluorescence of water samples after they have been frozen we studied previously frozen, and then melted samples of sea water (filtered and unfiltered). Both the fluorescence band-shape and fluorescence intensity of CDOM remain constant after the samples were first frozen, then melted, and final adjustment of the temperature to the initial value (see Fig. 5). Hence, freezing of samples does not affect the estimation of CDOM concentrations with fluorescence spectroscopy.

3.4 *Temperature dependence: protein-like fluorescence*

Protein-like fluorescence at 300…350 nm was observed with unfiltered seawater samples at 230 or 270 nm excitation (see Fig. 1b-c, 2b-c). The temperature coefficient of protein-like fluorescence was varying for different samples from -0.5 to -1.0% /°C. The estimated error in these measurements is higher than for gelbstoff fluorescence because, in addition to temperature-dependent fluorescence quenching, other processes (physical-chemical, biological) in natural water can change the true concentration of the fluorophores.

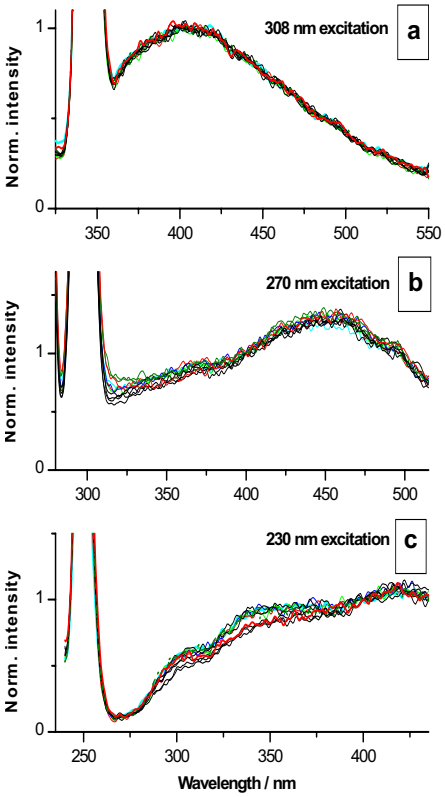

Figure 4. Fluorescence spectra of unfiltered seawater sample measured at different temperatures normalized by the intensity at 400 nm. Each graph presents 12 spectra measured at different temperatures within the range of 6…70 °C with the given excitation wavelength.

As with gelbstoff, the temperature coefficient of protein-like fluorescence is negative, but it differs from that for gelbstoff and is varying for different samples from -0.45 % to -1.0% /°C. Assuming a constant fluorophore concentration, a temperature variation through the water column of 10…15°C causes relative changes up to 15% in protein-like fluorescence intensity.

The difference in the temperature coefficient of gelbstoff and protein-like 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 using remote sensing, but could be noticeable when fluorescence data are used for DOM type characterization. The changes in ratio of gelbstoff to protein-like fluorescence can be seen from fluorescence spectra normalised by gelbstoff intensity at 400 nm (Fig. 4b-c).

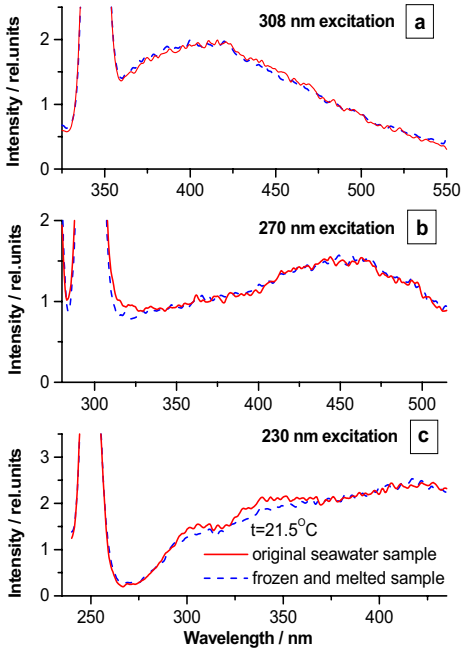

Figure 5. Fluorescence spectra of an original and previously frozen seawater sample.

4 DISCUSSION

A linear fit of the normalised fluorescence intensity of gelbstoff fluorescence versus temperature in the range of 6…70°C gives the mean value of temperature coefficient of –0.45% /°C for intensity changes compared to intensity measured at 20°C.

A correction of the temperature dependence of fluorescence intensities can be done with the following formula:

$$flu\,(\theta) / flu\,(\theta_0) = 1 + CTD\,(\theta - \theta_0)\,,$$

where $flu\,(\theta)$ and $flu\,(\theta_0)$ represent the fluorescence signal measured at temperatures θ and θ_0 in °C, and CTD is the coefficient of temperature dependence. If we set $\theta_0 = 0$°C, then it follows $CTD = 0.0042$. With $\theta_0 = 20$°C one has $CTD = 0.0045$.

Patsayeva (1995) showed that the temperature dependence of intensity both for emission and excitation spectra is well described by an exponential function $I=I_0 \exp(-a(\theta - \theta_0))$. At $\theta_0 = 20$°C one finds a=0.0082 for emission spectra (337 nm excitation) and a = 0.0078 for excitation spectra (425 nm excitation).

Fig. 6 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 published in 1995 (experiment 2).

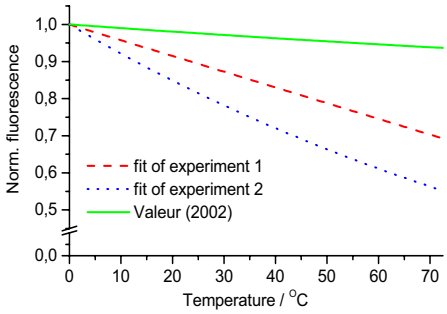

Figure 6. Fitting of experimental data and theoretical values for intensity in relative units. Further explanations are given in the text.

The slope of the curve for the experiment described in (Patsayeva, 1995) is higher than for the results given in this paper. This might be due to a different origin of the samples. In this paper we used an unfiltered sample of seawater from 1000 m depth, while a Baltic Sea sample with increased gelbstoff concentration prepared using ultrafiltration had been investigated in 1995.

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 given in Kelvin (Valeur, 2002). This is graphically shown in Fig. 6, where fluorescence intensity is set to 1 at temperature 0 °C. The data in the temperature range of 0…70 °C can be fitted by a linear function with good accuracy. 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 is much smaller than for the experimental data. Hence, in addition to a trivial thermal quenching of fluorescence other mechanisms might influence the fluorescence spectra of gelbstoff as well. An understanding of processes which take place in natural water and affect its fluorescence spectra requires further experimental work.

5 CONCLUSIONS

The fluorescence intensity of gelbstoff can be used in remote sensing with fluorescence lidar as a measure of gelbstoff concentrations with good accuracy, if the effect of fluorescence quenching by temperature is taken into consideration.

The intensity of gelbstoff fluorescence is, on the average, decreasing by 0.5% /°C. Intensity changes are practically reversible. The fluorescence band-shape remains invariant.

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

The intensity of protein-like fluorescence has a slightly different temperature coefficient compared to gelbstoff fluorescence. This causes variations of the UV-to-blue fluorescence ratio in spectra excited at 270 or 230 nm. The temperature coefficient of protein-like fluorescence at 230 or 270 nm excitation, with a maximum emission at 300…350 nm, is varying for different samples from 0.5 to 1.0% /°C. The estimated error of the temperature coefficient of protein-like fluorescence is higher than gelbstoff fluorescence because other processes than temperature-dependent fluorescence quenching (physical-chemical, biological) occurring in natural water disturb the fluorescence measurements.

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