# SEAWATER DUAL FLUORESCENCE ANALYSIS DURING THE ARCTIC SUMMER 2006 POLISH OCEANOGRAPHIC CAMPAIGN

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## ABSTRACT

The new spectrofluorometer, already developed at the ENEA FISLAS section, was implemented, for the first time, to marine seawater quality monitoring. The apparatus, installed onboard of the Polish oceanographic research vessel *Oceania* performed in-situ measurements along the scheduled transects in the 2006 arctic campaign.

The instrument has been equipped with two excitation lasers at 266 nm and 405 nm, respectively, in order to maximize the fluorescence emission of dissolved organic matter (CDOM, tryptophan, tyrosine) in seawater and phytoplankton pigments (chlorophyll-a, phycoerythrin, phycocyanin), respectively. Furthermore, two filtration stages have been added in the hydraulic circuit. The first acts to block larger interference hydrosols and allows to monitor phytoplankton cells in the sampling cuvette, while the second filter completely stops all particles and the dissolved matter can be analysed in the second cuvette. The protocol adopted allowed for discrimination between algal carotenoids pigments and concurrent organic matter fluorescence. Both cuvettes are optically matched via fibre optic cables to compact spectrum analysers.

The spectrofluorometric data, after proper fitting analysis, have been released in Raman units and successively transformed in absolute values upon comparison with in-situ calibration. Thematic maps of particulate and dissolved organic matter have been released for vertical transects and superficial distributions. Statistical analysis among Chlorophyll-a fluorescence and the other main components (CDOM, tyrosine, tryptophan and carotenoids) put in evidence, a part from a general trend to decrease versus depth for all the substances, the high correlation degree between Chl-a and retrieved carotenoids fluorescence respect to the others.

## INTRODUCTION

The Arctic region is characterized by one of the most extreme environments on the Earth, with limited sunlight, extreme temperatures, and a short growing season. Sea ice, snow cover, glaciers, tundra, permafrost, boreal forests are all case indicators of change, susceptible to subtle variations in sunlight, surface temperature, ocean heat transport, air and ocean chemistry, and the particulate loading of the atmosphere.

Global climate models indicate that global warming induced by the greenhouse effect will be most acute in polar regions, most likely resulting in changes in extent of sea ice, increased permafrost and polar ice masses melting, with profound societal impacts around the globe.

In addition to being an indicator of change in the coupled Earth system, the Arctic plays a key role in many global processes such as global atmosphere and ocean circulation and includes potentially important sources and sinks of trace gases.

In particular, the Arctic marine system is linked to the global ocean and atmosphere by both physical and biogeochemical mechanisms, such as the influence that freshwater outflows from the Arctic have on North Atlantic convection and thermohaline circulation of the world ocean. Arctic Ocean waters are

strongly influenced by biogeochemical processes occurring over the arctic shelves and a synoptic understanding of these processes is essential for trace and predicting the impacts of climate change.

The Arctic Ocean, despite to its economic and environmental importance, it is the smallest and shallowest of the world's five oceans. The greatest inflow of water comes from the Atlantic Ocean by way of the Norwegian Current, which then flows along the Eurasian coast. Water also enters from the Pacific Ocean via the Bering Strait. The East Greenland Current carries the major outflow. The Spitsbergen Current, with cool and fresh glacial water, strongly affects the mixing area of salty and warm water in the Norwegian Seas region up to Svalbard Islands.

Many terrestrial and marine components contain chromophoric chemical group that have the behaviour to emit fluorescence after proper excitation. In plants photosynthetic pigments (carotenoids and chlorophylls contained in leaves), when excited with UV – Visible light, show emission fluorescence in the VIS near Infra Red region, peculiar of the specific groups.

In water, small idrosol components are present, mainly consisting of liquid or solid suspended particles. They are featured by inorganic and organic matter, in last particulate (POM, i.e. phytoplankton) and dissolved organic matter (DOM) can be disentangled. Many of these components exhibit fluorescence emission upon UV excitation as phytoplanktonic pigment (Chlorophyll-a, Chl-a; phycocyanin and phycoerythrin) and chromophoric DOM (CDOM).

The ENEA (Italian National Agency for New Technologies, Energy and the Environment) laser remote sensing laboratory, along more than two decades of activities, has developed different sensors for environmental monitoring and one of its research branch is devoted to fluorescence of natural and pollutant components (1,2). Laser Induced Fluorescence (LIF) spectroscopy has been chosen for its intrinsic skills to be employed in real time water quality parameter determinations, with portable instruments, during intensive monitoring campaigns. This technique allows one to perform qualitative and quantitative in situ determination of dissolved (pollutants, humic and fulvic acids) or particulate (phytoplankton) organic matter.

Former experience in developing and operating a lidar remote sensing apparatus in different marine campaigns, either in European (Adriatic (3) and Swedish (4) sea) and in polar area (Southern Ocean (2) and Antarctic Ross sea (3), was recently applied to develop a new compact laser fluorometer in the frame a desertification project in southern Italy (5).

That compact instrument has been designed for in situ analysis thus allowing a dual laser excitation of sea water samples in order to match the fluorescence analysis of dissolved (protein like compounds, humic acids) and particulate (phytoplankton) organic matter. Furthermore, due to a newly implemented double filtration abetter discriminate between fluorescence contribution of dissolved components from overlapping phytoplanktonic pigments can be achieved. The peculiarities of this new instrument, mainly portable, friendly use, in its first application was employed in inland water quality monitoring campaign, enable its exploitation also for marine applications.

The recent scientific agreement signed between our laboratory and the Institute of Oceanology of the Polish Academy of Science in Sopot (6), put the bases to have the access to the polar research infrastructures (ships) and therefore to have a peculiar test site for new technologies developed in the frame national or international cooperation. But last and not least, it’s a interesting scientific challenge to monitor artic polar marine areas and to compare with the respective Antarctic peculiarities.

Thematic maps of the local data measured during the 2006 Polish arctic campaign, along the different transects, have been released in terms of the investigated seawater optical properties.

## ARCTIC POLAR CRUISE

The Polish Institute of Oceanology conducts regular scientific research in the shelf seas and coastal regions including the Baltic and European Arctic Seas and its main research strategic directions are mainly focused in the determination of the role of the oceans in climate change and its effects for the European Seas, in monitoring natural and anthropogenic variability of the Baltic Sea environment, to

observe contemporary changes of the coastal ecosystems in the shelf seas and finally to follow genetic and physiological mechanisms of functioning marine organisms; principles of marine biotechnology (6).

The area of the Nordic Seas, in the quadrant C4 of the Artic Ocean, from the outermost Norwegian costs passing to the left side of Svalbard isles up to the 80° latitude north was monitored during the 2006 summer oceanographic campaign with the Polish Research Vessel R/V Oceania.

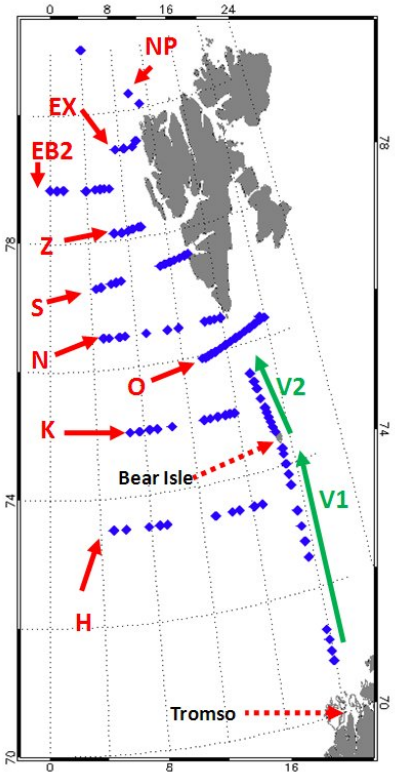


*Figure 1: Map of the stations measured along the 2006 summer cruise with the indication of the different transects.*

*Table 1: List of the transects performed during the arctic campaign.*

| Transect | Stations | Samples | Date |
|---|---|---|---|
| V | 15 | 32 | 20-23/06/06 |
| H | 8 | 29 | 24-27/06/06 |
| K | 11 | 52 | 28-29/06/06 |
| V2 | 11 | 23 | 30/06/06 |
| O | 16 | 50 | 01-02/07/06 |
| N | 13 | 48 | 06-08/07/06 |
| S | 12 | 38 | 09-10/07/06 |
| Z | 7 | 23 | 12/07/06 |
| EB2 | 3 | 12 | 12/07/06 |
| NP | 4 | 12 | 15-16/07/06 |
| EX | 3 | 9 | 17/07/06 |
| **Total** | **103** | **328** | **28 days** |

The R/V Oceania started its cruise from Tromso harbour 19th of June 2006 sailing towards the Svalbard isles performing the planned transects (Table 1). First stop was foreseen the 5th of July in the

Longyearbyen port for logistics and to allows a turn over of the scientific personnel. Afterwards, the cruise followed in the northern area and came back in Longyearbyen the 20th of July, where our colleagues left the vessel. The ship returned back in Tromso 5th of August 2006 ending the campaign.

Therefore our scientific activity was focused operating the portable fluorometer along 11 transects, with 103 stations and 328 samples collected and measured along 28 days of cruising. Details on the instrument and procedures adopted are described in the following. Data from transect N have been skipped because they didn't pass the quality procedure process adopted.

**METHODS**

At each station the oceanographic rosette was downloaded, being packed with Niskin bottles in order to collect water samples at different depths together with CTD profiling and other oceanographic activities. Samples of seawater was spilled from the bottles and used for laser fluorometric analysis and successively analyzed onboard.

Laser induced fluorescence (LIF) spectra were measured with the laser fluorometer. Briefly it is composed by two lasers emitting at 266nm and at 405nm. The beams emitted from the two laser sources following different paths are combined in a way that they arrive at the exit optical plane collinearly. The laser beams illuminates the sampling chamber made by two quartz cuvettes. The cuvettes have an in-out connection to fill them with seawater samples. When the laser beam passes trough the cuvette windows a laser induced fluorescence is emitted by the sample. Emitted light is collected and coupled by means of a fibre optic to OceanOptics spectrometer for spectral analysis. Seawater is allowed to flow on a system of interconnected pipes until it completely fills each cuvette.

*Table 2: Operating parameters for the laser fluorometer.*

| **Cuvette** | **Filter** | **Notes** |
|---|---|---|
| C1 | 30 µm | Chlorophyll detection |
| C2 | 0.22 µm | CDOM |
| Flush cycle | | 100 ml sea water + 100 ml fresh drinking water |
| Load cycle | | 100 ml sea water |
| Spectrometer | 200ms | Integration time |

*Figure 2: The portable laser fluorometer installed inside the Oceania wet laboratory.*

Seawater optical characteristics is accomplished by separating water constituents and allowing to enter in each cuvette for successive laser excitation. In particular the seawater sample entering the laser fluorometer is filtered with 30 µm porosity, which allows to pass only phytoplankton and nanoplankton. The filtered water is allowed to enter the first cuvette C1 for determination of pigments contained in algae (phycoerythrin, phycocyanin, chlorophyll), successively water exiting from the first cuvette passes a combination of two filters (0.8 µm porosity, to remove biggest algal cells, 0.22 µm to remove all other particles) and finally enters the cuvette C2 for determination of substances dissolved in water

(CDOM, tryptophan, tyrosine). Parameters used in the operation are specified in Table 2. During the oceanographic campaign, the fluorometer was installed inside a wet laboratory of the R/V Oceania (Figure 2).

## DATA ANALYSIS

The laser induced fluorescence signal of a complex target (like sea water samples) can be modelled to a first order approximation (no re-absorption or saturation) as a linear superposition of different emission bands excited by the laser beam.

$$LIF(\nu,\nu_{ex}) = K(\nu)\left(\sum_{m=1}^{M} k_m L_m(\nu_{ex},\nu) + k_R R_w(\nu_{ex},\nu)\right) \otimes S(\nu) \qquad (1)$$

where:

| | |
|---|---|
| $K(\nu)$ | system spectral constant (collection efficiency, detector spectral sensitivity, laser beam power, etc.) |
| $S(\nu)$ | spectrometer unit transfer function |
| $R_w(\nu_{ex},\nu)$ | water Raman emission when excited at $\nu_{ex}$ |
| $L_m(\nu_{ex},\nu)$ | *i*-th cromophore fluorescent spectral emission when excited at $\nu_{ex}$ |
| $k_m$ | concentration of *i*-th chromophore |
| $M$ | number of independent spectral bands. |

Note that in Eq. (1) the frequency has used rather than the wavelength, because from a physical point of view it is easier to justify Gaussian broadband peaks. A general-purpose curve-fitting utility with a graphical user interface was written in IDL (7) and implemented to the arctic data set selecting the components and their relative peak positions, as detailed in Table 3.

More than three hundred water samples were collected along the scheduled transects, thus resulting in more then 1300 spectra to be analysed (Table 1). For each water samples, four spectra are associated in filtered (30 µm) and double filtration (0.22 µm), both excited at 266 and 405 nm, respectively. Successively, after being processed, then data have been normalized to their respective water Raman signal and released in Raman units, in order to perform successive calibration and comparison with previous data. CDOM content have been extracted form double filtration water samples as excited by 266 nm, due to a better water Raman discrimination and less overlapping substance interference. As an example in Figure 3 is shown the resulting fit. Dissolved organic fluorescence intensities were extracted from double filtration. While carotenoids fluorescence pigments have been retrieved form the difference between the two blue fluorescence emission at 440 nm, before and after the second filtration determination.

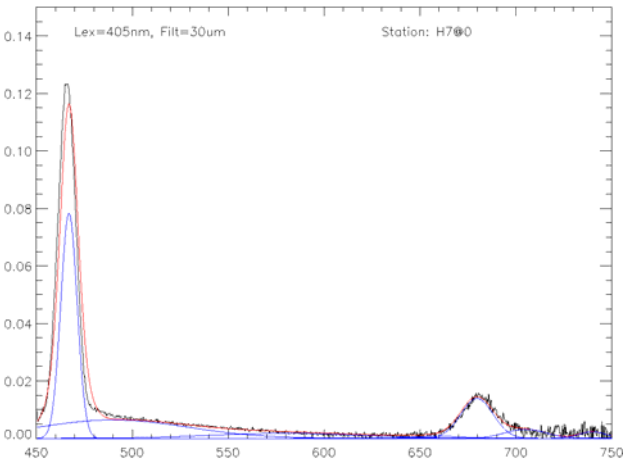


*Figure 3: Fitting procedure of measured fluorescence spectrum (black line), Gaussian functions (blue lines) and final fitted function (red line) ($\lambda_{exc}$ = 405 nm, filtration: 30 µm. Sample H7, superficial).*

*Table 3: Main fluorescence bands selection.*

| Component | Laser excitation | Fluorescence Band | Filtration |
|---|---|---|---|
| Tyrosine | 266 nm | 330 | Double |
| Tryptophan | | 360 | |
| CDOM | | 430 | |
| Carotenoids | 405 nm | 440 | Single |
| Phycoerythrin | | 575 | |
| Phycocyanin | | 630 | |
| CHL-a | | 680 | |

### Chl-a Calibration

As described previously, the R/V Oceania completed its first leg with the stop in the Longyearbyen, where the Polish spectrometer was put in operation and used for comparison with the laser fluorometer. Therefore only the second leg dataset was employed for calibration purpose and extended to the previous measurements. In Figure 4 the calibration curve is obtained from the intercomparison among the two datasets. The retrieved calibration has been applied to retrieve biomass (Chl-a) distributions in the investigated arctic transects.

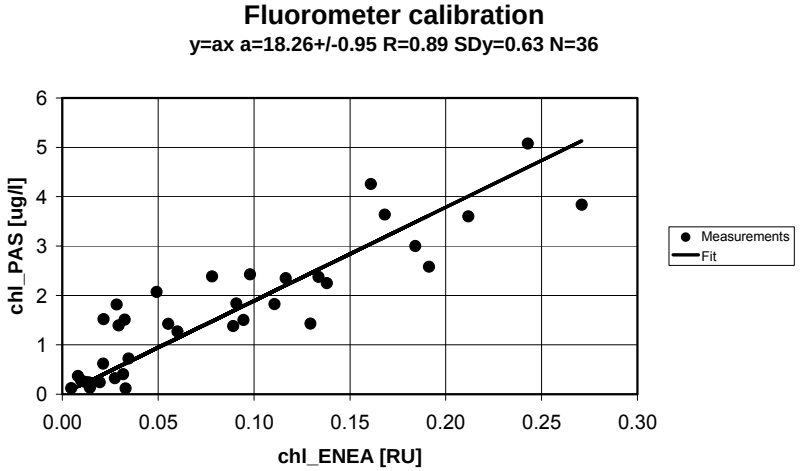


*Figure 4: Intercomparison between spectrometer (µg/l) and spectrofluorometer data (RU).*

## RESULTS

### Arctic campaign

Data measured during the Polish oceanographic campaign have been grouped in three main depth layers as superficial (100 samples; Figure 5), euphotic (up to 100m; 150 samples; Figure 6) and aphotic (from 100 up to bottom; 140 samples; not shown). The euphotic layer is the illuminated zone of aquatic ecosystems, above the compensation level and therefore it is the zone of effective photosynthesis. In marine ecosystems it is much thinner than the deeper aphotic zone (below the level of effective light penetration), typically reaching 30 m in coastal waters but extending to 100-200 m in open ocean waters.

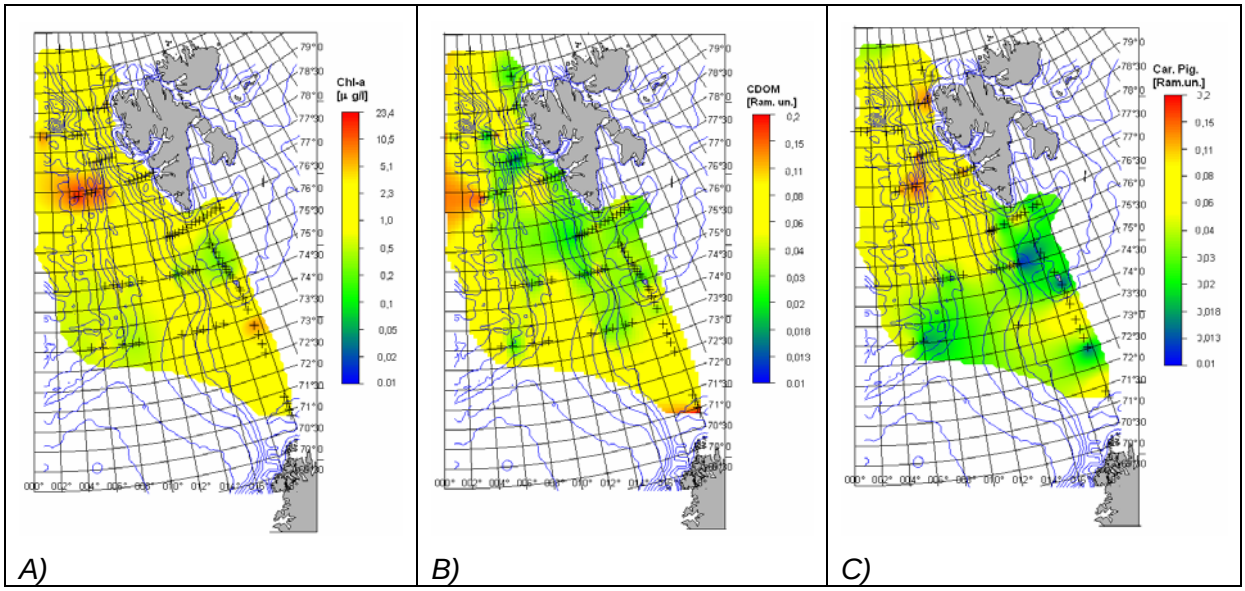


*Figure 5: Superficial distribution measured in the oceanographic campaign: a) Chl-a (µg/l); b)CDOM (Raman Units); c) Carotenoids (Raman Units).*

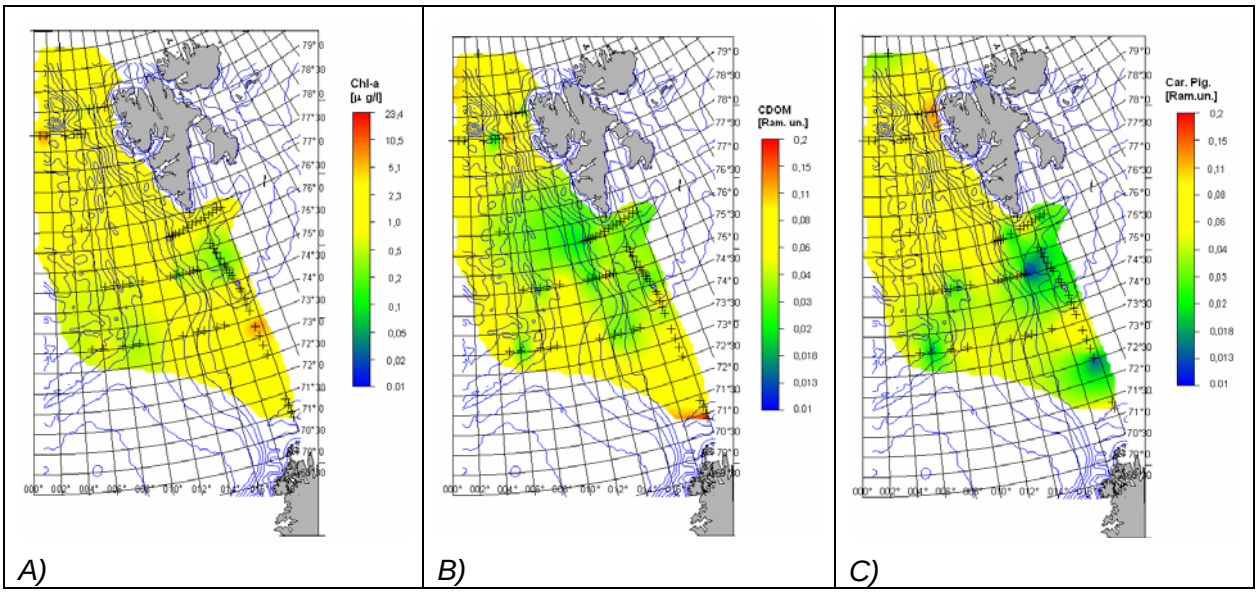


*Figure 6: Euphotic distribution (up to 100m depth) measured in the oceanographic campaign: a) Chl-a (µg/l); b)CDOM (Raman Units); c) Carotenoids Raman Units).*

The area investigated resulted affected by different algal blooms, with large patches mostly in the western area of the Svalbard Isles and in the first northern transect (Figure 5a). The superficial behaviour is confirmed by the concurrent high concentrations measured in the euphotic layer (Figure 6a).

In the CDOM distribution a large concentration spot is observed in the proximity of the Norwegian costs and in the western side of the Svalbard isles, not in coincidence with the algal bloom patch (Figure 5b). The same trend is confirmed along the euphotic zone (Figure 6b).

While carotenoids occurrence is spread in all the investigated area with remarkable higher concentrations in the western and northern zone respect to Svalbard Isles with strong connection with Chl-a pigment.

Last experimental evidence is confirmed from data analysis correlation matrix, where Chl-a index have been considered as most remarkable and compared to the others (Figure 7). As general trend a

decrease versus depth is observed for all the substances, but it's interesting to notice the strong correlation between Chl-a and retrieved carotenoids fluorescence, respect to others.

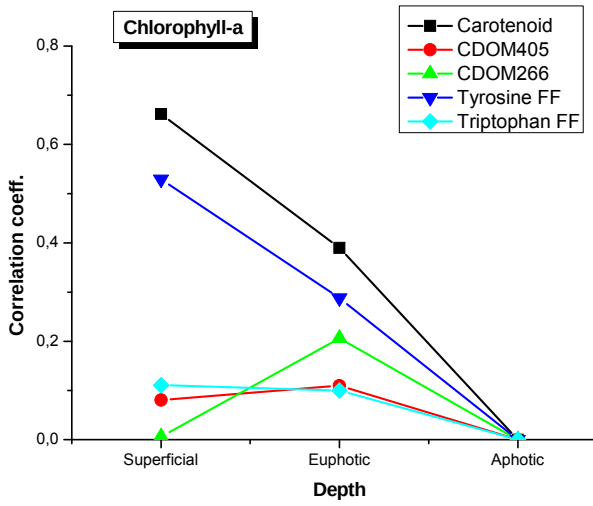


*Figure 7: Correlation coefficients retrieved from data analysis and comparison among chlorophyll-a and fluorescent parameters along three main depths (superficial, euphotic and aphotic).*

Passing to consider vertical transect *vs* depth distributions, we limited Chl-a display only to the most significant euphotic layer respect to the total behaviour for all the other measured quantities. In Figure 8, is displayed the distribution measured at different depths, in the scheduled stations along the V1 (from Tromso to the Bear Isle) and V2 (from Bear Isle to the Svalbard) transects, for CDOM (Figure 8a) and Chl-a (Figure 8b).

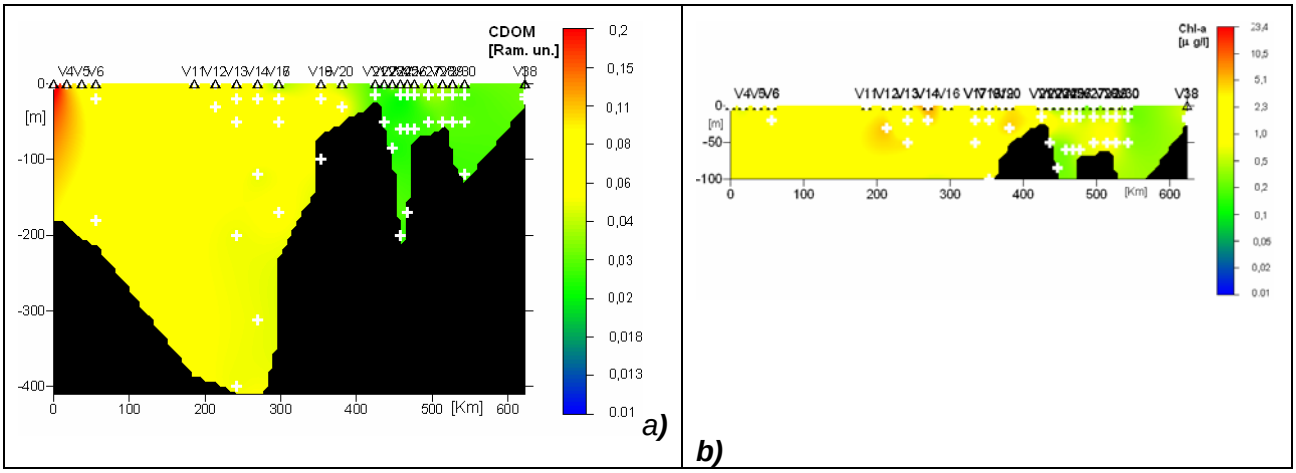


*Figure 8: Vertical distribution of fluorescent seawater components in V1 and V2 transects: a) CDOM (Raman Units); b) Chl-a (µg/l) up to the euphotic layer.*

As clearly observed from both figures (Figure 8a, b), the arctic shelf break acts as an wall between the two areas. More concentrated the zone close to the Norwegian respect to the depthless Svalbard costs. To corroborate this evidence, potential temperature measurements show a first warmer transect V1 (Figure 8a) respect to the more structured V2 (Figure 8b). The corresponding water masses shows similar trend also in salinity and density, more concentrated and salty in V1 transect (not shown).

This area is directly affected by the Gulf stream that originates in the Gulf of Mexico, exits through the Strait of Florida, and follows the eastern coastlines of the United States and Newfoundland before crossing the Atlantic Ocean. At about 30°W, 40°N, it splits in two, with the northern stream crossing to

northern Europe and the southern stream recirculating off West Africa. The Gulf Stream influences the climate of the east coast of North America from Florida to Newfoundland, and the west coast of Europe.

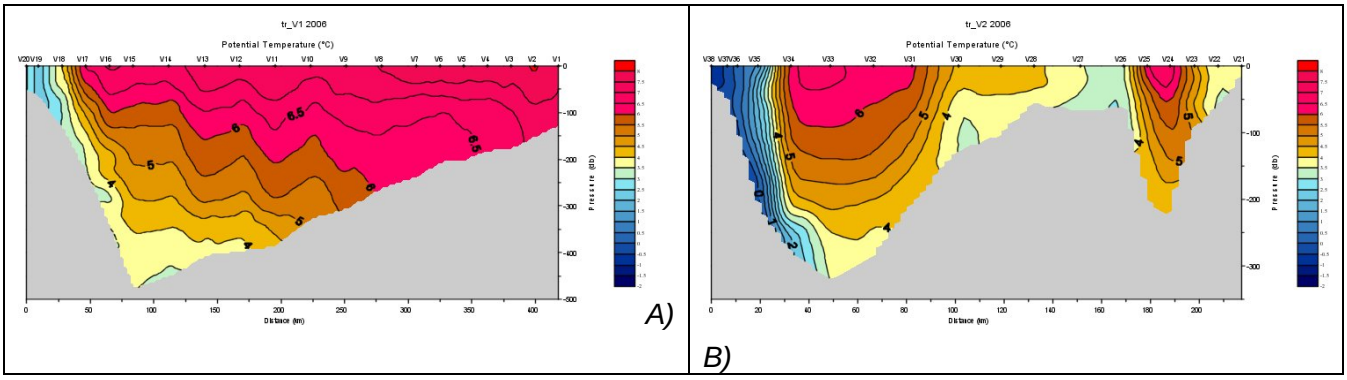


*Figure 8: Vertical distribution of potential temperature (°C) in: a) V1 and b) V2 transect.*

In Table 4 have been summarized the average values measured in previous Antarctic lidar fluorosensor campaign and data from the last arctic survey. As can be observed these extreme areas are affected by high CDOM and Chl-a concentrations due to the local upwelling and downwelling phenomena strongly induced by winds, ice melting and tides.

*Table 4: Comparison between Antarctic and Arctic Chl-a and CDOM fluorescence data, as collected during different campaigns. In case of Antarctic determinations also some relevant sites are indicated.*

| Campaign | Site | Date | Chl-a (µg/l) | CDOM (Raman Units) |
|---|---|---|---|---|
| Antarctic | Mooring B | 30/12/97 | 1.60 | 0.12 |
| | | 26/01/00 | 0.80 | 0.31 |
| | | 26/01/00 | 0.80 | 0.31 |
| | | 19/01/01 | 0.20 | 0.16 |
| | | 13/02/03 | 12.7 | 0.35 |
| | Cape Adare | 28/12/97 | 0.36 | 0.12 |
| | | 23/01/01 | 0.40 | 0.05 |
| | | 07/02/03 | 1.20 | 0.31 |
| | Terranova Bay | 03/01/98 | 2.56 | 0.43 |
| | | 08/02/01 | 2.80 | 0.13 |
| | | 15/01/03 | 17.5 | 0.39 |
| Arctic | | 07-08/06 | 0.0 ÷ 20 | 0.0 ÷ 0.38 |

## CONCLUSIONS

The most interesting result obtained from the 2006 Polish arctic campaign, with the new portable laser fluorometer, was the potential discrimination between dissolved and particulate organic matter in seawater components thus resulting in carotenoid algal pigment determination.

The broadband blue emission is usually ascribed as CDOM fluorescence and this is generally correlated with Chl-a algal pigment (8, 9) in particular when large patches of algal blooms occur. This phenomenon is particularly evident in coastal waters while the correlation decreases in off-shore waters. Carotenoids fluorescence (10) is particularly evident when concentrated algal solutions are observed.

This evidence will be better investigated in the following 2007 Polish artic campaign already scheduled, where a lidar fluorosensor and the portable spectrofluorometer will be together employed. In case of a positive proof this effect should be considered in remote lidar fluorosensor measurements.

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