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Spectrofluorometric phytoplankton and sea water characterization during the XIII Italian Antarctic mission

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ABSTRACT

Seawaters detailed spectroscopic analyses were carried out in the ENEA mobile fluorosensor laboratory participating to the oceanographic campaign within the XIII Italian Antarctic Mission (R/V Italice, Nov.'97-Jan'98). A complete set of in-vivo emission and excitation spectra was measured during cruises in the Ross Sea and along Southern Ocean transects up-to New Zealand. Measurements allowed for distinguishing among natural seawater components (tyrosine and tryptophan protein-like substances) and for identifying phytoplankton pigments (Chl-a, b, c, carotenoids, phycoerithrin, and phaeopigments). Georeferenced data have been collected at fixed time intervals and released on thematic maps.

Keywords: phytoplankton, fluorescence, Chl-a, phycobilins, DOM, tyrosine and tryptophan, Antarctica

1. INTRODUCTION

Marine ecosystems are mainly characterized by phytoplankton primary producers playing a fundamental role in the trophic relationships among different organisms. The sea water productivity is affected by chemical (salinity, nutrients, etc) and physical (temperature, solar radiance) parameters.

The Antarctic Ross Sea is a particular case of a large basin which presents rapid outgrowth during the pack-ice breaking and melting due to the seasonal temperature rise in combination with strong catabatic winds, with the occurrence of intense phytoplankton blooms, especially in polynya areas^{1/1}. These seawaters are characterized by high turbidity, low salinity and high nutrient concentrations which allow for the growth of natural phytoplankton during the period of intense sun illumination¹. The blooms are of special concern to the local biosystem since the Antarctic plankton population, ranging from pico to nano and micro-plankton species, is the first ring of the nutritional chain for zooplankton, fishes, whales, seals and birds. Humic substances are known to play a major role in many biogeochemical processes taking place in coastal and offshore waters, including transportation and speciation of trace metals². In the Antarctic area, most of the inorganic particulate, released after ice melting, is complexed by the humic substances, thus contributing to increase the phytoplankton productivity. In contrast, the Southern Ocean waters at lower latitudes and up to the Antarctic convergence, collect impurities from all the hemisphere, being characterized by strong mixing effects with water masses surrounding the Antarctica continent (*Circumpolar Current*). Finally, the study of biological and hydrological seawater parameters in these areas is also important for a better understanding of interactions with the surrounding environment, including the atmosphere, which in turn govern the climatic evolution of the planet.

To this connection, local methods suitable to investigate seawater chromophores *in vivo*, without sample concentration or extraction, are especially valuable for water classification. Spectrally resolved fluorescence measurements are among the most versatile tools, since signatures of dispersed impurities, such as crude oils, protein-like (amino acids) and humic-like substances (DOM) and phytoplankton, can be extracted from fluorescence excitation and emission spectra of seawater samples from the UV to the Visible range^{3, 4}. Data can be converted to absolute concentration values after normalization against conventional chemical measurements, and can be further used to calibrate remotely sensed measurements taken in the same spectral range by using either active (lidar) or passive systems⁵.

In the frame of the Italian National Research Programme for Antarctica (PNRA), the ENEA laser remote sensing group in Frascati has developed an integrated LIF system, whose heart includes a lidar fluorosensor and a lamp spectrofluorometer,

^{1/1} Polynyas, or areas of combined open water and thin ice surrounded by sea and/or land ice, are thought to play important roles in heat transfer from ocean to atmosphere, ice production, the formation of dense shelf water, spring disintegration of sea ice, and the sustenance of primary and secondary productivity in polar regions.

for continuous and automatic seawater quality and phytoplankton remote monitoring⁶. Spectrofluorometric determinations relevant to fluorescence excitation and emission spectra have been recorded at different UV-VIS excitation/emission wavelengths during all the oceanographic campaign. Distributions have been obtained of different water components, such as tyrosine and tryptophan forming the UV fraction of DOM, humic and fulvic acid forming the visible (blue) fraction of DOM, and phytoplankton pigments, including phycoerythrin and Chl a. To gain a deeper insight of algal blooms, four stations were selected in the polynya area of Terra Nova Bay (BTN) during the first period of the oceanographic campaign. Data relevant to all measured parameters have been used to build thematic maps of the regions, released on a digital cartographic base, along the Southern Ocean transect, in the Ross Sea and in the BTN area.

2. MATERIALS AND METHODS

The instrumentation, enclosed in a dedicated ISO 20" mobile laboratory and loaded on the upper deck of the Italian Oceanographic Research Vessel "Italica", has operated during the first leg of the oceanographic campaign (Nov.'97-Jan'98) within the XIII Italian Antarctic Mission.

A commercial lamp spectrofluorometer (PTI Quantamaster) has been used to collect excitation and emission spectra from water samples picked up every hour, both from the sea surface and 5m underneath. The instrument produces emission, excitation and synchronous spectra from liquid samples. A list of the main fluorescence channels is detailed in table 1. An example of fluorescence emission spectra at five different excitation wavelengths is plotted in Figure 1, for a surface seawater sample. Spectral signatures of the main pigments and fluorescing substances are represented by the indicated wavelength integration bands, whose contents are used to retrieve corresponding concentrations from. Note that DOM gives rise to several bands, which are dominated in the UV by tyrosine and tryptophan protein-like substances (Fig. 1a-b), and in the visible mostly by humic and fulvic acids (Fig. 1c). The most important phytoplankton pigment is Chl-a, which becomes evident upon near UV (Fig. 1c) and visible excitation (Fig 1d-e). Phycoyanin and phycoerythrin pigments, if present, are better discriminated upon excitation in the visible range (Fig 1e). Fluorescence spectra were processed starting with a background subtraction, and proceeding through a multiple Gaussian deconvolution of unresolved or overlapped structures. Resolved peaks were then integrated within a 10 nm bandwidth and normalized to the relevant water Raman peak.

Table 1. Main excitation and emission fluorescence bands used in the fluorescence determinations.

Natural Band	λ_{exc} [nm]	λ_{em} [nm]	Notes
Raman	230	249	Transparency
Tyrosine	230	305	Tyrosine-like fluorescence
Tryptophan	230	345	Tryptophan-like fluorescence
DOM	230	380	DOM fluorescence in the UV
Raman	266	292	Transparency
Tryptophan	266	345	Tryptophan-like fluorescence
DOM	266	380	DOM fluorescence in the UV
Raman	355	403	Transparency
Humic and fulvic acids	355	445	DOM fluorescence in the Blue
Phycoerythrin	355	580	Algal pigment
Allophycocyanin	355	650	Algal pigment
Chlorophylls	355	680	Chl- algal pigments
Blue-green pigments	480	510	Degradation algal pigments
Humic and fulvic acids	480	545	DOM fluorescence tail
Raman	480	571	Transparency
Chlorophylls-a	480	680	Chl-a algal pigments
Phycoerythrin	530	585	Algal pigment
Phycocyanin	530	630	Algal pigment
Raman	530	644	Transparency

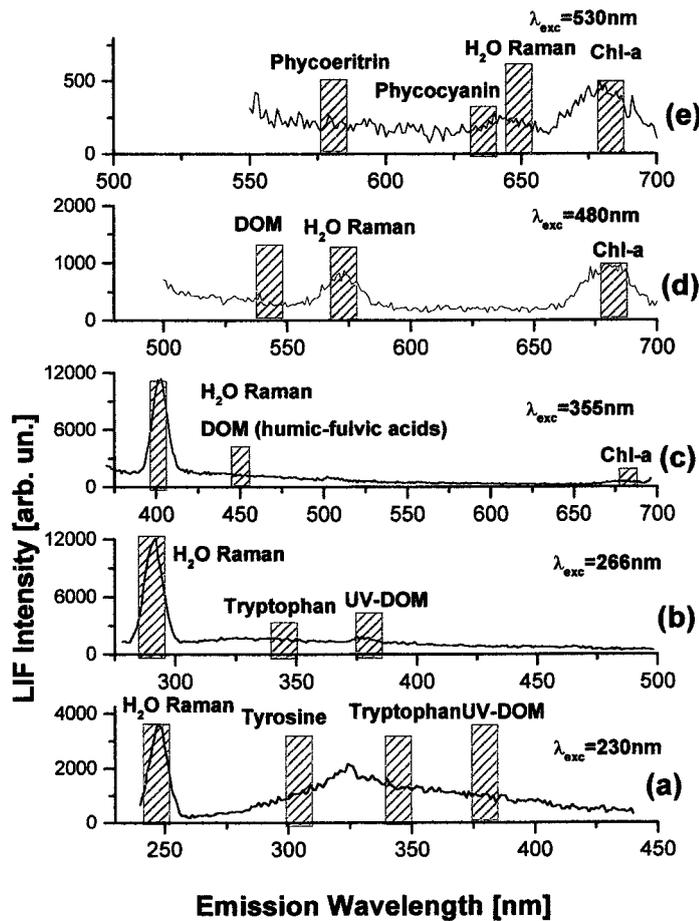


Figure 1. Fluorescence emission spectra of a surface Antarctic seawater sample: a) $\lambda_{exc}=230\text{nm}$; b) $\lambda_{exc}=266\text{nm}$; c) $\lambda_{exc}=355\text{nm}$ d) $\lambda = 480\text{nm}$; e) $\lambda = 530\text{nm}$. [75° 00' 57" S 164° 24' 65 "E, Dec. 10 '97].

Releasing data in “Raman units” allows to relate measurements performed on identical water samples by different local or remote instruments and to compare different seawaters. Fluorescence intensities in Raman units can be successively converted into absolute values of concentrations by calibrating them against chemical analytical determinations carried out upon the same seawater samples. The latter procedure is usually followed for Chl-a data. During the present campaign, local seawater sampling was performed by using Niskin bottles and the concentration of algal *chlorophaeopigments* (Chl-a and phaeopigments) in units of $\mu\text{g/l}$ was determined by means of a spectrophotometer⁸ and using the trichromatic equations^{9,10}. The spectrophotometer determinations on unfiltered seawater samples were used to calibrate our spectrofluorometer data @ 680nm. A good correlation coefficient ($R = 0.97$) was found to couple the two sets of Chl-a concentration measurements⁸ upon the same samples taken at a 5 m depth, which led to a conversion factor of 1.3 ± 0.1 [$\mu\text{g/l}$]/[Raman units].

3. RESULTS

3.1 Phytoplankton pigments recognition

Widely different seawaters masses, from the standpoint of dispersed impurities, have been monitored during the oceanographic campaign, ranging from offshore oceanic to inshore or polynya areas. A database of more than 700 spectroscopic determinations has been collected, including surface and subsurface (5 m depth) samples. Laboratory analyses of the major algal species concentration in [cell/l] and [%], with the relevant Chl-a and phaeopigments content in units of $\mu\text{g/l}$, are reported in table 2 for the selected samples. The dominance of the prymnesiophyte *Phaeocystis* sp. was observed at two stations (#2 and #4). The same species was absent in station #9, while in station #11 undetermined flagellates prevailed. The phaeopigments were identified in all samples in a quasi-constant ratio with respect to the Chl-a concentration, except for station #11. A low Chl-a concentration value was obtained in station #9 where *Phaeocystis* was not found.

Table 2. Phytoplankton composition and biomass characterization at four stations near BTN (minor species concentrations are not reported).

St.	Lat.	Lon.	Date	Phytoplankton Species	Conc. [cell/l]	Conch [%]	Chl-a [$\mu\text{g/l}$]	Phaeo [$\mu\text{g/l}$]
2	75°06.35' S	164°12.06' E	7-12-97	<i>Phaeocystis</i> sp	642000	97.3		
				<i>Thalassiosira antarctica</i>	8640	1.3		
				<i>Fragilariopsis curta</i>	4520	0.7		
				Total	659600		1.08	1.23
4	75°12.14' S	163°59.95' E	8-12-97	<i>Phaeocystis</i> sp.	1276000	98.1		
				<i>Thalassiosira</i> sp.	6920	0.5		
				<i>Fragilariopsis curta</i>	4960	0.4		
				Total	1300080		1.65	1.40
9	74°46.71' S	164°48.74' E	9-12-97	<i>Thalassiosira antarctica</i>	10400	55.0		
				Undetermined flagellates	2640	14.0		
				Total	18920		0.43	0.37
11	74°59.82' S	164°25.28' E	9-12-97	<i>Phaeocystis</i> sp.	184000	34.7		
				<i>Thalassiosira</i> sp	28000	5.3		
				<i>Thalassiosira gravida</i>	5600	1.1		
				Undetermined flagellates (<10 μm)	308000	58.1		
				Total	530000		0.91	0.30

Excitation (figure 2) and emission spectra (not shown) were directly measured in-vivo, by means of the spectrofluorometer. Fluorescence excitation spectra ($\lambda_{em}=680\text{nm}$) of seawater samples collected in the BTN area are depicted in figure 2, after being normalized to the Chl-a excitation peak @ 430nm. Contributions of Chl-a (430nm), Chl-c (460nm), Chl-b (480nm), carotenoids (380nm, 520nm) and phaeopigments (410nm, 505nm, 590nm) bands are to be found at spectral locations marked by the arrows. Fluorescence spectra at St #2 and St #4, with maxima at 470 nm, appear different from those at Sts. #9 and #11, since the phytoplankton at the former two stations is dominated by *Phaeocystis*, which is characterized by Chl-c and is absent at St. #9, and overwhelmed by undetermined flagellates at St. #11. A clear indication of the phaeopigments bands is observed in the excitation spectra of all the samples, excluding sample #11 where their content is much lower (about one third) than Chl-a.

Excitation spectra collected during the cruise along the Southern Ocean down to BTN are reported in figure 3, approximately at increments of 5° of latitude. Changes of pigment compositions of the crossed seawaters become evident by looking at spectra in sequence. Namely, the spectrum collected at 50° S (and to a minor extent the spectrum at 70°) appears to be the most structured of the whole ensemble, due to the presence of all the chlorophyll bands and of carotenoids, which corresponds to a mixed phytoplankton population. On the other hand, the spectrum at 55° S seems to be dominated by a diatoms-like phytoplankton, due to the absence of the Chl-b band, while the spectrum at 65° does not present the Chl-c feature but is dominated by Chl-a and Chl-b, as expected in the case of the *chlorophytes*. The spectrum at 75° seems to be dominated by Chl-c excitation, with a second peculiar peak at 520 nm which can be attributed to *xanthophylls* excitation, both features supporting the presence there of a *Phaeocystis* dominated phytoplankton as for St. #2 and #4 (tab. 2, fig.2).

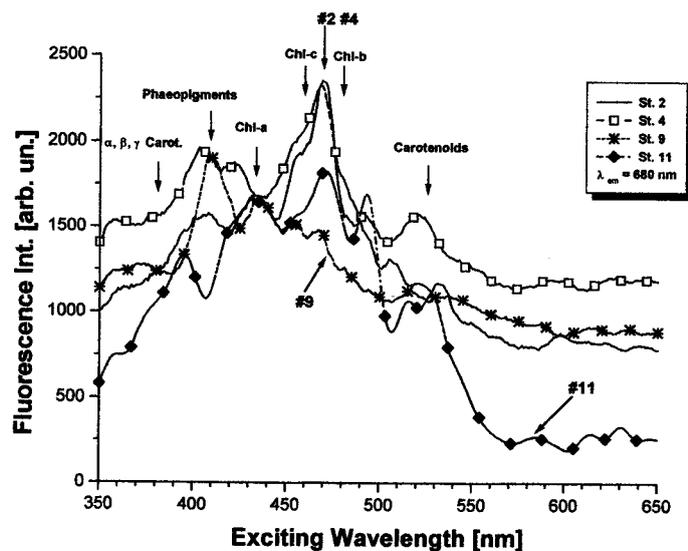


Figure 2. Excitation spectra at the hydrographic stations of table 2 [$\lambda_{em}=680\text{nm}$].

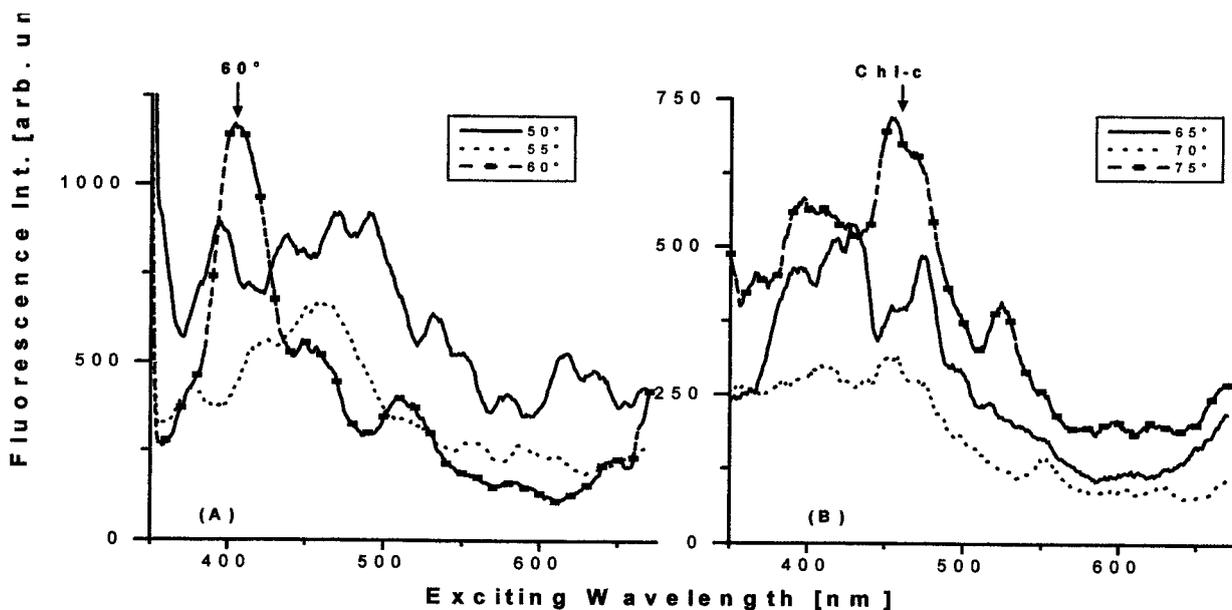
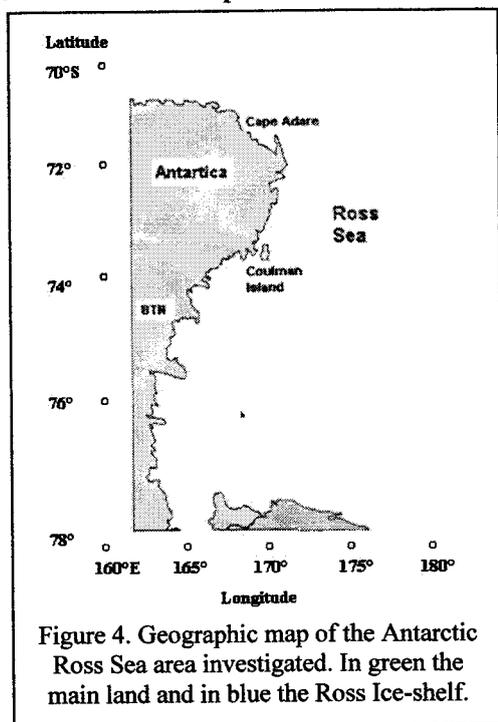


Figure 3. Excitation spectra of the seawater crossed in the Southern Ocean: a) $50^{\circ} 54' 22''\text{S } 172^{\circ} 52' 41''\text{ E } 25/11/97$; $54^{\circ} 57' 14''\text{S } 173^{\circ} 09' 55''\text{ E } [26/11/97]$; $60^{\circ} 21' 48''\text{S } 175^{\circ} 36' 06''\text{ E } [27/11/97]$; and in the Ross sea: b) $65^{\circ} 00' 00''\text{S } 179^{\circ} 10' 37''\text{ E } [28/11/97]$; $70^{\circ} 30' 42''\text{S } 179^{\circ} 00' 45''\text{ E } [30/11/97]$; $74^{\circ} 59' 36''\text{S } 169^{\circ} 29' 04''\text{ E } [01/12/97]$.

From a comparison between figure 2 and figure 3, the spectrum at 60°S turns out to be much similar to the one recorded at station #9, in that both spectra are dominated by the band at 410 nm, accompanied by the peak at 515 nm. These peaks are related to the same phaeopigments in both cases.

3.2 Fluorescence maps



Fluorescence measurements were started on 24th November '97, at the departure from the Littleton harbor (NZ), and were completed on 14th January '98 by returning to the Dunedin harbor (NZ). Data were collected during 53 days of cruise in the Antarctic Ross Sea and along the Southern Ocean transect. A detailed geographic map of the Antarctic coast is reported in figure 4, with the indication of some relevant Ross sea sites such as BTN sea area, the Ross Iceshelf, Cape Adare and the Coulman Island.

Time series of fluorescence intensities observed in the main spectral channels during the whole cruise are plotted in figure 5. Corresponding latitude coordinates are indicated in the top x-axis.

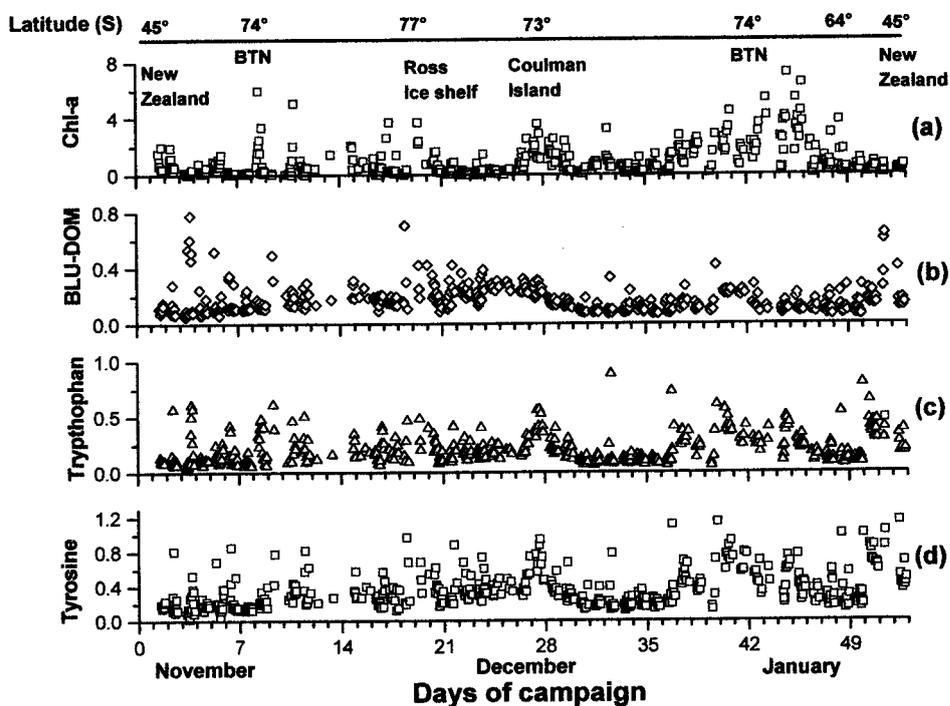


Figure 5. Time scale relative to the fluorescence intensities monitored during the seawater analyses: a) Chl-a ($\lambda_{exc}=480\text{nm}$); b) DOM ($\lambda_{exc}=355\text{nm}$); c) Tryptophan and d) Tyrosine ($\lambda_{exc}=230\text{nm}$). Fluorescence intensities are given in Raman units.

Chl-a concentrations, obtained according to the elaboration and calibration discussed in sect. 2, have been finally reported upon a digital cartographic chart in order to obtain thematic maps such as the one shown in Figure 6, which represents the along-route colour coded Chl-a distribution, in the Mercator projection. The corresponding DOM, tyrosine and tryptophan fluorescence distribution maps [Raman un.] are presented in Figures 7 to 9. In all of the figures main latitudes and the scale bar are given, the latter valid only in the horizontal axis (longitude), for easier localization. Zooms of Western Ross Sea and BTN areas are also added together with the longitudinal scale bar.

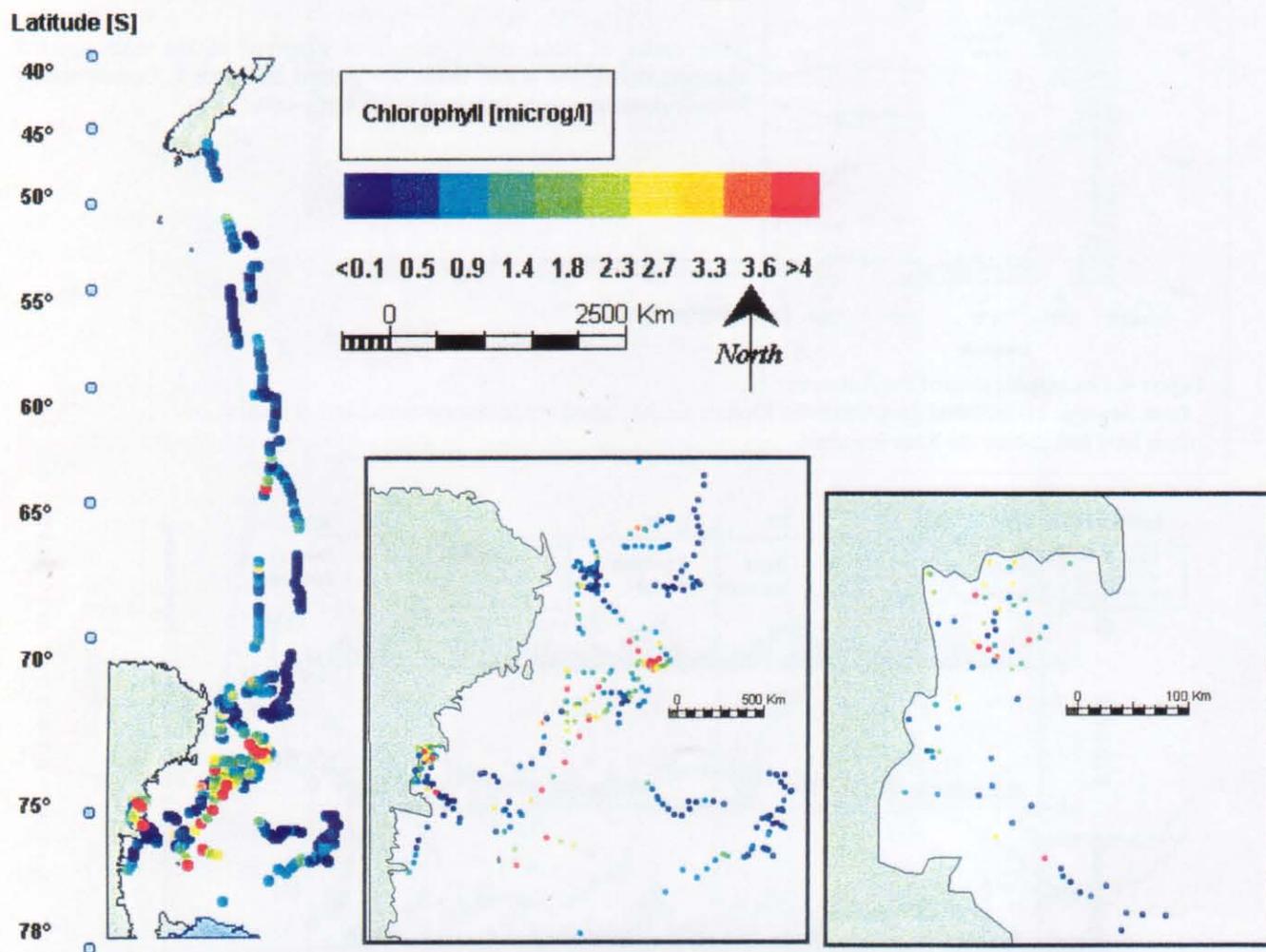


Figure 6. Thematic map of the Chl-a distribution measured during the oceanographic campaign (left). In the first inset (middle) a zoom of the Antarctic Ross Sea area, second (right) a more expanded zooming of the BTN area. The color code scale is given in $\mu\text{g/l}$. The horizontal scale bar is limited to the longitudinal axis (see text).

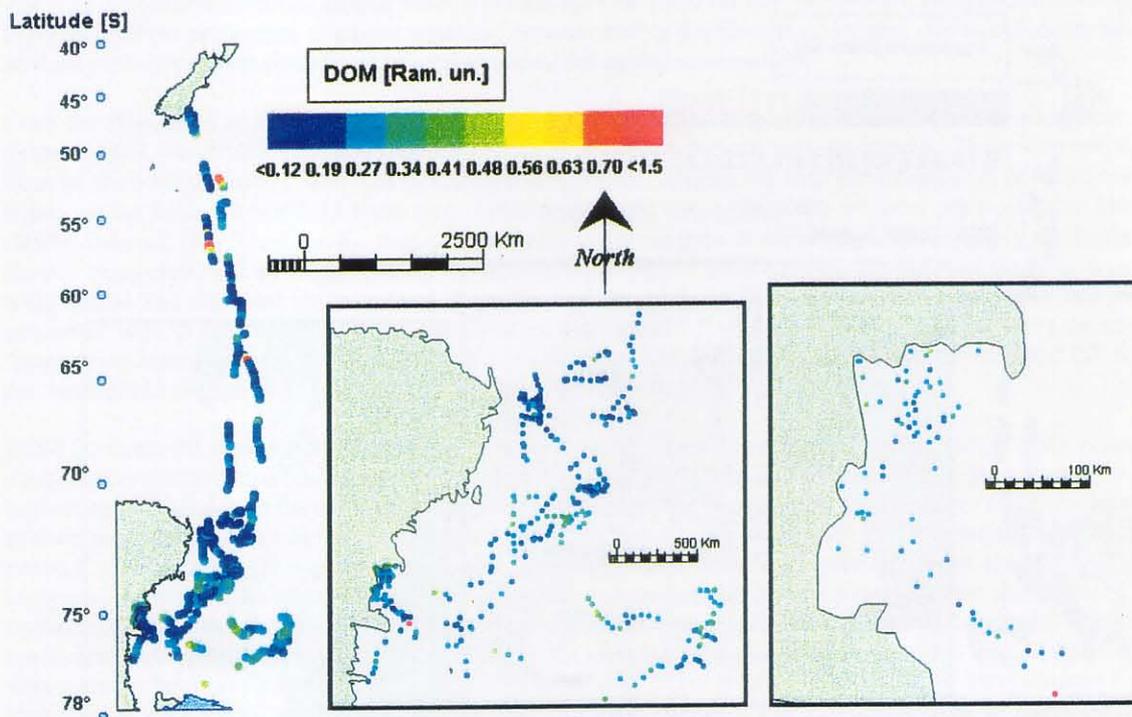


Figure 7. Thematic map of the DOM distribution. Details as in fig. 6. The color code scale is given in Raman units.

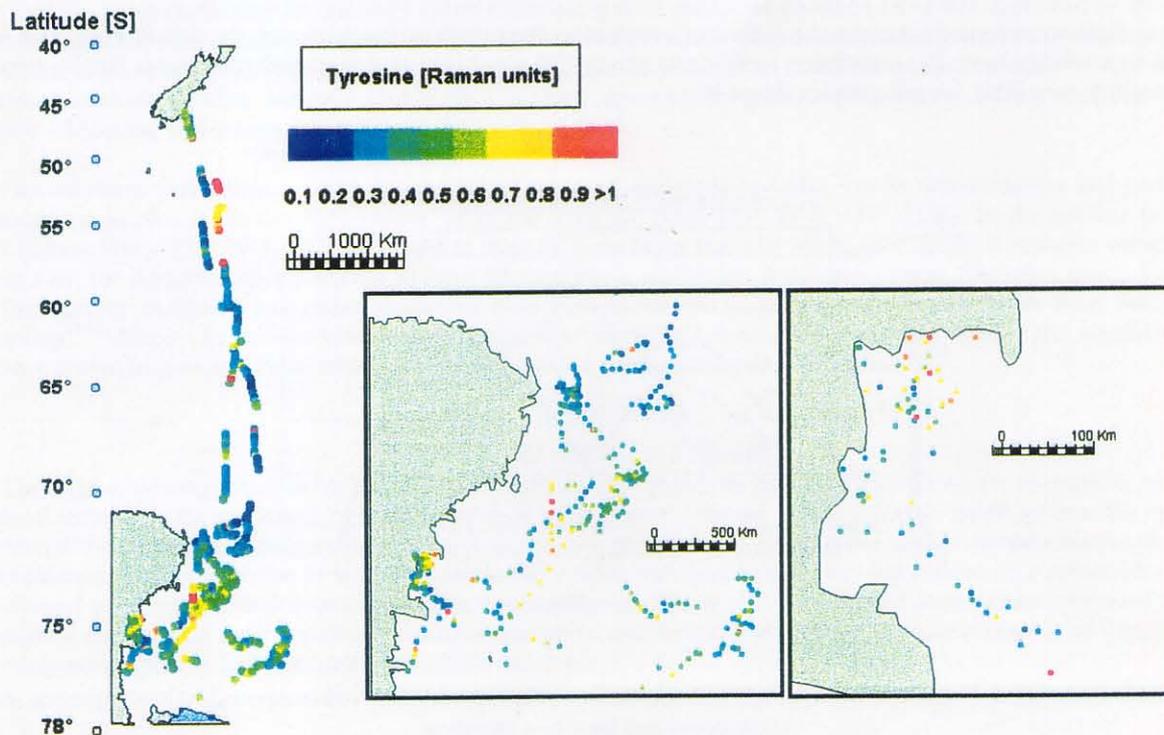


Figure 8. Thematic map of the protein-like fluorescence (tyrosine) distribution. Details as in fig. 7.

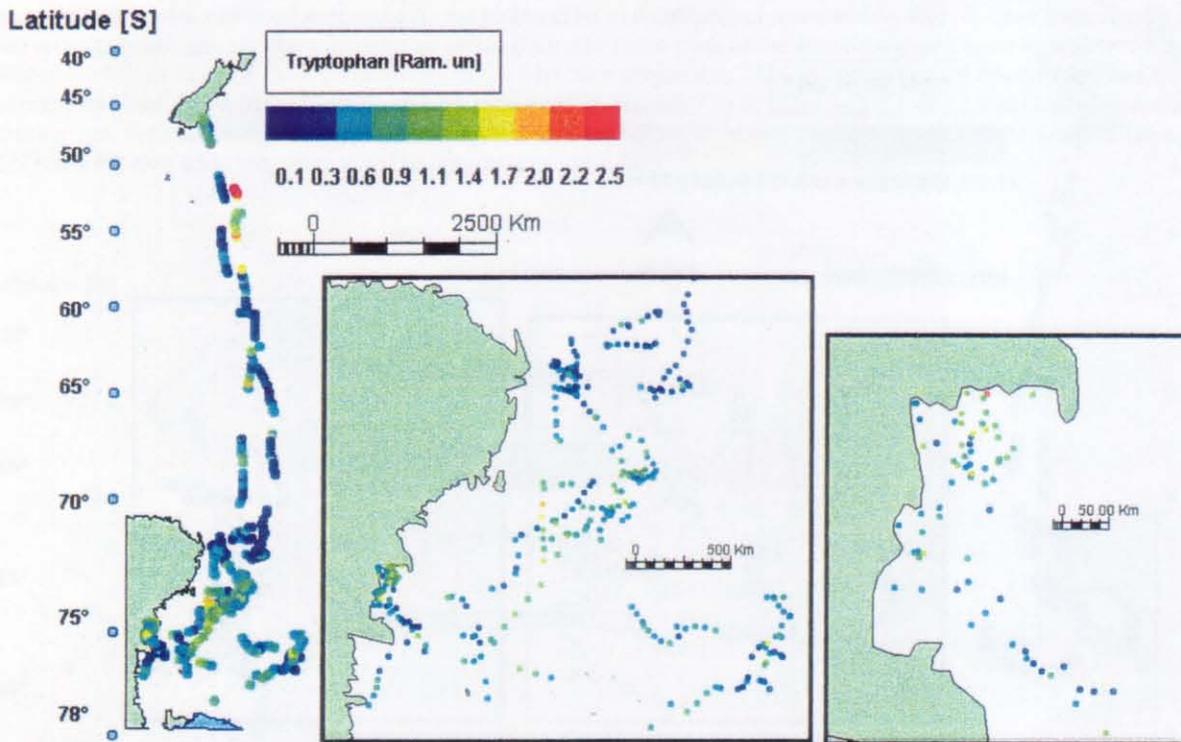


Figure 9. Thematic map of the protein-like fluorescence (tryptophan) distribution. Details as in fig. 7.

In order to discuss connections between the different fluorescence channels in emission spectra, the data of Fig 5 have been averaged on a weekly base. The correlation coefficients among Chl-a and the other identified compounds (DOM, tyrosine and tryptophan) emissions, are presented in figure 10.

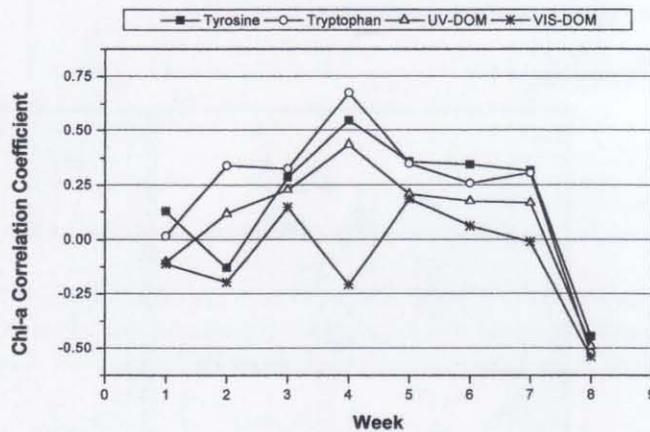


Figure 10. Correlation diagram of weekly averaged data between the organic dissolved compounds (DOM, tyrosine and tryptophan) and the Chl-a emission.

The high correlation observed around week 4 among the Chl-a and the UV protein-like components emissions supports the hypothesis of the production of amino acids and proteins during the blooms end phase, due to cell degradation and to algal photodegradation effects (essudates) occurring during the austral summertime.

From the inspection of the correlation diagram (fig. 10) and of the maps (fig. 6 to 9), few peculiar areas appear as very dynamic sites, where biological and physical processes are connected and strongly interact. These sites are: the Marginal Ice Zone of the central western Ross Sea (southward of Coulman Island), the well known polynya of Terra Nova Bay and the region of the Ross Ice Shelf. In these three areas remarkable late spring-early summer phytoplankton blooms have been clearly observed (fig. 5 and fig. 6), with surface Chl-a concentrations of up to 7µg/l. These highly productive regions, have already been identified as characterizing the Ross Sea ecology^{1,11} also through Ocean Color Remote Sensing¹³. Also the wide spatial and temporal extensions of these blooms, respectively hundreds of miles and up to one month, are quite consistent with previous reports from the Western Ross Sea^{1,14}. As far as offshore waters are concerned, chlorophyll fluorescence increases were observed (Fig. 6) just in front of and inside the melting ice areas (64° and 67° S), after crossing the Antarctic Convergence (58°S) and near the New Zealand coasts (52°S), respectively.

DOM fluorescence values in the blue emission band (figure 7) from 5m underneath water samples, are generally lower than what we previously measured in other seawaters¹². Here, DOM specific structures may be assigned to the complexed impurities emerging from the ice melting, whose contributions can be separated from those of algal accessory pigments and of their photo-degradation products. In general the dissolved organic substances have the tendency to be stratified and to produce surface layers (few microns). Actually, low underwater DOM concentrations were also confirmed by systematic comparisons between simultaneous analyses of surface and underneath seawater samples (not shown here). In contrast, the surface and underwater levels of Chl-a fluorescence have a similar magnitude and a rather concurrent behavior (Correlation coefficient R=0.8), thus supporting the hypothesis of a constant algal distribution along the first sublevel layers. However, discrepancies between DOM and Chl-a have been monitored in the return transect next the New Zealand coasts, where the blue fluorescence signal could be mostly related to DOM coming from the degradation of natural (like lignine) and anthropogenic substances produced in the main land.

Based on the data of Fig.5, protein-like (tyrosine and tryptophan) fluorescence emissions appear to be strongly correlated (R=0.97) between themselves and only loosely correlated with the DOM emission (R=0.43). Increases in these channels, with respect to normal values, has been previously observed in surface waters from the Sargasso Sea during a phytoplankton bloom and in phytoplankton culture media¹⁵, as well as in the presence of metal contamination (Cu)¹⁶. The solar radiation hitting seawaters during Antarctic summertime is also responsible for photodegradation of DOM protein-like and humic-like substances, to a greater extent than in the Southern Ocean.

Phycocerythrin fluorescence shows generally low values if compared to Chl-a, but in few occasions and just for shortly extended patches it reaches high values, as in the Ross Ice Shelf (75° 59'S, 171° 35'E), in the melting ice zone near Coulman Island (73° 39'S, 172° 47'E) and in front of Terra Nova Bay (74° 45'S, 164° 55'E). It could be considered rather unusual for Antarctic phytoplankton to have Phycocerythrin containing organisms, but in few other cases, Phycocerythrin fluorescence excitation and emission spectra have been measured in Marginal Ice Zones of the Ross Sea, during late spring^{17,18}. Moreover, summer blooms of cryptophytes, mainly in coastal and ice-melting areas of the Antarctic Peninsula, have shown to give important contributions to the total phytomass and primary production¹⁹.

4. CONCLUSIONS

The large database of fluorescing quantities obtained during the present campaign has shown the potentiality of fluorescing local techniques for continuous monitoring of large marine areas. Namely, for fluorescence based systems the capability has been demonstrated of releasing data in relative or absolute units suitable for seawater quality characterization. A satisfactory correspondence between the in-vivo fluorescence @ 680nm and the Chl-a content determined by a spectrophotometer, has allowed releasing spectrofluorometric data in absolute values. The *in vivo* fluorescence analysis has confirmed to be able to rapidly characterize natural phytoplanktonic community and discriminate among the miscellaneous of dissolved organic components, both at different trophic situations and depth.

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