

DNA and RNA Concentrations in the Atlantic Ocean, Between 49° and 67°S, in Relation to the Chlorophyll Biomass

(DNA | RNA | chlorophyll)

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INTRODUCTION

DNA, which carries the basic genetic information, is relatively independent of the environment while RNA, directly involved in protein synthesis, is dependent on environmental factors. The DNA concentration was proposed as a biomass indicator (Holm-Hansen, 1968) and the RNA/DNA ratio as an index of physiological state of the phytoplankton population (Dortch *et al.*, 1983, 1985). Furthermore, chlorophyll *a* is used as an indicator of the presence of photoautotrophic population. Measurement of the chlorophyll *a* concentration is widely used to estimate phytoplanktonic biomass.

The aim of the present study is to estimate the abundance and the metabolic activity of microplanktonic population in the ocean environment in the Indian sector of the Antarctic region, during the summer period, through the analysis of the nucleic acids, and the relation to the phytoplankton biomass, through the quantification of chlorophyll *a*.

The biomass and the metabolic activity of phytoplanktonic microorganisms communities were analysed with a new biochemical technique

(Berdalet & Dortch, 1991; Mordy & Carlson, 1991): DNA and RNA fluorimetric measurement after marking by two fluorochromes, a very sensitive and easy to use method. Data on nucleic acid concentrations have been published in the field of oceanography, in the Pacific (Takahashi *et al.*, 1974), in the Atlantic (Dortch *et al.*, 1985; Paul *et al.*, 1985), in the Mediterranean (Berdalet & Estrada, 1993), and in the Antarctica (Bailiff & Karl, 1991; Fabiano *et al.*, 1993 and Fabiano *et al.*, 1996).

MATERIALS AND METHODS

Field sampling

During the Antares 2 cruise (France-JGOFS), from 24 February to 9 March 1994, on board of R/V "Marion Dufresne", on a north-south transect of the Indian region of the Austral Ocean (Fig. 1), between 49 and 67°S, west of Kerguelen Plateau, four stations (A18, A12, A10 and A6) were studied between 0 and 100 m for DNA, RNA and chlorophyll measurements. The hydrological aspects of Antares 2 can be found in Fiala (1994).

Sampling was performed by means of Niskin-bottles rosette and a CTD-fluorescence probe.

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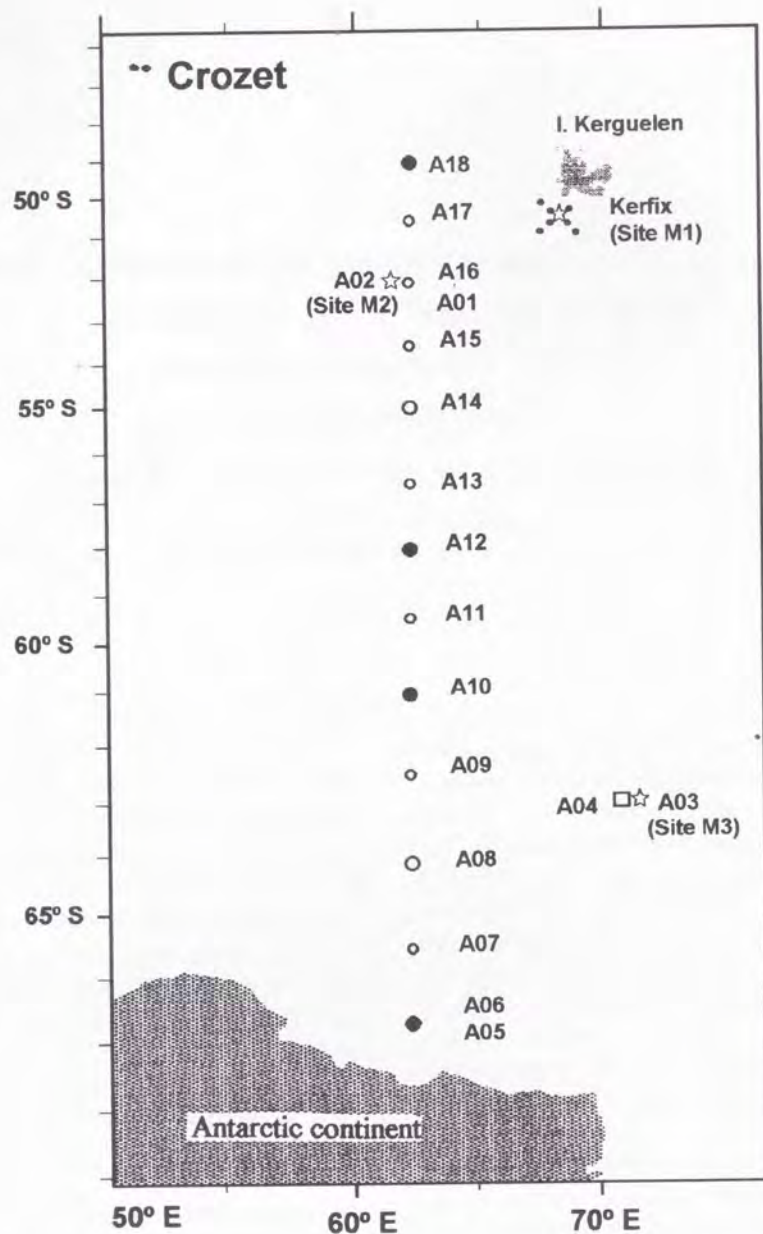


Fig. 1 — Position of 4 stations (black dots) studied during the Antares 2 Cruise (JGOFS-France) in the Antarctic Ocean (February-March 1994).

Chlorophyll a measurements

500 ml of sea water was filtered on GF/F and the pigments were extracted in acetone 100%. Chlorophyll *a* concentrations were analyzed according to the spectrofluorimetric method by Neveux & Panouse (1987). Fluorescence was measured with a Perkin-Elmer MPF 66 spectrofluorimeter using 6 pairs of wavelengths.

Nucleic acids measurements

Sampling: one to two litres of sea water were filtered under low vacuum on pre-combusted (420°C, 24 h) GF/F filters and stored in liquid nitrogen until the moment the analysis was performed.

Treatments: the filters were placed in glass centrifuge tubes on ice with 6.0 ml of PBS (phos-

phosphate buffered saline) buffer. Cells on GF/F filters were disrupted on ice with pulsed sonication using a Branson W-350 at 5.0 output with 60% duty, for time periods of 30 sec. Heparin (5 mg ml^{-1}), tris-HCl (0.5 v/v), and proteinase K (4 mg ml^{-1}) were added to the homogenates. The tubes with the homogenates were placed on a rotary shaker at 4°C for 15 min. Refrigerated centrifugation was done at $6000 \times g$ for 15 min.

Measurement: DNA and RNA concentrations were determined spectrofluorometrically, according to the double fluorochrome method, using Hoechst 33258 and Thiazole Orange as stains (Berthelot & Dortch, 1991). Extraction and calculation of nucleic acids were performed according to Machado (1994).

RESULTS

Distribution of DNA, RNA, chlorophyll, and RNA/DNA ratio

During the austral summer the concentration of DNA is small and its average does not exceed $2.6 \mu\text{g l}^{-1}$ for nucleic acids and $0.2 \mu\text{g l}^{-1}$ for chlorophyll *a* (Table I). The RNA/DNA (Fig. 3) ratio is low, indicating the presence of not very active populations.

At station A18, located in the subantarctic region, DNA concentrations ($2.5\text{-}3.0 \mu\text{g l}^{-1}$) are almost constant between 0 and 100 m (Fig. 2). RNA concentrations are homogeneous at the surface (0-20 m), increasing with depth ($2.0 \mu\text{g l}^{-1}$ at 100 m). The RNA/DNA ratio (between 0.2 and 0.7) suggests the presence of a population with low metabolic activity (Fig. 3). Chlorophyll *a* concentrations are homogeneous ($0.2\text{-}0.3 \mu\text{g l}^{-1}$) in the euphotic zone.

Further south (58°S), at station A12, concentrations of nucleic acids increase slightly at 30 m (Fig. 2). Deeper, DNA concentration varied from

1.3 to $2.1 \mu\text{g l}^{-1}$, as RNA and RNA/DNA ratio (Fig. 3) increase progressively ($1.3 \mu\text{g l}^{-1}$ and 1, at 100 m depth, respectively). Concentrations of chlorophyll *a* are homogeneous down to 75 m ($0.09 \mu\text{g l}^{-1}$).

Station A10 (61°S) presents the highest DNA and RNA values. Concentrations are higher at the surface (Fig. 2), reaching a maximum at 30 m ($4 \mu\text{g DNA l}^{-1}$ and $4.2 \mu\text{g RNA l}^{-1}$). Chlorophyll *a* is very homogeneous down to 80 m, reaching its maximum at 100 m ($0.3 \mu\text{g l}^{-1}$). Populations are more active at the surface, where the RNA/DNA ratio (Fig. 3) is higher than 1, between 0 and 50 m.

At station A06, close to the Antarctic continent (67°S), the vertical profile of the three parameters is homogeneous (Fig. 2), down to 50-60 m. Then the values decrease with depth. Maximum concentrations of DNA and RNA are respectively 3.6 at 20 m and $2.5 \mu\text{g l}^{-1}$ at 40 m, whereas the concentration of chlorophyll *a* is $0.4 \mu\text{g l}^{-1}$ between 20 and 60 m. Populations are more dynamic at 60 m, according to the analysis of the RNA/DNA ratio (0.9, Fig. 3).

DNA, RNA and chlorophyll a integrated values

The integrated values of DNA, RNA and chlorophyll *a* between 0 and 100 m showed minimum concentrations at the open ocean station (A12), and higher values in the continental margin (Fig. 4), either Kerguelen or the Atlantic continent.

Nucleic acids and chlorophyll a ratio

The data set for the three parameters ($n=31$) shows that only DNA is correlated with chlorophyll *a* ($r=0.51$; $\alpha < 0.01$).

For each station treated separately (Table II), nucleic acids are only correlated with chlorophyll *a* at the coastal region (A06).

TABLE I
Average and maximum concentrations ($\mu\text{g l}^{-1}$) of DNA, RNA and chlorophyll *a* on a transect of the Indian sector of the Antarctic Ocean ($n=31$; \pm standard deviation)

Parameter ($\mu\text{g l}^{-1}$)	Average	Minimum	Maximum
DNA	$2.59 (\pm 0.68)$	1.19	4.01
RNA	$1.74 (\pm 0.94)$	0.42	4.20
Chl <i>a</i>	$0.23 (\pm 0.12)$	0.09	0.43

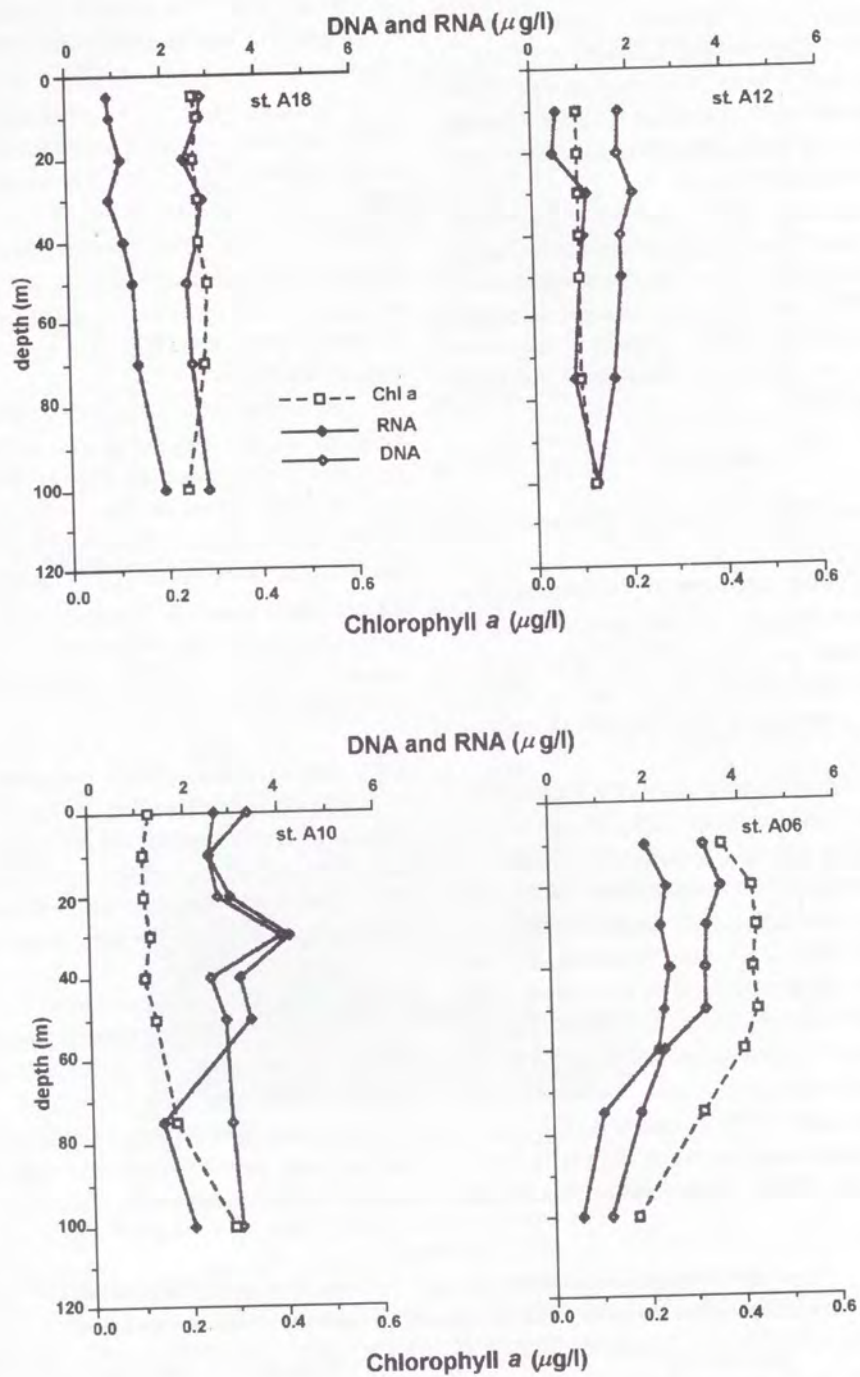


Fig. 2 — Variations in DNA, RNA and chlorophyll *a* concentrations on a transect between 49 and 67°S of the Indian sector of the Antarctic Ocean in February-March 1994 (Antares 2 Cruise).

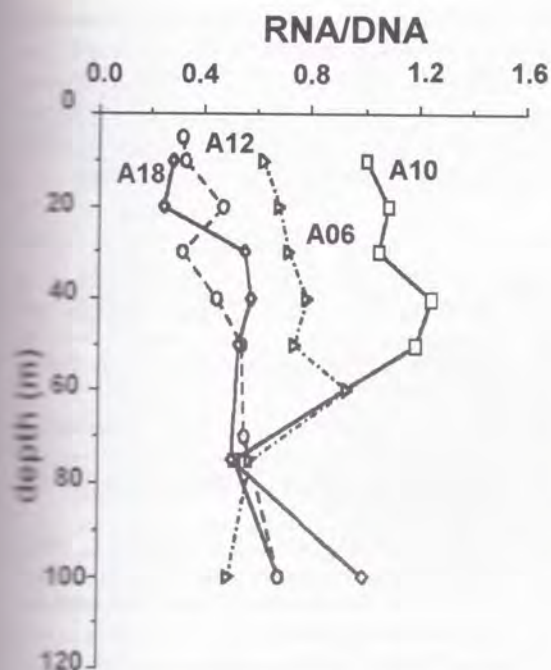


Fig. 3 — RNA/DNA ratio as a function of depth, for 4 stations of the transect between 49 and 67°S in the Indian sector of the Antarctic Ocean in February-March 1994 (Antares 2 Cruise).

DISCUSSION

DNA, RNA and chlorophyll *a* concentrations were used in the present paper to assign phytoplankton biomass and physiological state of this population. Particulate DNA and RNA, and RNA/DNA ratios, have been measured in the coastal

TABLE II
Simple linear correlation coefficients between nucleic acid concentrations and the chlorophyll *a* concentration for each station ($n=8$). The correlation is only significant in station A06 ($\alpha > 0,001$)

Parameters	Stations			
	A18	A12	A10	A06
DNA vs Chl <i>a</i>	-0.49	0.15	0.11	0.88
RNA vs Chl <i>a</i>	-0.34	-0.52	-0.52	0.96

waters of the Southern Ocean in this last decade by several methods (Bailiff & Karl, 1991; Fabiano *et al.*, 1993 and Fabiano *et al.*, 1996). Bailiff & Karl (1991) indicate DNA concentrations of the same order-of-magnitude as in this present study for oligotrophic waters or post-bloom conditions in the Antarctic Peninsula region, while Fabiano *et al.* (1993, 1996) showed higher nucleic acids values, but in more coastal areas.

Comparison is also possible with other regions, particularly with other oligotrophic sites. In the study, DNA concentrations are of the same level as those obtained in other oligotrophic regions by Paul *et al.* (1985), Boehme *et al.* (1993), Takahashi *et al.* (1974), Boucher *et al.* (1991), Berdalet & Estrada (1993). RNA concentrations are lower than those found in other oligotrophic sites (Takahashi *et al.*, 1974; Berdalet & Estrada, 1993). Chlorophyll *a* concentrations are of the

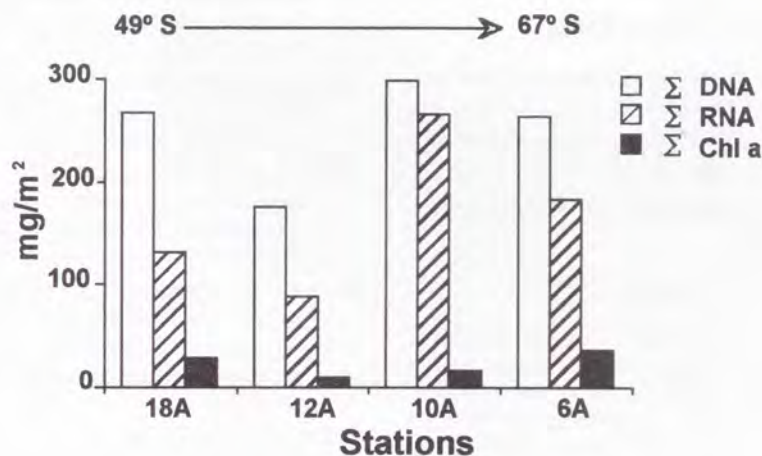


Fig. 4 — Integrated values between 0 and 100 m of the concentrations ($\text{mg}\cdot\text{m}^{-2}$) of DNA, RNA and chlorophyll *a* along the transect between 49 and 67°S in the Indian sector of the Antarctic Ocean in February-March 1994 (Antares 2 Cruise).

same order as those previously found in the same region (Le Jehan & Tréguer, 1983; Jacques, 1989; Jacques & Panouse, 1991).

The integrated values of DNA, RNA and chlorophyll *a* between 0 and 100 m show a particular distribution that seemed related to the vicinity of an enrichment source, like nearness to the continent.

The low DNA/RNA ratio and RNA concentrations indicate the presence of communities with slow metabolic activity. Delille (1993), working at a coastal region in the Antarctic (Adélie Land), showed that the bacterioplankton is not very active (low frequency of diving cells) during the summer period.

The station further south (A06) rich in nucleic acids and chlorophyll *a* is the only one where these parameters are correlated. Correlation between nucleic acids and chlorophyll *a* is present when phytoplankton biomass is relatively rich and absent when it is poor, implying a variation in proportion between autotrophic and heterotrophic along the transect, during the summer. The variation in proportion between autotrophic and heterotrophic is also indicated by direct observations (Fiala & Delille, 1992; Back *et al.*, 1992). The classic structure of the antarctic trophic chain (Diatoms \Rightarrow Euphausiaceas \Rightarrow Whales) has been shaken in the last years and different authors confirm the significance of the picoplankton and of the bacterioplankton in the oceanic region (Hewes *et al.*, 1990; Jacques & Panouse, 1991; Becquevort *et al.*, 1992; Delille, 1992, 1993).

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SUMMARY

Nucleic acids (DNA and RNA) have been proposed as biochemical indicators of biomass and physiological state in natural microplanktonic populations. We present correlations between these indicators and the chlorophyll *a* concentration, a more standard measure of autotrophic biomass. Sampling was undertaken during the Antares 2 cruise (France-JGOFS programme) in the Indian sector of the Antarctic Ocean, at 62°E, on a transect between 40°S and 67°S in the summer. The vertical profiles (0-100 m) indicated almost homogeneous waters in

the open ocean. In the vicinity of the Antarctic continent, chlorophyll *a* showed deep maxima at 80-100 m (0.43 $\mu\text{g l}^{-1}$), but the nucleic acids exhibited higher concentrations at the subsurface waters (30-40 m), with 4.01 $\mu\text{g DNA l}^{-1}$ and 4.20 $\mu\text{g RNA l}^{-1}$. Greatest integrated values of nucleic acids and chlorophyll *a* were found in the proximity of the Antarctic continent.

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