Journal of Plankton Research Vol.22 no.6 pp.1185–1201, 2000

Size-fractionated phytoplankton carboxylase activities in the Indian sector of the Southern Ocean

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Abstract. During the ANTARES 3 cruise in the Indian sector of the Southern Ocean in October–November 1995, the surface waters of Kerguelen Islands plume, and the surface and deeper waters (30–60 m) along a transect on 62°E from 48°36′S to the ice edge (58°50′S), were sampled. The phytoplankton community was size-fractionated (2 µm) and cell numbers, chlorophyll biomass and carbon assimilation, through Rubisco and β-carboxylase activities, were characterized. The highest contribution of <2 µm cells to total biomass and total Rubisco activity was reported in the waters of the Permanent Open Ocean Zone (POOZ) located between 52°S and 55°S along 62°E. In this zone, the picophytoplankton contributed from 26 to 50% of the total chlorophyll ($a + b + c$) with an average of 0.09 ± 0.02 µg Chl l^{-1} for <2 µm cells. Picophytoplankton also contributed 36 to 64% of the total Rubisco activity, with an average of 0.80 ± 0.30 mg C mg Chl a^{-1} h^{-1} for <2 µm cells. The picophytoplankton cells had a higher β-carboxylase activity than larger cells >2 µm. The mixotrophic capacity of these small cells is proposed. From sampling stations of the Kerguelen plume, a relationship was observed between the Rubisco activity per picophytoplankton cell and apparent cell size, which varied with the sampled water masses. Moreover, a depth-dependent photoperiodicity of Rubisco activity per cell for <2 µm phytoplankton was observed during the day/night cycle in the POOZ. In the near ice zone, a physiological change in picophytoplankton cells favouring phosphoenolpyruvate carboxykinase (PEPCK) activity was reported. A species succession, or an adaptation to unfavourable environmental conditions such as low temperature and/or available irradiance levels, may have provoked this change. The high contribution of picophytoplankton to the total biomass, and its high CO_2 fixation capacity via autotrophy and mixotrophy, emphasize the strong regeneration of organic materials in the euphotic layer in the Southern Ocean.

Introduction

The open Antarctic Ocean is known to be only moderately productive, even in summer, but typically contains high concentrations of dissolved nitrate, phosphate and silicate (Priddle *et al*., 1995). The contrast between high nutrient levels and relatively low production has been called the Antarctic paradox (Tréguer and Jacques, 1992) and is usually considered as a high nutrient low chlorophyll (HNLC) area like the Equatorial Pacific or Sub-Arctic Pacific Ocean. Numerous studies have observed the relative importance of water column stability, light limitation (by low irradiance and length of day), grazing pressure, sedimentation, and macro- and micronutrient concentrations in limiting primary production in

the Southern Ocean throughout the autumn, winter and early spring [reviewed by (Jacques, 1983; Priddle *et al*., 1986; Priddle *et al*., 1996)]. On the other hand, several studies [Antiprod I, II, III cruises (Blain *et al*., 1997)] showed an increase of biomass near the Kerguelen islands located on 49°S in the Southern Ocean (Kerguelen plume). One hypothesis to explain this enhancement is an iron input from rivers. Moreover, the Kerguelen plume is affected by a complex hydrodynamic system of surface waters with the Polar Front in this area (Park *et al*., 1997). Tréguer and Jacques described the Polar Front as the area where trophic structures are controlled by hydrological structures with the presence of strong ergoclines, sharp nutrient gradients and numerous mesoscale eddies (Tréguer and Jacques, 1992). This hydrological front plays an essential role in determining the spatial heterogeneity and dynamics of plankton (Koubbi *et al*., 1991).

The discovery of picoplankton and its importance in the food web of oceans changed the view on the structure and function of pelagic ecosystems (Johnson and Sieburth, 1979, 1982; Waterbury *et al*., 1979). Globally, picophytoplankton contributes at least 10% of net primary production, accounting for at least 3 Pg C fixed per year (Fogg, 1986; Falkowski and Raven, 1997). Several studies have shown that nano- (<20 μm and >2 μm) and pico- (<2 μm) phytoplankton quantitatively prevail in the Southern Ocean (Weber and El-Sayed, 1987; Ning *et al*., 1996). Picoplankton accounts for up to 40% of the integrated chlorophyll *a* in the waters of the Indian area (Weber and El-Sayed, 1987; Fiala *et al*., 1998a). Although several studies have revealed that picoplankton are ubiquitous in their distribution (Johnson and Sieburth, 1979) and may be responsible for a major portion of primary production (Shapiro and Guillard, 1987), the ecophysiological capabilities of such organisms are not well known.

Under the drastic environmental conditions in the Southern Ocean, the photosynthetic activity of picophytoplankton, their metabolic activities and the carbon fixation pathways used, are poorly known. Phytoplankton in marine systems fix inorganic carbon by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), an enzyme from the Calvin–Benson cycle which uses light as an energy source. The reaction catalysed by Rubisco is not the only carboxylation reaction in autotrophic cells; β-carboxylation reactions catalysed by phosphoenolpyruvate carboxylase (PEPC), phosphoenolpyruvate carboxykinase (PEPCK) or pyruvate carboxylase (PYRC) also fix inorganic C using organic components as co-substrates from the Calvin–Benson cycle or from outside the cell (Appleby *et al*., 1980; Glover, 1989; Falkowski and Raven, 1997). The interest in β-carboxylation reactions is to account for the marked incorporation of inorganic carbon into amino acids and intermediates of the tricarboxylic acid cycle (Mortain-Bertrand, 1988), which is coupled with incorporation of nitrate and ammonium (Yentsch, 1977). Kremer (Kremer, 1981) suggested that β-carboxylation reactions are more profitable in terms of energy than the Calvin–Benson (C_3) pathway, especially at low-light intensities, because it permits the regeneration of an ATP molecule for each inorganic carbon atom assimilated (Appleby *et al*. 1980). Mortain-Bertrand hypothesized that in Antarctic waters, which are characterized by low light and low temperatures, a high rate of dark fixation by β-carboxylation stimulated by high NO_3 or NH_4 may be an important ecological advantage to compensate for

low photosynthesis due to the other environmental factors (Mortain-Bertrand, 1988). The relationship between N assimilation and β-carboxylase activity has been confirmed by Turpin *et al.* (Turpin *et al.*, 1991). We focused our study on the picophytoplankton, especially on C fixation by these small cells. In this hydrologically-perturbed area, the classical ^{14}C incubation (Steemann Nielsen, 1952) is not the most suitable method to observe variations in high frequency processes. Incubation of the samples does not permit the study of rapid variations in carbon assimilation. Moreover, interpretation of the processes involved during incubation are difficult (Leftley *et al.*, 1983; Glover, 1989) and the classical ^{14}C method does not permit distinction between the two CO_2 fixation pathways used by phytoplankton. Enzymatic activity can be measured instantaneously *in situ*, and the estimate of the enzymatic pool involved in a metabolic pathway allows the maximal rate of a particular physiological function to be determined (Descolas-Gros and Oriol, 1992).

This study will allow us to estimate the picophytoplanktonic contribution to total C fixation in the Indian Sector of the Southern Ocean in early spring, to determine which predominant C pathway is used and to follow *in situ* variations at the cellular scale (expressed by Rubisco activity per picophytoplankton cell).

Method

Sampling stations and depths

Data were obtained during the ANTARES 3 cruise (JGOFS-France) in October–November 1995 and sampling was carried out in October 1995. During the ANTARES 3 cruise in early spring, two different areas were studied. First, the surface waters of the Kerguelen plume were sampled through the Polar Front (Figure 1A). This Front is defined by Park *et al.* as the northernmost extent of the winter remnant of Antarctic surface waters with a subsurface temperature minimum of 2°C (Park *et al.*, 1993). Secondly, the Permanently Open Ocean Zone [POOZ (Tréguer and Jacques, 1992)] was studied along a transect on 62°E from 49°S to ice limit located at 58°50′S (Figure 1B).

From 5–8 October 1995, 13 stations were sampled at 5 m depth through the Kerguelen plume (Table I). Afterwards, from 10–25 October 1995, seven stations (A18, A17, A16, A15, A14, A12 and A11) were sampled along a transect on 62°E from 49°S to the ice limit (Table II) at two depths: between 5 and 10 m (near-surface waters) and deeper, at 30, 40, 50 or 60 m. A day/night cycle occurred at two stations (A16 and A11) at two depths (near-surface and 50 or 60 m).

Underwater pumping

Large volumes of sea water were filtered (between 50 and 250 l h^{-1}) on 142 mm diameter glass fibre filters (Gelman) by underwater pumps (Challenger Oceanic INSU-CNRS) on the transect from 48°S to the ice edge. Clamped on the hydrological cable, the pump offers quick sampling at various depths, at *in situ* temperatures and light intensity, which decreases the physiological stress on the organisms and speeds up sample storage.

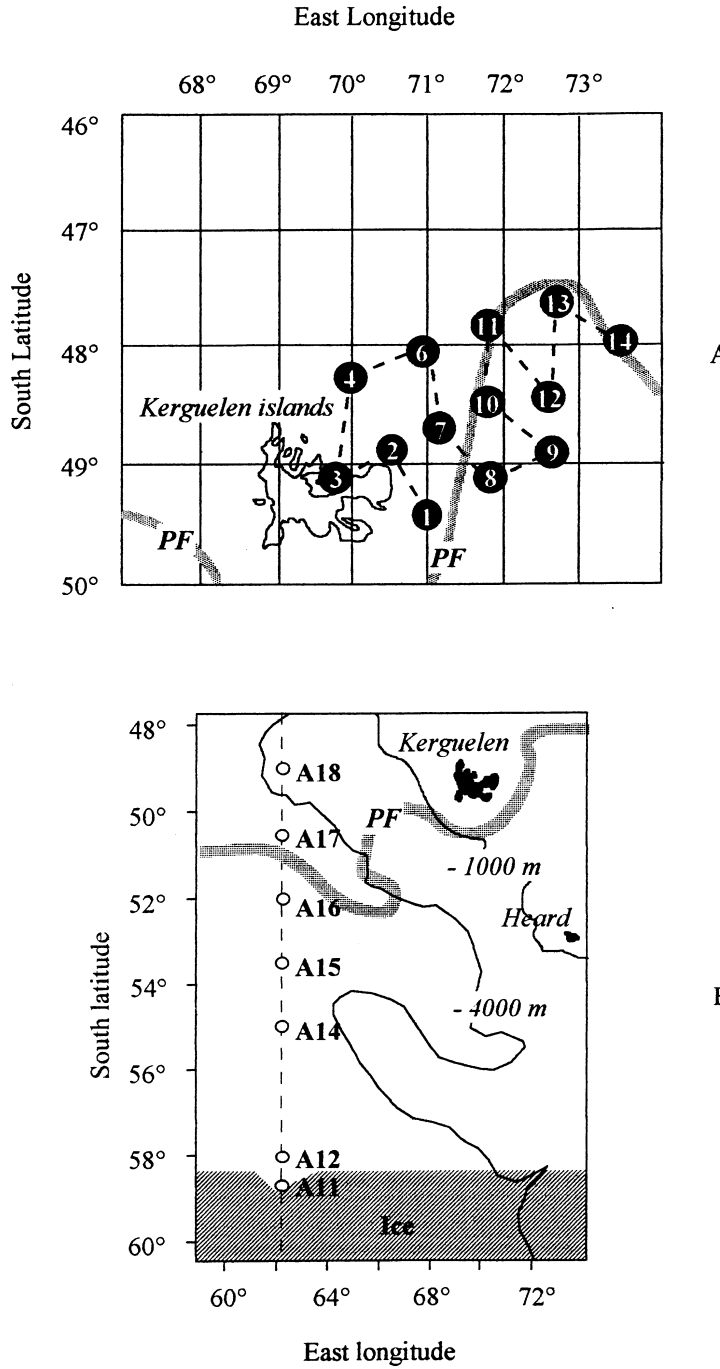

Fig. 1. Map of sampling stations at near-surface waters of the Kerguelen plume (**A**) and along 62°E transect (**B**) during ANTARES 3 cruise from 29 September to 8 November 1995. The position of the Polar Front (PF) is indicated according to Park *et al.* (Park *et al.*, 1997).

Table I. Date of sampling and location of the Kerguelen stations

Stations	Sampling date	Sampling hours (local time)	South latitudes	East longitudes
1	5/10/1995	00:10	49°20′	71°00′
2	5/10/1995	04:45	48°55′	70°30′
3	5/10/1995	10:15	49°10′	69°45′
4	6/10/1995	02:15	48°20′	70°00′
6	6/10/1995	08:20	48°00′	71°00′
7	6/10/1995	14:00	48°43′	71°10′
8	6/10/1995	22:30	49°09′	71°50′
9	7/10/1995	04:45	48°55′	72°40′
10	7/10/1995	10:10	48°30′	71°48′
11	7/10/1995	19:00	47°48′	71°40′
12	8/10/1995	02:15	48°28′	72°39′
13	8/10/1995	08:30	47°44′	72°40′
14	8/10/1995	14:40	48°00′	73°35′

Table II. Location and depths of sampling stations along the 62°E transect during the ANTARES 3 cruise (10–25 October 1995)

Stations	South latitude	East longitude	Sampling depths in m
A18	48°59′	62°00′	5 and 30
A17	50°30′	62°00′	5 and 40
A16	52°12′	62°00′	5 and 50
A15	53°29′	62°00′	5 and 50
A14	54°59′	62°00′	5 and 50
A12	58°00′	62°00′	5 and 50
A11	58°50′	62°00′	10 and 60

During the day/night cycle at the A16 station, *in situ* samples were collected every 4 h close to the sampling time of size-fractionated filtrations. The glass fiber filter was stored in the dark in liquid nitrogen for carboxylase activity measurements.

Size-fractionated filtrations

From Niskin bottle or on-board pump sampling, size-fractionated filtrations were performed at every station to determine the picophytoplanktonic contribution in the phytoplanktonic assemblage. From two size-fractionation filtrations (2 µm threshold), we simultaneously measured chlorophyll ($a + b + c$), *in vitro* carboxylase activity and cell numbers from filtrates. We followed the variations in Rubisco activity per cell for the <2 µm fraction in the different environmental conditions. Sea water (5 or 8 l) was sampled with a shipboard pump (5 m depth sample) or Niskin bottles (5–10 m and 30, 40, 50 or 60 m) and pre-filtered through a 200 µm mesh nylon net. Filtration and size-fractionation were carried out with 2 µm Nuclepore and GF/F Whatman filters within 1 h of sampling, using a Millipore pump with a vacuum (<0.3 atm) to preserve small algal cells.

At each station, two filters (2 µm Nuclepore and GF/F Whatman) for chlorophyll (*a*, *b* and *c*) analysis and carboxylase activity measurements were stored in the dark in liquid nitrogen. Subsamples were taken to determine cell abundance in each fraction (seawater sample, after 2 µm filtration and after GF/F filtration). The >2 µm and <2 µm cell concentrations were calculated by substracting the filtrate results. Samples for flow cytometric analysis were preserved with formaldehyde (2% final concentration) and stored in liquid nitrogen following the protocol described in Troussellier *et al.* (Troussellier *et al.*, 1995).

Biomass analysis

Biomass expressed as chlorophyll (*a* + *b* + *c*) concentration (Chl) was determined by a spectrofluorimetric method (Neveux and Lantoine, 1993).

Flow cytometric measurements

Flow cytometry analyses (FCM) were run with a FacsCalibur flow cytometer (Becton Dickinson, San Jose, CA). Phytoplanktonic cells were detected according to their right-angle light scatter (related to cell size and structure) and their orange and red fluorescence emissions (due to phycoerythrin and chlorophyll pigments, respectively). Beads (1 µm; Polysciences, Warrington, PA) were used as an internal standard and were analysed simultaneously with samples in order to normalize and compare the cytometric signatures defined as the cell fluorescence emission (expressed in bead fluorescence unit: bfu) and the apparent cell size (expressed in bead scatter unit: bsu) of each different cell group. FCM allowed us to enumerate the whole picophytoplanktonic cells only and not the debris or broken cells due to the size-fractionation protocol.

Size-fractionated carboxylase activity and <2 µm Rubisco activity expressed per cell

The *in vitro* carboxylase activity was determined by measuring the incorporation of radioactive bicarbonate into stable products under standard conditions, following the protocol described in Descolas-Gros (Descolas-Gros, 1983) and Descolas-Gros and Oriol (Descolas-Gros and Oriol, 1992). Triplicate assays were automated with a Gilson auto-analyser (222 Autosampler Injector interfaced with a Dilutor 401). Enzyme activities (Rubisco, PEPC, PEPCK and PYR) were analysed and expressed as nmol CO_2 fixed l^{-1} h^{-1} or normalized per chlorophyll *a* (Chl *a*) unit. The quantification of *in vitro* Rubisco and β-carboxylase activity (sum of the PEPC, PEPCK and PYRC activities) was performed on each phytoplanktonic size class. The β-carboxylase/Rubisco ratio (βC/R expressed in %) allowed us to distinguish the two pathways (Calvin-Benson cycle and β carboxylation) that were preferentially used and which are related to the phytoplankton species, physiological state of the cell, and the environmental conditions such as light regime or nutrient concentration (Glover, 1989).

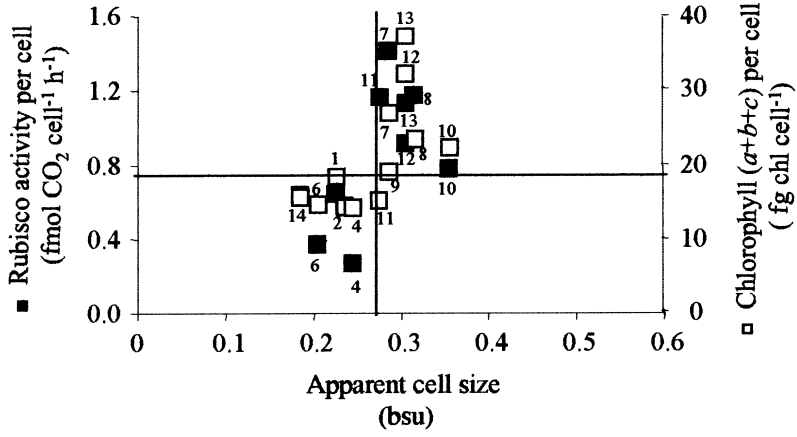

Fig. 2. Average of the apparent size estimated by FCM of the picophytoplankton cells related to the Rubisco activity and chlorophyll ($a + b + c$) per cell for each station sampled in near-surface waters across the Kerguelen plume.

Results

Variations of picophytoplankton apparent cell size from Kerguelen sampling

From the flow cytometry analysis of the picophytoplanktonic fraction, the lowest apparent cell size, from 0.19 to 0.25 bsu, was measured at stations located north of the Polar Front (stations 1, 2, 4, 6 and 14). The larger picophytoplankton cell sizes, from 0.28 to 0.31 bsu, were observed at stations located south of the Polar Front (stations 7, 8, 9, 10, 11, 12 and 13).

Variations of the picophytoplankton biomass

The picophytoplankton contribution to the total biomass varied from 11 to 40% in the Kerguelen samples. The highest values of chlorophyll per picophytoplankton cell (>18 fg Chl cell^{-1}) were measured at stations where the highest values of picophytoplankton apparent cell size (≥0.29 bsu) were reported (Figure 2). The variation in <2 µm chlorophyll content seemed to be related to the apparent size of the picophytoplankton cells.

Along the 62°E transect, at stations A18 to A14, the picophytoplankton contributed about 40% of the total biomass. At station A14, during the day/night cycle, the chlorophyll content for picophytoplankton cells varied from 19.6 to 58.8 fg Chl cell^{-1} at both sampling depths. The southern stations, A12 and A11, differed from the other stations, with picophytoplankton contributing less to total chlorophyll (20–30%). At station A11, during the day/night cycle, an average of 45.8 ± 12.5 (standard deviation) fg Chl cell^{-1} was measured at both sampling depths.

Table III. The average and range of values of the Rubisco activity per chlorophyll *a* and βC/R ratio for both size class from samples at Kerguelen stations, stations A18 to A14 and near-ice stations (A12 and A11) for both sampling depths

	Kerguelen stations		Stations A18–A14		Near-ice stations	
	>2 µm	<2 µm	>2 µm	< 2 µm	>2 µm	<2 µm
Rubisco activity per chlorophyll *a* (nmol CO_2 µg Chl a^{-1} h^{-1})	58 32–90 *n* = 12	53 24–101 *n* = 12	24 12–37 *n* = 21	69 10–136 *n* = 22	35 25–56 *n* = 11	39 14–82 *n* = 9
βC/R ratio (%)	14 8–23 *n* = 12	62 20–120 *n* = 12	17 7–42 *n* = 22	35 6–195 *n* = 23	11 5–23 *n* = 11	59 22–127 *n* = 9

Size-fractionated phytoplankton carboxylase activities

Kerguelen sampling. The picophytoplankton contribution to total Rubisco activity per litre varied from 6 to 36%. The same range of Rubisco activity per Chl *a* unit for the two size fractions was measured in the surface waters of the Kerguelen plume (Table III). However, a higher βC/R ratio, averaging 62%, was measured for picophytoplanktonic cells, compared with the >2 µm cells which had an average of 14% (Figures 3A, B and Table III).

The highest values of picophytoplankton Rubisco activity per cell (>0.7 fmol CO_2 $cell^{-1}$ h^{-1}) were measured at stations where the highest values of apparent cell size (≥0.29 bsu) were reported (Figure 2). As for chlorophyll content, variations in autotrophic activity in the <2 µm cells also seemed to be related to the apparent size of the picophytoplankton cells.

Transect 62°E. The results of Rubisco activity per litre from underwater pumping were compared with the summed size-fractionated Rubisco activity. We compared the values from seven samples at two depths performed during the day/night cycle at station A16. For surface waters, an average of 8.4 ± 2.7 nmol CO_2 l^{-1} h^{-1} was measured from underwater pumping and 7.9 ± 2.3 nmol CO_2 l^{-1} h^{-1} in size-fractionated samples. For deeper waters, an average of 8.4 ± 1.4 nmol CO_2 l^{-1} h^{-1} was measured from underwater pumping and 7.8 ± 1.5 nmol CO_2 l^{-1} h^{-1} from size-fractionated samples. The average value from underwater pumping was thus equivalent to the mean value from the size-fractionated samples. We assumed that the values from size-fractionated filtrations were representative of *in situ* values and that the shipboard filtrations did not perturb *in vitro* carboxylase activity measurements.

At stations A18 to A14, the picophytoplankton contributed about 60% of the total Rubisco activity for both sampling depths. At these stations, the Rubisco activity per Chl *a* for picophytoplankton varied greatly, with an average of 69 ± 29 nmol CO_2 µg Chl a^{-1} h^{-1}, and the βC/R ratio varied from 6 to 195% with an average of 35% (Figure 3A and Table III). The >2 µm phytoplankton had lower Rubisco activity per Chl *a*, with an average of 24 ± 7 nmol CO_2 µg Chl a^{-1} h^{-1},

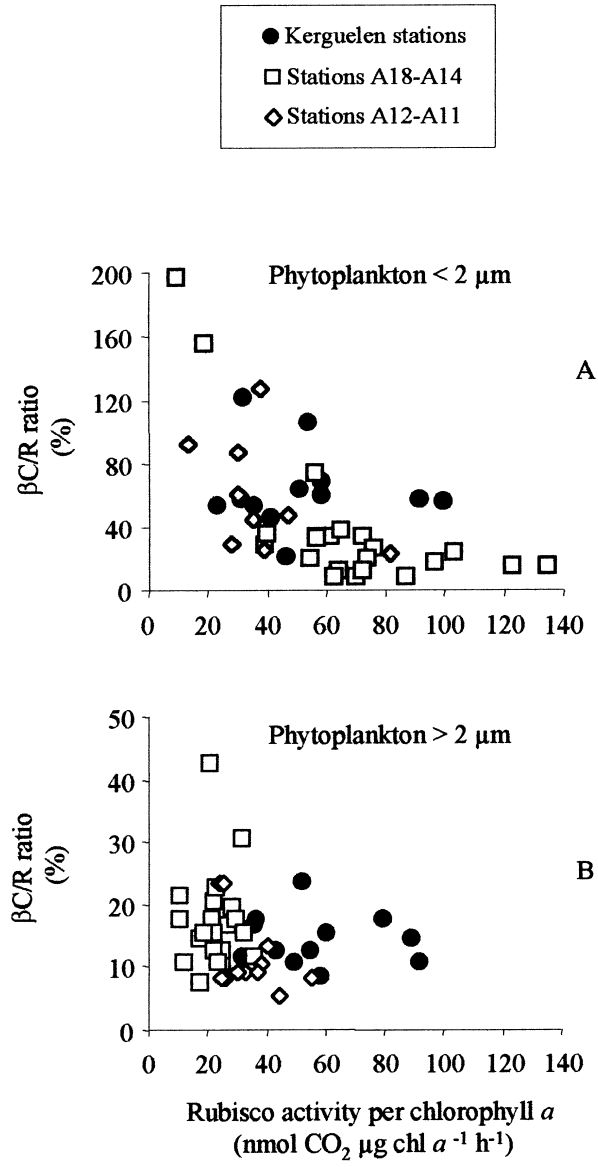

Fig. 3. Rubisco activity per chlorophyll *a* versus βC/R ratio (the % ratio of β-carboxylase to Rubisco activity) at both sampling depths for the <2 µm fraction (**A**) and for the >2 µm fraction (**B**). Circles: Kerguelen stations; squares: stations A18, A17, A16, A15 and A14 along 62°E; diamonds: stations A12 and A11 near ice edge.

and a lower βC/R ratio, which varied from 7 to 42% with an average of 17% (Figure 3B and Table III). Strong co-variations in the Rubisco activity per cell and the chlorophyll content of the cell were noticed along the transect during the day/night cycle at station A16 (Figure 4). During this cycle, the Rubisco activity

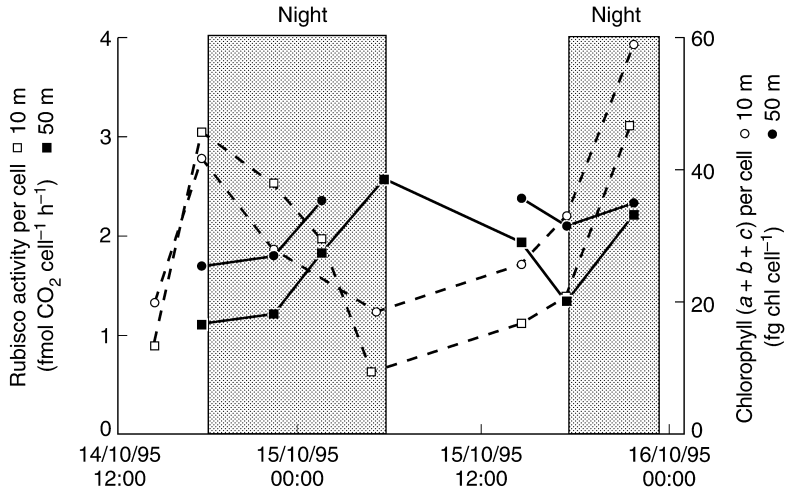

Fig. 4. Depth-dependence of diel variations of Rubisco activity per cell and chlorophyll per cell for <2 µm cells during a day/night cycle at station A16 along the 62°E transect. Open symbols: samplings at near surface (5 m); closed symbols: at 50 m depth. The sampling times (in local time) were October 14: 14:15 h, 17:15 h, 22:00 h; October 15: 01:15 h, 04:45 h (50 m), 5:30 h (5 m), 14:30 h, 17:30 h, 21:45 h.

Table IV. The average and standard deviation of the contribution in percentage of PEPC, PEPCK and PYRC activity to the total β-carboxylase activity measured from size-fractionation filtrations and from *in situ* pumps along the different studied geographical zones during the ANTARES 3 cruise (10–25 October 1995)

	Antarctic waters (POOZ) 50°S to 55°S at 5 and 30–50 m depth			Near-ice waters 57°S to 59°S at 5–10 and 50–60 m depth		
	in situ pumps and summed size-fractionations (n = 40)	>2 µm fraction (n = 21)	<2 µm fraction (n = 22)	*in situ* pumps and summed size-fractionations (n = 20)	>2 µm fraction (n = 9)	<2 µm fraction (n = 9)
PEPC	45 ± 25	37 ± 27	45 ± 26	14 ± 14	23 ± 15	19 ± 18
PEPC K	33 ± 27	30 ± 25	37 ± 29	68 ± 19	38 ± 21	72 ± 19
PYRC	23 ± 12	33 ± 20	18 ± 12	19 ± 12	39 ± 10	9 ± 7

for picophytoplanktonic cells varied from 0.9 to 3.1 fmol CO_2 cell^{-1} h^{-1} at both sampling depths. Different maxima for diel variation were observed between the near surface and 50 m depth. The highest Rubisco activities were observed at the beginning of the night at the sea surface (at 17:15 h, October 14 and at 21:45 h, October 15) and at the beginning of daylight at 50 m depth (at 05:30 h, October 15). The irregularity of sampling hours during the day/light cycle may explain why two different maxima were observed at the sea surface between the first day (October 14) and the second day (October 15).

The southern stations A12 and A11 differed from the other stations, with a lower contribution of picophytoplankton to total Rubisco activity (20–30%) for both sampling depths. At these stations, the Rubisco activity per Chl *a* for <2 µm

and >2 µm were similar, i.e., 38 ± 19 nmol CO_2 µg Chl*a*$^{-1}$ h^{-1} and 35 ± 10 nmol CO_2 µg Chl *a*$^{-1}$ h^{-1}, respectively (Table III). A higher βC/R ratio was reported for <2 µm phytoplankton, varying from 22 to 127% with an average of 59%, compared with the βC/R ratio for >2 µm phytoplankton which varied from 5 to 23% with an average of 11% (Figure 3A,B and Table III). From the β-carboxylase activity results, we observed a high PEPCK activity from the whole phytoplanktonic community, especially for the <2µm fraction, (Table IV). At station A11, the diel variations were not marked because of the perturbed spatial sampling and because only a few Rubisco activity values were obtained at this station. An average of 1.4 ± 0.5 fmol CO_2 cell^{-1} h^{-1} was measured during the day/night cycle for both sampled depths.

Discussion

The variations of picophytoplankton Rubisco activity and chlorophyll per size-dependent cell

A picophytoplanktonic species succession in the waters of the Kerguelen plume could explain the variations in Rubisco activity and chlorophyll per cell. The highest values of Rubisco activity and chlorophyll per cell for the <2 µm fraction seemed to be related to the picophytoplankton fraction with the highest apparent cell size. The picophytoplanktonic community from the surface waters of each side of the Polar Front was different. The smallest values of the average cell size (<0.29 bsu), the Rubisco activity per cell (<0.7 fmol CO_2 cell^{-1} h^{-1}) and the chlorophyll content (<18 fg Chl cell^{-1}) were observed in waters north of the Polar Front in the Kerguelen plume. The Polar Front seems to represent a sharp transition for picophytoplanktonic species between the sub-Antarctic and Antarctic waters like the subtropical frontal zone between the Indian ocean and sub-Antarctic waters (Fouilland *et al*., 1999).

Contribution of the picophytoplankton autotrophic capacity

From 50°S to 55°S, the picophytoplankton contribution was very high in the first 50 m of the homogenous Antarctic water column, contributing up to 40% of the total biomass and 60% of the total Rubisco activity. In the same area, Fiala *et al.* on the ANTARES 2 cruise observed that the picophytoplankton fraction was clearly dominant, contributing about 47% of the total biomass during the late austral summer of 1994 (Fiala *et al.*, 1998b). Moreover, the Rubisco activity per Chl *a* for picophytoplankton in our study was higher than the values for >2 µm cells. The autotrophic capacity of picophytoplankton was thus higher than that of nano- and micophytoplankton during our study. This observation confirms those from productivity measurements by Ning *et al.* (Ning *et al.*, 1996) who reported the highest productivity for picoplankton in Antarctic waters. Such size dependence of photosynthetic parameters is not consistent with the findings of Shiomoto *et al*. (Shiomoto *et al*., 1997), who observed that at low temperatures, picoplankton productivity is not significantly higher than that of larger-sized phytoplankton in the sub-Arctic region and off the South Shetland Islands

(Shiomoto *et al*., 1998), and in the surface waters across the Indian subtropical frontal zone (Fouilland *et al*., 1999). Such differences in productivity between the areas studied could be due to the seasonal variations in environmental factors, such as light intensity (Shiomoto *et al*., 1998) and micronutrient limitations, which could influence differently the physiology of sized-fractionated phytoplankton as suggested by Fouilland *et al.* (Fouilland *et al*., 1999).

Jacques reported that a significant proportion of primary production in the Southern Ocean is due to <2 µm organisms and corresponds to the microbial loop (Jacques, 1987). In this microbial loop model, material can be recycled efficiently within the euphotic zone and is responsible for most remineralization of organic carbon and nitrogen in the upper water column. This system induces a poor export of organic matter and is dominated by high floating organisms (Jacques, 1987). In the POOZ, along the 6°E in the austral spring, Jochem *et al.* (Jochem *et al*., 1995) reported a contribution of 36–75% by <2 µm cells to primary production, which is close to our values. These authors suggested a light limitation by the vertical deep mixing in these waters governing phytoplankton production in favour of small cells. In contrast to previous studies which used the classical ^{14}C method, from Rubisco activity measurements we have considered only one physiological process, the autotrophic CO_2 fixation capacity of phytoplankton. The dominance of the picophytoplankton autotrophic capacity measured in these waters suggests that most of the CO_2 fixed by primary producers contributes to organic material recycling in the euphotic layer.

During the day night/cycle at station A16, a slight diel variation in the <2 µm Rubisco activity per cell appeared with a depth-dependent periodicity. Maxima in Rubisco activity per cell were observed at the beginning of the night near the surface and at the beginning of daylight at 50 m depth. Paul *et al.* showed clear evidence of diel regulation of gene expression (rbcL) for the Rubisco C fixation enzyme, with a greater concentration of rbcL transcripts in cells in the light than in the dark (Paul *et al*., 1998). Several studies have shown that C fixation, assimilation number and photosynthetic efficiency of natural communities changes during the day, with minimum values during the night and maximal values occurring either before or after solar noon [in (Legendre *et al*., 1988)]. The possible diel periodicity of Rubisco activity per cell reported in our study can be explained by a depth-dependent photoperiod (length of day and night periods) which also influenced the chlorophyll content in the small cells. We did not observe a change in cytometric picoeukaryote signatures (in apparent chlorophyll and apparent cell size) at 50 m, because our sampling time was certainly not well adapted to characterise a diel cell cycle by FCM. However, we hypothesize that diel variations in chlorophyll and Rubisco activity per cell reflect the different diel periodicity in the cell cycle of picoeukaryotes between the surface and deeper waters at this station.

Antarctic phytoplankton β-carboxylase activities

For both sampling areas during the cruise, a βC/R ratio smaller than 40% was measured for >2 µm phytoplankton. A similar ratio was reported for Antarctic

diatoms (Descolas-Gros and Fontugne, 1990a). The low βC/R ratio is characteristic of a high proportion of C fixed by the Rubisco pathway and of strictly autotrophic cells (Descolas-Gros and Oriol, 1992). For the <2 µm fraction, we did not distinguish picophytoplankton groups with a very high βC/R ratio, similar to some heterotrophic dinoflagellate species (Descolas-Gros and Oriol, 1992), and groups with a very low βC/R ratio, similar to the strict autotrophs using principally the Rubisco pathway. The wide range in the observed βC/R (%) ratio for this fraction suggested a continuum between autotrophy and heterotrophy for these small cells. It leads to the hypothesis that the autotrophic picophytoplankton probably also have a mixotrophy potential or a facultative photoautotrophy capability as defined by Falkowski and Raven (Falkowski and Raven, 1997). In fact, the high βC/R ratio for the <2 µm fraction measured in our study was due to the high β-carboxylase activity per Chl *a*, about 21 ± 13 nmol CO_2 µg Chl a^{-1} h^{-1}. The β-carboxylase activity per Chl *a* for >2 µm cells was about 5 ± 3 nmol CO_2 µg Chl a^{-1} h^{-1}. A high βC/R ratio has also been observed by Descolas-Gros and Fontugne (Descolas-Gros and Fontugne, 1990b) in the St Lawrence estuary where flagellates dominated in contrast to the other stations characterized by a diatom dominance. The β-carboxylation reaction is required to provide several essential amino acids and to replenish intermediates of the tricarboxylic acid cycle (TCA cycle). This cycle is coupled with nitrate and ammonium incorporation (Yentsch, 1977). The β-carboxylation pathway provides essential compounds for growth, such as several essential amino acids, tetrapyrols, pyrimidines, purines and lipids (Falkowski and Raven, 1997). Higher N uptake rates in phytoplankton have small cells has been observed in Antarctic surface waters (Probyn and Painting, 1985; Koike *et al.*, 1986). We suggest that higher N assimilation might explain the higher rate of β-carboxylation found in picophytoplankton cells, leading to a higher synthesis of amino acids. An enhancement of β-carboxylation reactions may confer an important ecological advantage, allowing cells to reach a higher maximal rate of photosynthesis than that which would be expected with only the C3 pathway (Calvin-Benson cycle) (Kremer, 1981). A βC/R ratio >100% for small cells was sometimes measured in our study. A βC/R >100 % in autotrophic dinoflagellates was also measured by Descolas-Gros and Oriol (Descolas-Gros and Oriol, 1992). They concluded that these species can use organic compounds as co-substrates for inorganic carbon assimilation. The picophytoplankton from our study seemed to have a high C assimilation which used both C fixation pathways, especially β-carboxylation. Such C metabolism allows cells to rapidly supply themselves with the necessary compounds, such as amino acids (Yentsch, 1977; Falkowski and Raven, 1997), from substrates (phosphoenolpyruvate) derived from reserve carbohydrates (probably very low in such small cells) or by the incorporation of external organic (dissolved or undissolved) carbon, probably endogenous compounds, as hypothesized by Fontugne *et al.* (Fontugne *et al.*, 1991). This latter possibility suggests a heterotrophic capability as shown by Rivkin and Putt (Rivkin and Putt, 1987) for some Antarctic diatoms. They suggested that the assimilation of dissolved organic substrates could supplement light-limited growth during the austral spring.

At the near ice stations, higher PEPCK activity than PEPC or PYRC was

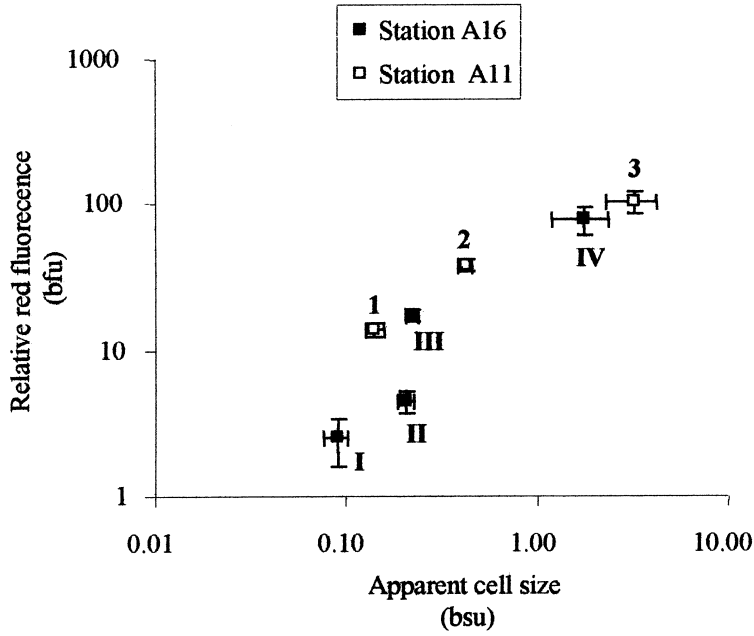

Fig. 5. Flow cytometry characterization of the picophytoplanktonic assemblage according to cell properties (red fluorescence and light scatter normalized by 1 µm beads: cytometric signature) from stations A16 and A11 along 62°E transect. I, II, III and IV are the four different picophytoplanktonic signatures observed at station A16; 1, 2 and 3 are the three different picophytoplanktonic signatures observed at station A11.

observed, especially for the picophytoplankton. Appleby *et al.* hypothesized that PEPCK could be an adaptation to low light intensities because it permits the regeneration of an ATP molecule for each inorganic C atom assimilated (Appleby *et al.*, 1980). This may confirm the hypothesis of low irradiance as a limiting factor at these stations. Descolas-Gros also observed such PEPCK activity dominance for the whole community from the APSARA cruise from 35°S to 75°S in the Atlantic sector of the Southern Ocean (Descolas-Gros, 1983). In our study, we observed different picophytoplanktonic cell signatures by cytometric detection at station A16 (four picophytoplanktonic cytometric signatures) located in the POOZ and at station A11 (three picophytoplanktonic cytometric signatures) near the ice limit (Figure 5). Changes in the βC/R ratio may also be due to species succession, with different β-carboxylase enzymes in the <2 µm fraction as reported for a whole community during an algal bloom (Descolas-Gros and Fontugne, 1988; Collos *et al.*, 1992).

In conclusion, the high picophytoplanktonic contribution to the total autotrophic activity in the Indian sector of the Antarctic waters suggested strong carbon recycling in the euphotic zone in early spring. We also suggest that the Antarctic picophytoplankton had a very active C metabolism and used two pathways (Calvin-Benson cycle and β-carboxylation) to rapidly supply themselves

with amino acids and, finally, proteins. We sometimes noticed a βC/R ratio >100% for small cells which suggests mixotrophic activity. Such mixotrophy could also be correlated with the presence of unknown heterotroph non-photosynthetic cells using β-carboxylation enzymes in this <2 µm fraction. This mixotrophic capability could be explained by the picophytoplankton condition, which appears to be derived and polyphyletic in both prokaryotes and eukaryotes (Raven, 1998). Such activities differ from the gross primary production characterized by CO_2 fixation using light as an energy source. From the enzymatic essays, we could not distinguish the *in vivo* enzyme activity from the potential activity, so we can only conclude that the picophytoplankton seem to have a stronger metabolic adaptability than larger cells. If such activity is confirmed for picophytoplankton in other oceanic areas, it could explain their greater competitiveness in unfavourable environmental conditions.

Acknowledgements

This work is part of the Programme ANTARES within JGOFS-France. It was supported by the Centre National de la Recherche Scientifique-Institut National des Sciences de l'Univers (CNRS-INSU) and the Institut Français de Recherche et Technologie Polaires (IFRTP), and led by Stéphane Blain, Patrick Mayzaud and Paul Tréguer. We are grateful to the officers and crew of the Marion Dufresne for their efficient assistance at sea. Contribution ISEM 2000–2036.

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Received on April 20, 1999; accepted on December 12, 1999