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Prochlorococcus contribution to phytoplankton biomass and production of Takapoto atoll (Tuamotu archipelago)

Contribution des Prochlorococcus *à la biomasse et à la production phytoplanctonique de l'atoll de Takapoto (archipel des Tuamotu)*

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RÉSUMÉ

Les *Prochlorococcus* sont mis en évidence pour la première fois dans un lagon d'atoll. La répartition du phytoplancton au sein de la colonne d'eau était hétérogène. On distinguait une couche superficielle (0-10 m) moins riche en phytoplancton où les picoeucaryotes étaient le groupe dominant et une couche sous-jacente plus riche où le groupe dominant était des cyanobactéries du genre *Synechococcus*. Les maxima de biomasse, d'abondance et de production étaient systématiquement localisés dans la couche sous-jacente. Les pourcentages intégrés des *Prochlorococcus*, des *Synechococcus* et des picoeucaryotes étaient respectivement 20 %, 78 %, 2 % pour les abondances, et 2 %, 57 % et 41 % pour les fluorescences. Les abondances maximales étaient respectivement 1,1 x 10^4, 7,5 x 10^4, 0,3 x 10^4 cellules par ml, et les parts respectives de la production primaire étaient 2 %, 55 % et 43 %. Dans les eaux océaniques avoisinantes, les importances relatives des groupes étaient totalement différentes avec une nette dominance en abondance des *Prochlorococcus*. En valeurs intégrées, la part des *Prochlorococcus*, des *Synechococcus* et des picoeucaryotes était respectivement 98 %, 1 %, 1 % pour les abondances et 42 %, 6 % et 52 % pour les fluorescences. Les abondances maximales étaient 16 x 10^4, 0,15 x 10^4 et 0,2 x 10^4 cellules par ml. Pour des raisons encore inconnues, le milieu lagunaire privilégie les *Synechococcus* alors que le milieu océanique privilégie les *Prochlorococcus*. ▲

Mots clés : prochlorococcus, *lagon d'atoll, phytoplancton,* Synechococcus, *Polynésie française.*

ABSTRACT

Prochlorococcus *was observed for the first time in an atoll lagoon. Phytoplankton distribution was heterogeneous in the water column. In the upper 10 m, total biomass was lower and dominated by picoeukaryotes. Below, biomass was higher and dominated by* Synechococcus. *Biomass and production maxima were always below 10 m. In average (0-25 m), the percentage of* Prochlorococcus, Synechococcus *and picoeukaryote abundance were respectively 20%, 78%, 2% and percent of fluorescence was 2%, 57% and 41%. Maximum abundances were respectively 1.1 x 10^4, 7.5 x 10^4, 0.3 x 10^4 cells per ml and respective contributions to phytoplankton production were 2%, 55% and 43%. In surrounding oceanic waters (0-150 m), percentage of* Prochlorococcus, Synechococcus *and picoeukaryote abundance was respectively 98%, 1%, 1% and percentage of fluorescence 42%, 6% and 52%. Maximum abundances (cells ml^{-1}) were 16 x 10^4* Prochlorococcus, *0.15 x 10^4* Synechococcus *and 0.2 x 10^4 picoeukaryotes. Lagoonal conditions seem to be preferred by* Synechococcus *and oceanic conditions by* Prochlorococcus. ▲

Key words: *Prochlorococcus,* atoll lagoon, phytoplankton, *Synechococcus,* French Polynesia.

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VERSION ABRÉGÉE

Notre équipe en utilisant la microscopie à épifluorescence a rapporté la grande abondance des *Synechococcus* dans les lagons d'atolls ouvert et fermé, et démontré que la fraction < 1 µm était le siège de plus de 60 % de la biomasse et de la production phytoplanctonique. Les questions que nous nous posons concernent la présence des *Prochlorococcus* dans les lagons d'atolls, et le cas échéant leur contribution à la biomasse et à la production phytoplanctonique. Les eaux lagunaires étant d'origine océanique, une étude comparative de l'abondance des différents groupes taxinomiques entre le lagon et l'océan nous a paru indispensable.

Au cours d'une mission d'une semaine en novembre 1994, l'étude du phytoplancton a été entreprise à 6 stations du lagon de Takapoto et à 1 station océanique. La biomasse phytoplanctonique était estimée à partir de la chlorophylle-a (Chl-a) avec fractionnement de taille (> 3 µm, 3-1 µm, < 1 µm) et de comptage au cytomètre de flux. La production primaire était estimée par mesure *in situ* du taux d'incorporation du ^{14}C. Les fractionnements de taille étaient réalisés après l'incubation en utilisant les mêmes filtres que pour la chlorophylle.

Le phytoplancton se répartissait en 2 couches distinctes. Une couche superficielle (0-10 m) où les picoeucaryotes dominaient en fluorescences *in vivo* (54 %, n = 54), et une couche sous jacente (15-25 m) où les *Synechococcus* dominaient en fluorescence (70 %, n = 42). Cette opposition ne se retrouvait pas au niveau des fractionnements de taille de chlorophylle. Dans les 2 couches, la fraction inférieure dominait nettement. On avait pour les fractions < 1 µm, 1-3 µm, >3 µm respectivement pour la couche supérieure 68 %, 15 %, 17 % (n = 45) et pour la couche inférieure 71 %, 16 %, 13 % (n = 42). Sur l'ensemble de la colonne d'eau, la contribution des *Prochlorococcus* mesurée par la fluorescence *in vivo* était faible, représentant 2 % de la biomasse totale et 4 % de la biomasse < 1 µm.

Dans les eaux océaniques, ce sont les *Prochlorococcus* qui dominaient le phytoplancton en abondance. En valeurs intégrées, il y avait une nette dominance en effectif des *Prochlorococcus* (97,9 %) par rapport aux *Synechococcus* (0,8 %) et aux picoeucaryotes (1,3 %). Par contre, en fluorescence, on trouvait pour ces 3 taxa respectivement 42,3 %, 5,3 % et 52,4 %. Les abondances maximales étaient de 160 000 *Prochlorococcus*, 1500 *Synechococcus* et 2000 picoeucaryotes par ml.

Les maxima de production ont été systématiquement observés à 20 m de profondeur, ce qui correspond à la profondeur du maximum de photoprocaryotes. Cela diffère des résultats publiés précédemment où les maxima de production se situaient en surface. Les couches 0-10 m et 15-25 m avaient des rapports production/biomasse élevés et assez proches, respectivement 14,3 ± 1,5 µg C µg^{-1} Chl-a h^{-1} et 12,5 ± 1,4 µg C µg^{-1} Chl-a h^{-1}. Sur l'ensemble de la colonne d'eau (N = 30), les contributions relatives des différentes classes de taille (< 1 µm, 1-3 µm et > 3 µm) à la production primaire étaient respectivement de 57 ± 2 %, 23 ± 1 % et 20 ± 2 %. La contribution des *Prochlorococcus* à la production a été estimée à 2 %. La production moyenne du lagon à la station 9, intégrée sur 25 m était de 0,9 g C m^{-2} jour^{-1}.

En conclusion, les *Prochlorococcus* étaient présents dans le lagon de Takapoto mais ils ne contribuaient qu'à 2 % de la biomasse et de la production phytoplanctonique. Les *Synechococcus* constituaient le groupe largement dominant. Par contre, dans les eaux océaniques avoisinantes, les *Prochlorococcus* et les picoeucaryotes dominaient nettement la biomasse phytoplanctonique. L'épuisement en phosphate des eaux lagunaires et la possibilité pour les *Synechococcus* de fixer l'azote moléculaire peuvent être à l'origine des changements observés dans les populations phytoplanctoniques. ▲

In the 1980's, the great abundance and extensive occurrence of cyanobacteria of the genus *Synechococcus* was reported in the different oceans with the increasing use of epifluorescence microscopy in biological oceanography [1, 2]. At the end of the 1980's, *Prochlorococcus* were discovered [3] with the use of flow cytometers on board oceanographic vessels. Using epifluorescence microscopy, our laboratory recognized the great abundance of *Synechococcus* in closed and open atolls [4, 5], and we found that the fraction < 1 µm was responsible for more than 60 % of the phytoplanktonic biomass and production [5]. The presence of *Prochlorococcus* in atoll lagoons is investigated as well as their contribution to phytoplanktonic biomass and production. Because lagoonal waters are of oceanic origin, a comparative study of the abundance of the different taxonomic groups between lagoon and ocean is important.

Materials and methods

During a 1 week field study in November 1994, the picophytoplankton was enumerated and recognized at the group level by flow cytometry at 6 stations in the lagoon of Takapoto and at 1 oceanic station (one mile from the atoll) *(Fig. 1)*. In the lagoon, samples were taken every day every 5 m, at 9:00 AM, 1:30 PM, and 6:00 PM. A 24 h cycle with samples every 4 h was also taken at station 9. Oceanic water was sampled twice, 2 km from the atoll (station Ext) at the following depths: 0, 10, 20, 30, 40, 50 m, then 5, 20, 30, 50, 60, 80, 90, 100, 110, 120, 150 m. Samples were taken with a 5 l Niskin bottle fitted with a teflon spring. Samples for enumeration and for chlorophyll-a analysis (Chl-a) were stored in the dark in an ice box. Filtrations and separation by size (> 3 µm, 3-1 µm, < 1 µm) were carried out in the hour following the sampling. Nuclepore 3 µm and 1 µm and GF/F for the total fraction were used. Chl-a has been estimated by fluorimetry [6]. The method for enumeration has been described by Blanchot and Rodier [7]. Briefly, samples were analyzed live, in the hour following the sampling, with the Becton-Dickinson FACScan. The excitation source was a blue laser beam (15 mV, 488 nm), the red fluorescence (RF) of the chlorophyll was analyzed with wave lengths > 650 nm. In order to calibrate the fluorescence and estimate the volume analyzed, a known quantity of fluorescent beads (Polyscience, 2 µm) were added in each sample. In the near-surface nitrate-depleted samples, *Prochlorococcus* populations are too dim to be completely resolved by the FACScan system. Since the distribution of *Prochlorococcus* is a nomal distribution, we have calculated indirectly the approximative concentration of the dim *Prochlorococcus* by extrapolation of the missing part, by multiplying the right part of the distribution by 2, as reported by Blanchot and Rodier [7]. Samples for measure of primary production were incubated *in situ* in 200 ml polycarbonate bottles with 2 µCi of ^{14}C at

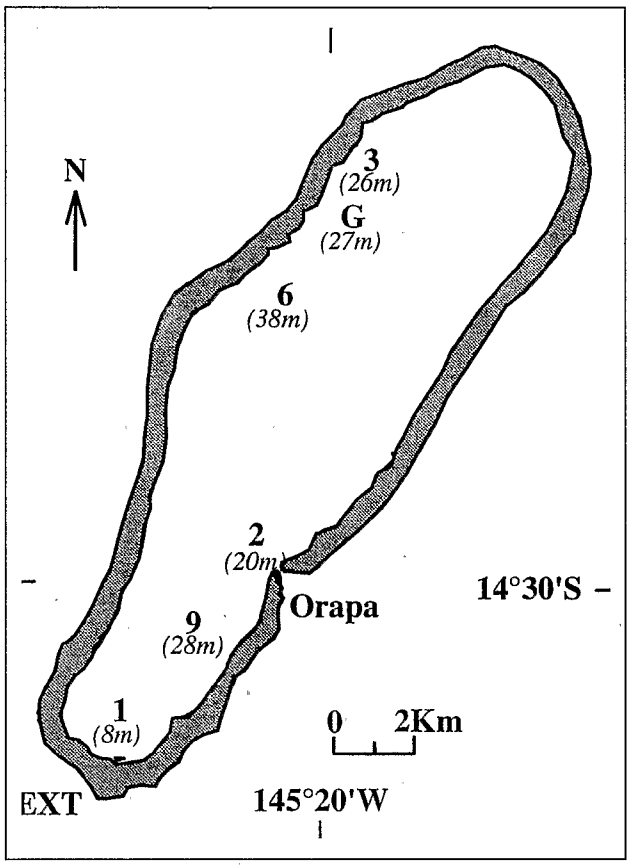

Figure 1. ***Location of sampling stations.***

0, 5, 10, 15, 20, 25 m depth at station 9. Filtrations and size fractionations were realized using the same filters as for the chlorophyll. Radioactive counts were realized using liquid scintillation (Packard).

Results and discussion

Means are given ± SE and the number of measurements is in parentheses.

Lagoon waters

Results of biomass are sumarized in *Table I*. RF = *in vivo* red fluorescence. We could distinguish 2 layers with respect to phytoplanktonic biomass: the upper layer (from the surface down to 10 m depth) where the biomass was smaller and the integrated RF dominated by picoeukaryotes (54%) and the deeper layer (15-25 m) largely dominated in RF by *Synechococcus* (70%) *(Fig. 2* to *Fig. 5)*. This opposition between the upper and the deeper layers did not exist for the chlorophyll size fraction. Repartition of Chl-a by size class < 1 µm, 1-3 µm and > 3 µm was quite constant with depth, respectively 70%, 16%, 14% for the water column. Enumerations made in 5 < 1 µm filtrates showed that 100% of *Prochlorococcus* and 89 ± 1% of *Synechococcus* passed through a 1 µm pore size filter. *Prochlorococcus* and *Synechococcus* contributions to the < 1 µm biomass, estimated using their contribution to RF (respectively 2% and 57%) were respectively $\frac{2 \times 100}{(2 + 57 \times 0.89)} = 4\%$ and 96%. At the surface, there was a difference between the percentage of Chl-a < 1 µm (68%) and the percentage of RF of photoautotroph prokaryotes, *Prochlorococcus* and *Synechococcus* (46%) whereas at deeper levels, the percentages were very close (71% and 72%). There is usually a better correlation between the total Chl-a and -b and RF, than between Chl-a and RF [8, 9]. Without a sorting analyzer, the *in vivo* fluorescence of the different taxonomic groups give direct measurements of each cell of the different groups. The contribution of the *Prochlorococcus* to RF was small (2.0 ± 0.1%, n = 87) on the entire water column.

Table I
Average ± SE of chlorophyll concentration (Chl-a), percentage of Chl-a in different size fractions, picophytoplankton abundance and percentage of *in vivo* red fluorescence (RF) in Takapoto lagoon

	0-10 m (n = 45)	15-25 m (n = 42)	0-25 m (n = 87)
Chl-a			
total (µg l^{-1})	0.20 ± 0.01	0.31 ± 0.02	0.25 ± 0.01
% < 1 µm	68.0 ± 1.0	71.2 ± 1.2	69.5 ± 0.8
% 1-3 µm	16.9 ± 0.6	15.5 ± 0.8	16.2 ± 0.5
% > 3 µm	15.2 ± 0.7	13.3 ± 0.7	14.2 ± 0.5
Prochlorococcus			
cells ml^{-1}	16,455 ± 1,354	35,634 ± 1,642	25,714 ± 1,474
% RF	1.5 ± 0.1	2.6 ± 0.2	2.0 ± 0.1
Synechococcus			
cells ml^{-1}	66,283 ± 2,219	130,152 ± 7,450	97,116 ± 5,091
% RF	44.8 ± 1.8	69.5 ± 1.7	56.7 ± 1.8
Picoeukaryotes			
cells ml^{-1}	2,542 ± 72	2,318 ± 127	2,433 ± 72
% RF	53.8 ± 1.8	27.9 ± 1.7	41.3 ± 1.9

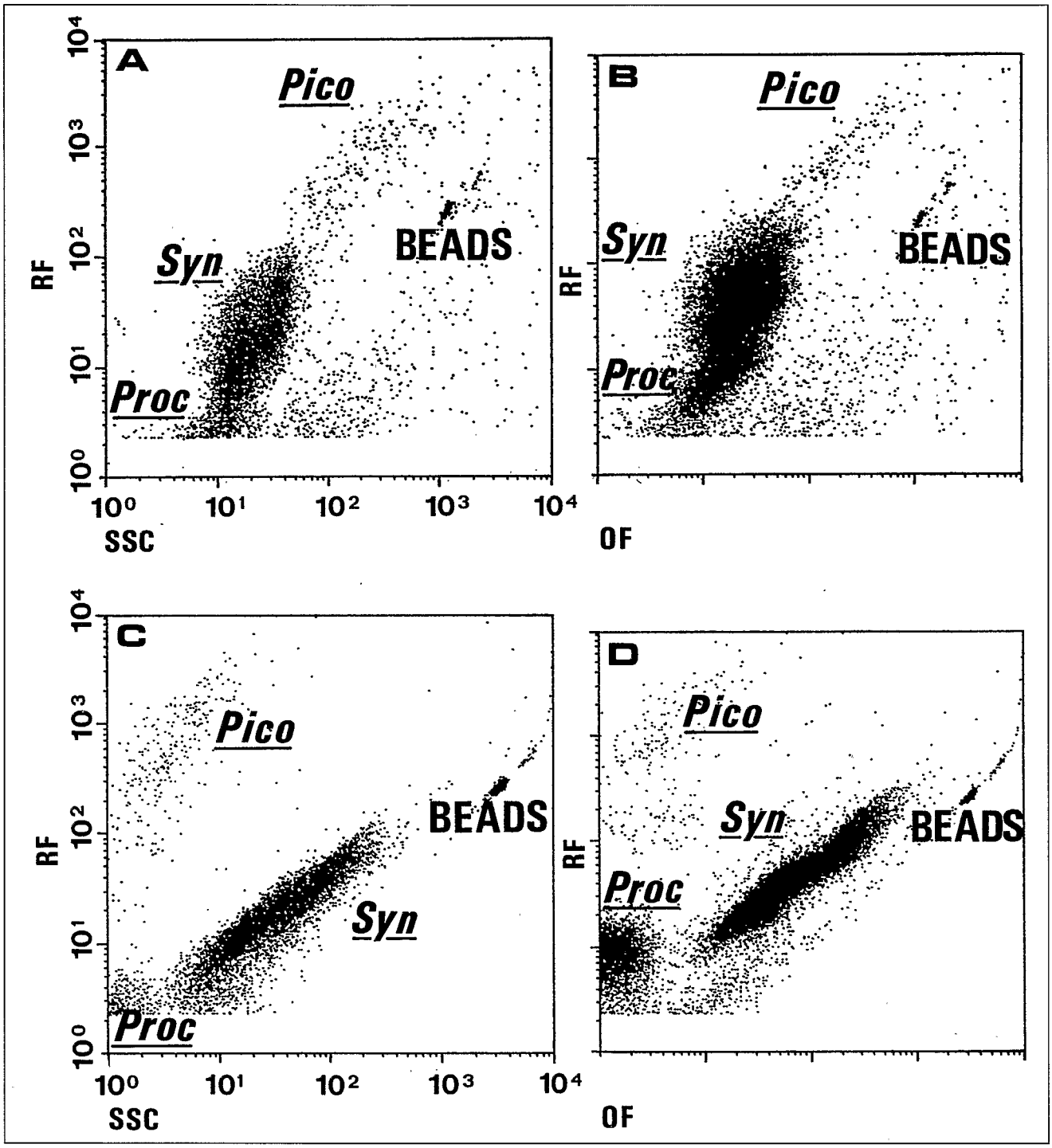

Figure 2. ***FACScan cytograms of Takapoto atoll lagoon water. (A and C)*** *Surface.* ***(B and D)*** *20 m. OF: orange fluorescence (phycoerythrin); RF: red fluorescence (chlorophyll a and b); SSC: side size scatter;* Proc*: Prochlorococcus;* Syn*: Synechococcus; Pico: picoeukaryote.*

Comparison with oceanic waters

In oceanic waters, *Prochlorococcus* dominated the phytoplankton *(Fig. 6* and *Fig. 7).* By integration, there was an effective dominance of the number of *Prochlorococcus* (98%) compared to *Synechococcus* (1%) and to picoeukaryotes (1%). On the other hand, using fluorescence *(Fig. 8),* we found for these taxa, respectively 42%, 6% and 52% of their effective dominance. The maximal abundances were 160,000 *Prochlorococcus,* 1,500 *Synechococcus* and 2,000 picoeukaryotes per ml. *Prochlorococcus* dominance in numerical abundance and carbon biomass was observed in Pacific subtropical ocean regions [10, 11]. This dominance is particularly important in nitrate-depleted layers and in the convergence areas [7]. In the upper layers of offshore tropical Pacific Ocean waters, *Synechococcus* constitute only a small percentage of integrated carbon values [7]. Therefore the community structures of the

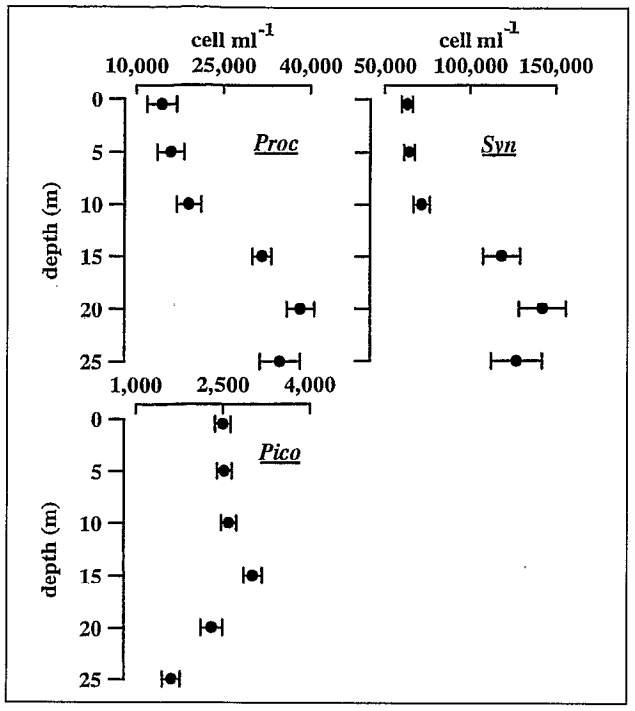

Figure 3. ***Average ± SE of phytoplankton abundance in Takapoto lagoon.*** Proc: Prochlorococcus; Syn: Synechococcus; Pico: *picoeukaryote.*

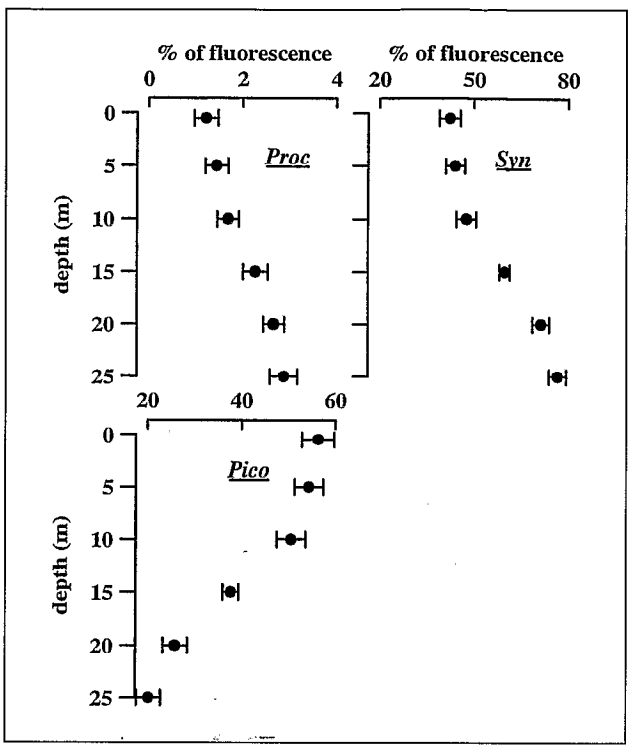

Figure 4. ***Percentages of in vivo red fluorescence in Takapoto lagoon.*** Proc: Prochlorococcus; Syn: Synechococcus; Pico: *picoeukaryote.*

lagoon and of the surrounding ocean are very different. In lagoonal environments the community is largely dominated by the *Synechococcus* and the second by *Prochlorococcus* and picoeukaryotes. Considering that the time for renewal of 99% of the lagoonal water is 18.4 years [12], the lagoonal picophytoplankton is indigenous.

Production

The production maxima were systematically observed at 20 m depth *(Fig. 9)*, which corresponds to the depth of the *Prochlorococcus* maximum, which is different from the previous published results where the production maxima were at the surface [5]. The 0-10 m and 15-25 m layers have high production/biomass ratios and these are relatively close to each other, respectively 14.3 ± 1.5 µg C µg^{-1} Chl-a h^{-1} et 12.5 ± 1.4 µg C µg^{-1} Chl-a h^{-1}. High production/biomass ratios of this kind were observed in Takapoto [5] and Tikehau [13]. On the entire water column (n = 30), the relative contributions of the different size classes (< 1 µm, 1-3 µm and > 3 µm) to the primary production were respectively 57 ± 2%, 23 ± 1% and 20 ± 2%. Taking into account that *Prochlorococcus* represented 4% of the < 1 µm biomass, their contribution to total phytoplankton production would be close to 57 x 0.04 = 2%. The lagoonal mean production at station 9, integrated over 25 m was 0.9 g C m^{-2} day^{-1}. This value is close to the one found previously (0.8 g C m^{-2} day^{-1}) [5], but twice as high as the one estimated 20 years ago at Takapoto [14] and 5 years ago at Tikehau [13]. The utilization of polycarbonate incubation bottles and one Niskin bottle fitted with a teflon spring might be one of the reasons for the observed differences during this present study and the one realized in June 1991.

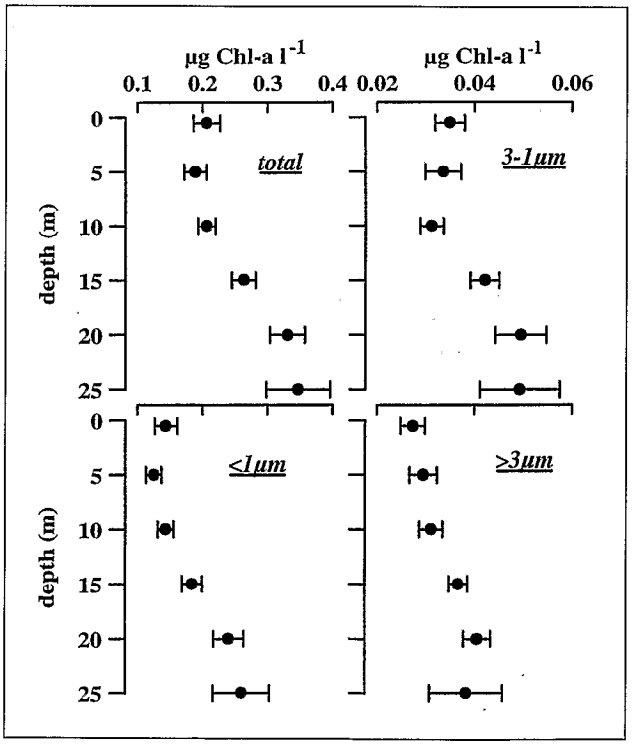

Figure 5. ***Average ± SE of Chl-a in different size fractions in Takapoto lagoon.***

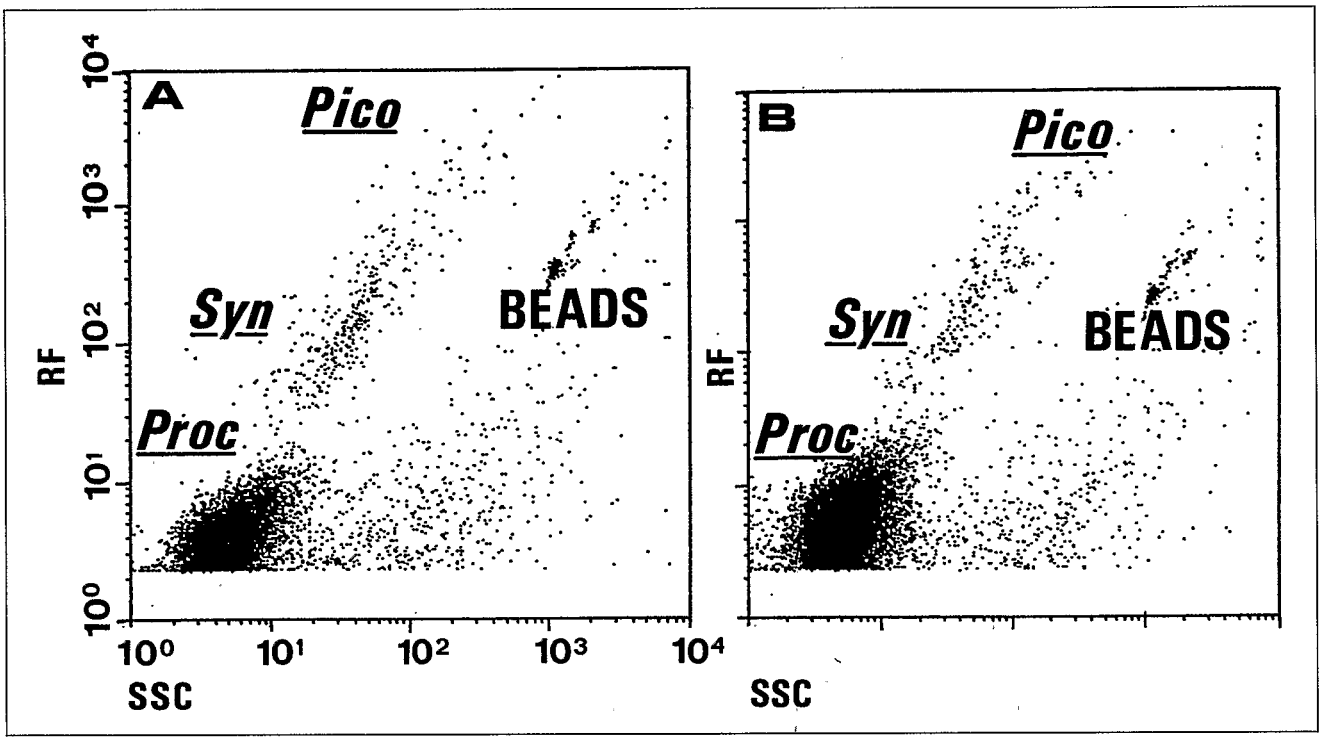

Figure 6. ***FACScan cytograms of oceanic water.*** *(A) Surface. (B) 50 m. RF: red fluorescence (chlorophyll a and b); SSC: side size scatter;* Proc: Prochlorococcus; Syn: Synechococcus; Pico: *picoeukaryote.*

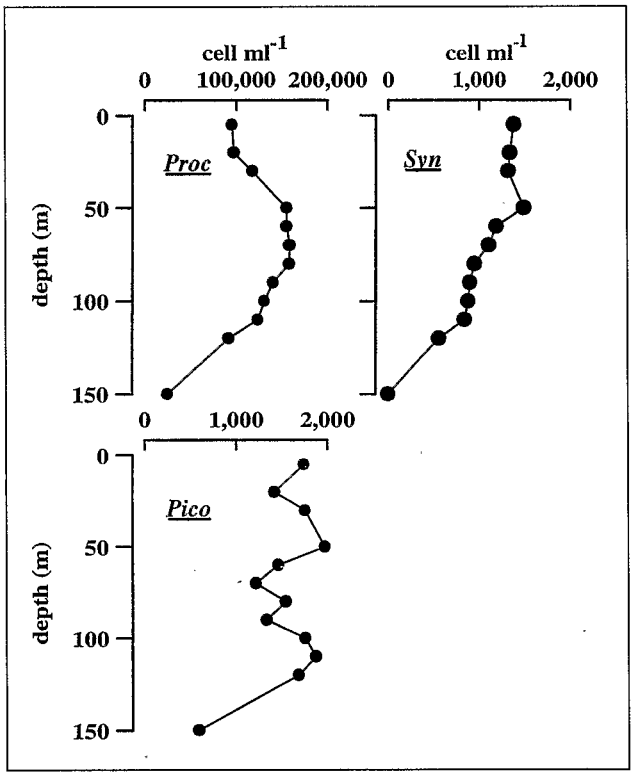

Figure 7. ***Phytoplankton abundance in oceanic waters.*** Proc: Prochlorococcus; Syn: Synechococcus; Pico: *picoeukaryote.*

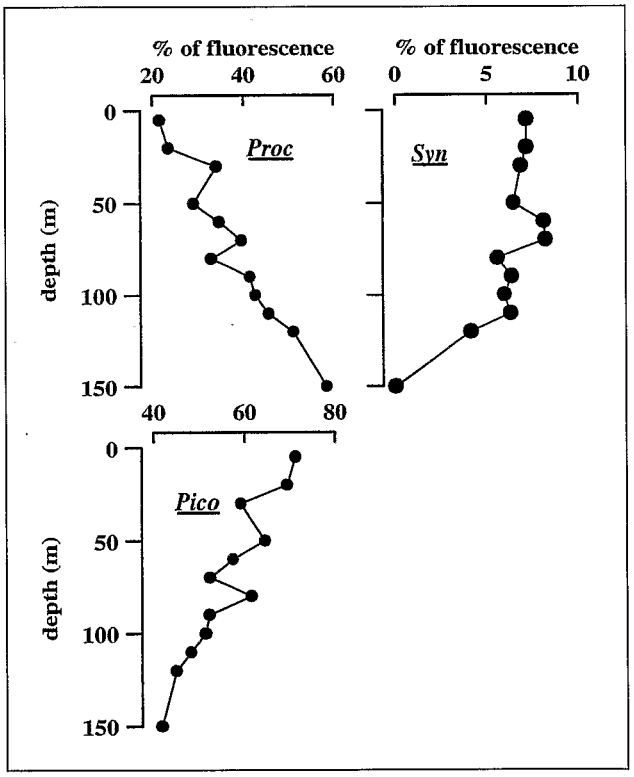

Figure 8. ***Percentages of* in vivo *fluorescence in oceanic waters.*** Proc: Prochlorococcus; Syn: Synechococcus; Pico: *picoeukaryote.*

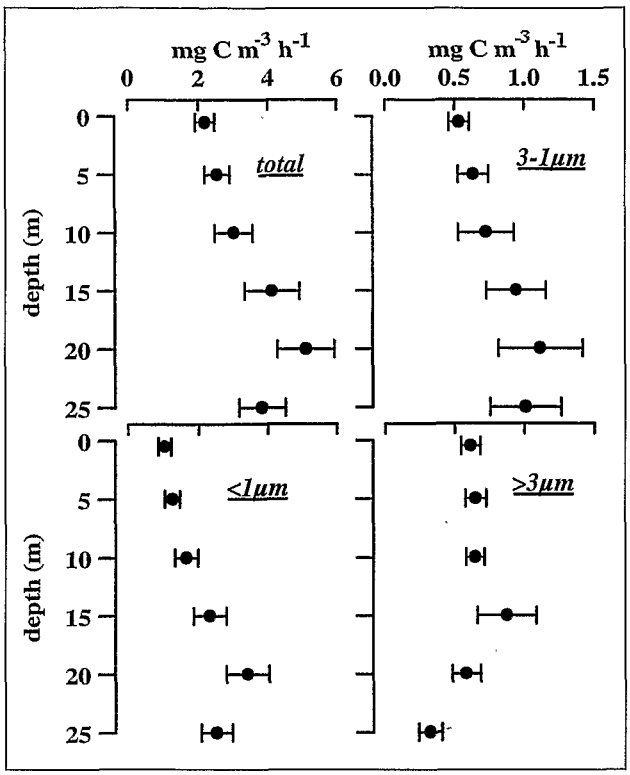

Figure 9. ***Average ± SE of C uptake in different size fractions in Takapoto lagoon.***

Conclusion

The typically offshore presence of *Prochlorococcus* seems to have one noticeable exception, the presence of a lagoonal population in the closed lagoon atoll of Takapoto. However, they contributed to only 2% of the RF and phytoplanktonic production. *Synechococcus* constituted the dominant group as this has already been reported in the open atoll of Tikehau [4] and in the closed atoll of Takapoto [5]. On the other hand, in the surrounding oceanic waters, *Prochlorococcus* and picoeukaryotes substantially dominated the phytoplanktonic biomass. The ecological constraints which favor *Synechococcus* abundances are unknown, but it has been reported on several occasions that the peaks of abundance of *Prochlorococcus* and *Synechococcus* were not concomitant [15], as if the great abundance of a *coccus* group excludes one from the other, and this for reasons still unknown [7, 9]. The depletion of phosphorus nutrient in the lagoon of Takapoto [16] suggest that dinitrogen fixation could be an important factor in this atoll. The well known fixation of the epilithic cyanobacterial community [17] is probably adequate to explain such a depletion, but dinitrogen fixation could also occures in the water column. Mitsui *et al.* [18] reported that a marine strain of *Synechococcus* fixes dinitrogen, it is possible that the dominance of *Synechococcus* in the inner water column is due to such an ability. ▼

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