Report of picophytoplankton study during the astro cruise In the Great Astrolabe Reef (18°45'S-178°30'E) Fiji.

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Abstract

Prochlorococcus were studied with a flow cytometer, in the lagoon of the Great Astrolabe Reef (Fiji) the distribution of pico- phytoplankton within the water column was mostly homogeneous. The *Prochlorococcus* (Proc.) and *Synechococcus* (Syn.) were the most abundant groups and the picoeukaryotes (Peuk.) were an order of magnitude less abundant. The *Synechococcus* dominated the integrated : cell number, *in vivo* fluorescence and carbon biomass. In the surrounding ocean, the phytoplankton distribution was heterogeneous. Proc. and Peuk. had a subsurface maximum near the nitracline; Syn. was only abundant in surface layers and decreased drastically downward. No group clearly dominated. *Prochlorococcus* dominated in integrated cell numbers, whereas *Synechococcus* in integrated fluorescence, and picoeukaryotes in integrated carbon biomass. By comparing the present results with the results reported from offshore oligotrophic oceans and from a closed atoll, we conclude that active exchanges occur between the lagoon and the surrounding ocean.

1. Introduction

The plankton includes all heterotrophic bacteria, plants and animals that are passively drifting along with water movements. In the subtropical and oligotrophic Pacific ocean, the main components of plankton in numerical abundance and carbon biomass are the heterotrophic-bacteria and the picophytoplankton (<2µm), (Campbell and Vaulot, 1993; Campbell *et al.*, 1994). In order to estimate the fertility and the biomass of the Great Astrolabe Reef Lagoon water column. We decided to study the following main components. The heterotrophic bacteria are reviewed in chapter X and the results of the picophytoplankton are reported in this paper. The organisms of picophytoplankton are composed of: 1) the prokaryotes or phytobacteria and 2) the eukaryotes. The phytobacteria are dominated by two genera *Prochlorococcus* (≈ 0.6µm)and *Synechococcus* (≤1µm). The prokaryotes are mainly micro-flagellates. We studied the picoplankton by using an on board flow cytometer (FCM) which allowed us to count the dimly fluorescent *Prochlororoccus*, which are too dim to be observed with an epifluorescent microscope.

2. Material and methods

Observations were made during the ASTRO cruise, aboard R/V "L'ALIS", 16 April - 1 May 1994, in the lagoon of the Great Astrolabe Reef (18°45'S-178°30'E) FIJI and in the surrounding ocean (Fig. 1). The cruise was organised by the ORSTOM-groups from Papeete (French Polynesia), and from Noumea (New Caledonia), in collaboration with the USP Suva. This work was supported in part by the French Embassy in Suva.

2.1 Sampling

Water samples from discrete depths were collected with 1.7 l Niskin bottles (General Oceanic) attached to a wire. In the lagoon the selected stations were the same as the benthic stations, selected in respect of the depth and of the substratum.

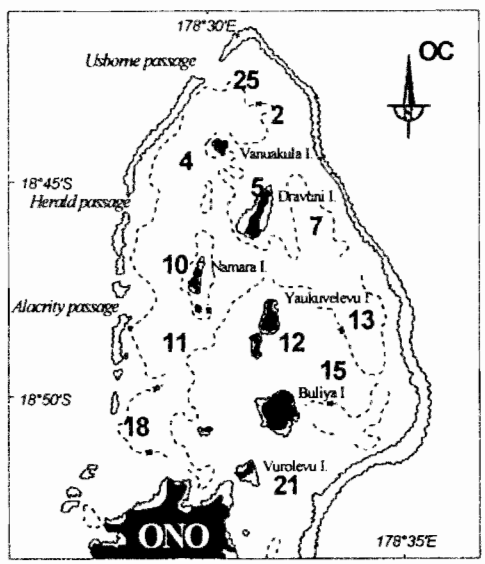

Figure 1: Station locations in GAR lagoon (OC = oceanic station

The standard depths are shown in the depth profiles at the selected stations: 10 in the lagoon

and one in the surrounding ocean near D'Urville Channel. In the lagoon samples were collected from the surface to the bottom (20m to 40m) and in the ocean from the surface to 200m.

2.2 Flow-cytometry

Samples for flow cytometer analysis were run at sea as reported by (Blanchot and Rodier, *in press*). Briefly, 0.1 ml was analysed. FCM data acquisition was performed *in vivo* without delay after sampling. A FACScan flow cytometer (Becton-Dickinson), equipped with argon laser (power = 15mW, at 488 nm) was installed in a dimly lit and temperature controlled laboratory. Sea-water filtered through GF/F filters was used as sheath fluid. For each cell, five signals were recorded on 4-decade logarithmic scales: two light scatters: and three fluorescence. The scatters are respectively: the size side scatter (SSC, link to cell absorbency), and the forward light scatter (FLS, link to cell size). The photomultipliers were set up to quantify : the red fluorescence (RF) from Chl (wavelength > 650 nm), the orange fluorescence (OF) from phycoerythrin PE (564-606 nm), and the green fluorescence (GF) from phycourobilin PUB (515-545 nm), following (Wood *et al.*, 1985; Olson *et al.*, 1988). Cellular fluorescence was always expressed relatively to the fluorescence of the beads (in arbitrary units, AU), by dividing the mean cell fluorescence by the mean bead fluorescence.

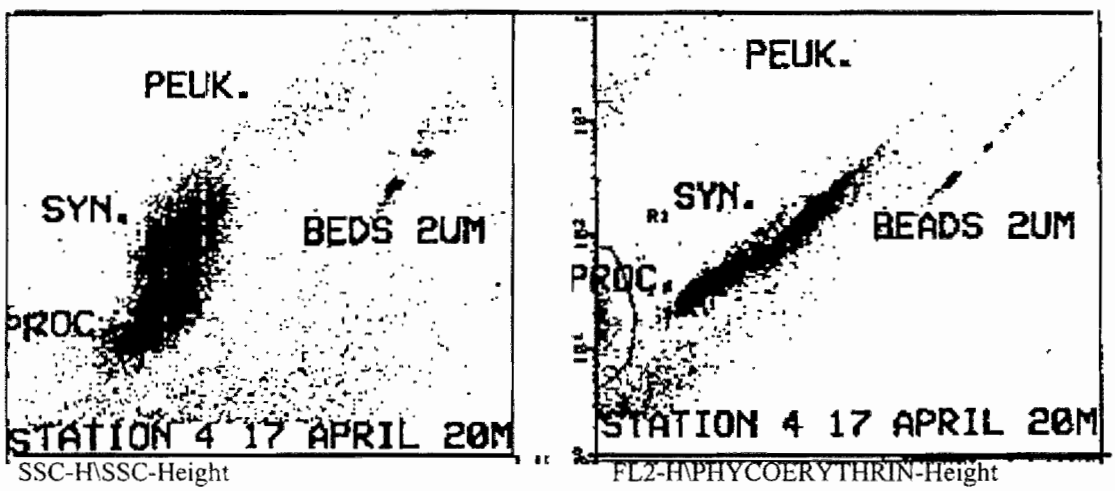

Figure 2: FACScan cytograms of Greeat Astrolabe Reef lagoon water. . St-4 fluorescence orange FL2(phycoerythrin), FL3= red fluorescence (Chlorophyll a and b); SSC = side size scatter; Proc. = Prochlorococcus; Syn. = Synechococcus; Peuk. = picoeucaryotes, Beads 2µm;

Cell groups (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) were determined, using their optical properties; Figure 2. Prochlorophytes, referred to as *Prochlorococcus marinus*, were easily discriminated from the picoeukaryotes by their much smaller red fluorescence and scatter. *Synechococcus* spp., have intermediate RF and SSC signals between those of *Prochlorococcus* and picoeukaryotes and were distinguished clearly by their OF. Picoeukaryotes always have the largest RF and SSC. In order to estimate numerical abundance, we used the histograms provided by the FACScan analysis system (number versus red fluorescence intensity; Lysis II software). At all stations, the *Prochlorococcus* populations were sufficiently bright to be completely resolved by the FACScan system.

2.3 Enumeration of cells by epifluorescent microscopy

FCM, trouble the last day constrained us to use epifluorescent microscopy to count cells for the cycle experiment. Only Syn. and Peuk. were enumerated. A time series of cast was performed at Station 5, 10m in order to study short-term variability of cell abundance. One depth was sampled at 1h interval during 21 h time series. Sample for cells counts were performed following (Blanchot et al., 1992). Briefly, cells were harvested by filtration on to black Nuclepore filter 0.2 µm (Ref. 110656). The coefficient of variation varied from for 200-800 cells counted on 20-80 fields was 12% .

2.4 Carbon biomass estimates-

The conversion factors used for carbon estimates were computed by Blanchot and Rodier *(in press)*. We used respectively: 61 fgC/ *Prochlorococcus*, 104 fgC/ *Synechococcus* and 3110 fgC/ picoeukaryotes.

2.5 Nutrients and chlorophyll determination

NO_3 + NO_2 analyses were performed with a delay of few hours on the field station of Dravuni. as described by (X?) chapter. In this paper, for convenience, NO_3 + NO_2+ NH_4 will be referred to as Nitrogen nutrients (N_n).
Samples for chlorophyll *a* (Chl *a*) were harvested by filtration onto Whatman GF/F filters. Chl *a* was determined fluorometrically on a methanol (95%) extract using a Turner model 112 fluorometer calibrated with commercial pure Chl *a* (Sigma). Details of the fluorometric method and size fractionation's are given in Charpy.(1996).

3. Results and discussion

3.1 Environmental setting

Our study occurred during a June 1994. In the lagoon, the sum of nitrogen nutrients (N_n=NO_3+NO_2+NH_4) was always (≥ 0.1µm), but the nitrate concentration varied from poor stations (10, 2) to rich stations (7, 18), Figure 3.
At the external station, N_n was always (≥ 0.1µm). But the surface layers were nitrate depleted and the nirtracline occurred between 50 and 60m, Figure 4.

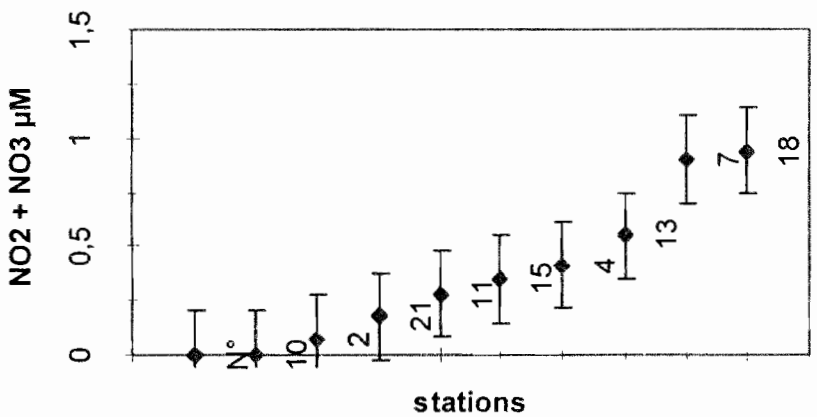

Figure 3: : Mean concentration off Nitrogen nutrients in the lagoon

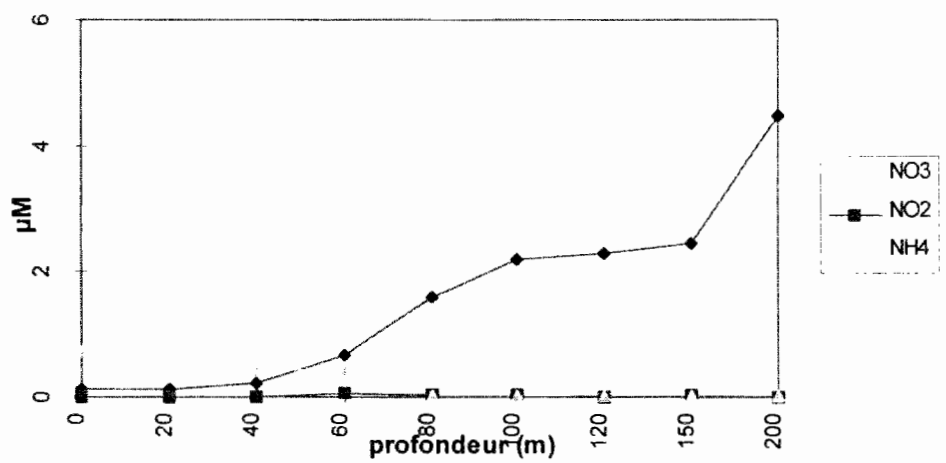

Figure 4: Vertical profiles of Nitrate at the St-Ext

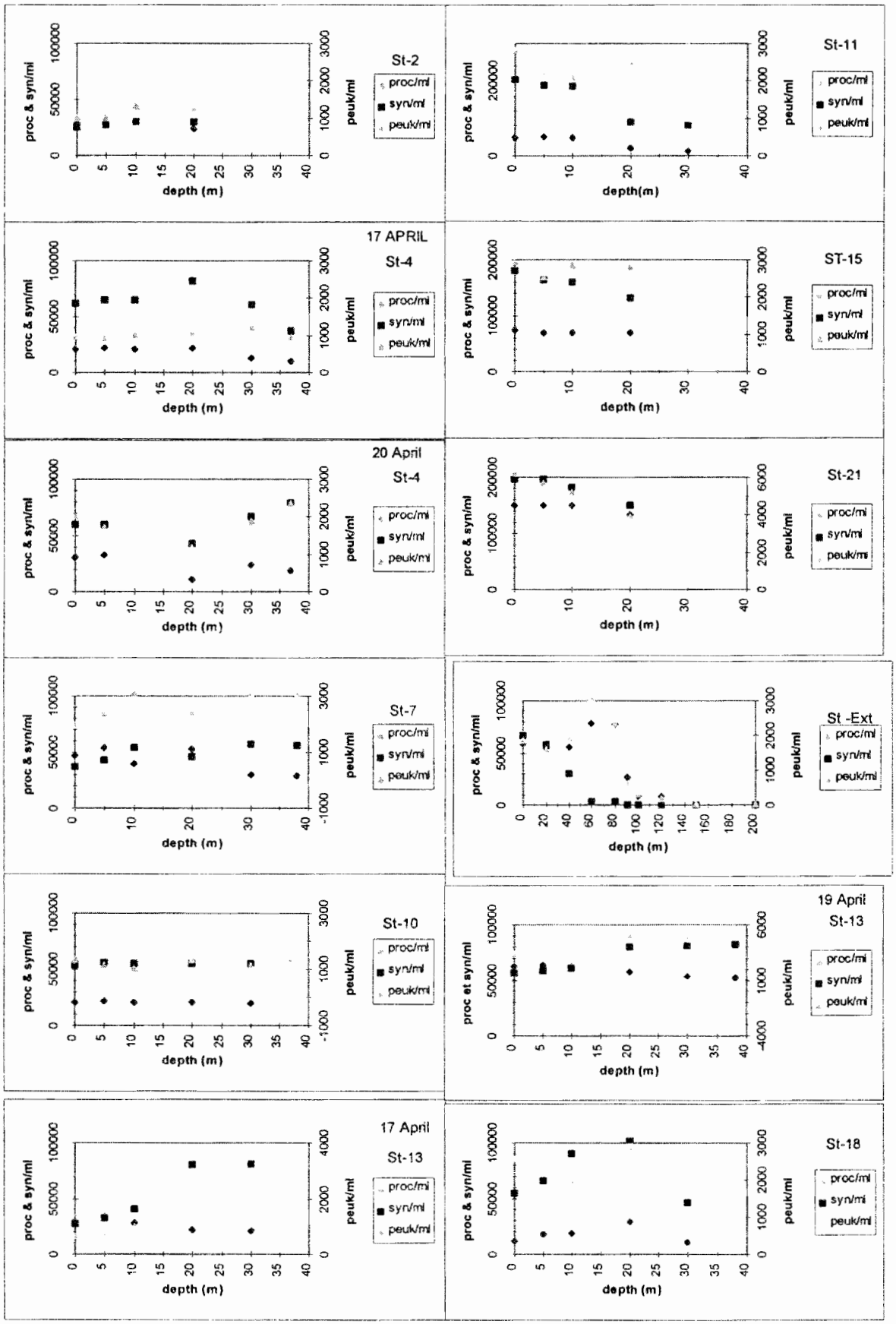

Figure 5: Depth profiles of Proc. (♦), Syn. (■) and Peuk. (∇) abundance at the lagoonal stations , and at the St-Ext.

3.2 Cell abundance distribution

In the lagoon, the phytoplankton distribution was roughly homogeneous in the water column. The depth profile of cells are shown Figure 5. In the lagoon the Syn. are the most abundant group whatever the station. The Proc. were less abundant but had the same order of magnitude as the Syn. The Picoeukaryotes were an order of magnitude less abundant. The mean abundance of cells (station 21 excluded), from the surface to 30m are respectively ($4.1 \pm 3.2\ 10^4$, $8.5 \pm 5.5\ 10^4$, $2.2 \pm 1.3\ 10^3$ cells ml^{-1}, n=11). The great sd are essentially due to the large abundance of cells at St21. At this station the maximum abundances occurred and were respectively $1.51x10^5$, $2.0x10^5$, $6.2\ x10^3$ cells ml^{-1}.
The stations belong to three sub-categories the relative poor and rich lagoonal stations and the intermediate stations. The lagoonal stations are characterized by homogeneous depth-profiles. The intermediate stations are characterized by heterogeneous depth-profiles. The relatively poor lagoonal stations. had in average a cell number Proc. and Syn. ($<10^5$ ml^{-1}), the mean Peuk. cell number is ($<310^3$ ml^{-1}), St (2, 4, 7, 10). The relative lagoonal rich stations had in average a cell number of Proc. or of Syn. ($>10^5 ml^{-1}$) and), the mean Peuk. cell number is ($>310^3 ml^{-1}$), St (11, 15,21). The intermediate stations, had a two layers., with cell number in surface layers less abundant than in subsurface layers St (13, 18). The maximum abundances occurred at station 21 and were respectively for Proc. Syn. and Peuk. $1.51x10^5$, $2.0x10^5$, $6.2\ x10^3$ cells ml^{-1}. Therefore the lagoonal community is dominated by Syn. But the lagoonal abundances of cells reported here shown a north/south or a relative poor/rich gradient. In the south, near Ono Island the abundances could thrive to an order of magnitude more that the mean cell number of all the other stations.
In the surrounding ocean, the phytoplankton distribution was heterogeneous. The depth-profiles of cells are shown Figure 5. The *Prochlorococcus* are the most abundant group, the *Synechococcus* are less abundant but had the same order of magnitude and the Picoeukaryotes were an order of magnitude less abundant. The Proc. and the Peuk. had a subsurface maximum near the nitracline. The Syn. were abundant in surface layers and decreased drastically downward. Maximum abundances (cells ml^{-1}) were 7.710^4 Proc., $6.7x10^4$ Syn. and $3.1x10^3$ Peuk. The typical Proc. dominance in numerical abundance was observed in Pacific subtropical ocean regions (Campbell and Vaulot, 1993; Campbell et al., 1994; Blanchot and Rodier, *in press*) This dominance is particularly important in nitrate-depleted layers and in the convergence areas (Blanchot and Rodier, *in press*). In the upper layers of offshore tropical Pacific Ocean waters, *Synechococcus* constitute only a small percentage of integrated values (Blanchot and Rodier, *in press*). As reported in the environmental setting the surrounding ocean is not N_n depleted, therefore the three components are well represented in the well illuminated layers.

3.3 In vivo cellular fluorescence

3.3.1 Increase of in vivo fluorescence per cell

The increases of fluorescence with depth are presented Figure 6. In the lagoon the increase of fluorescence values normalised to surface values for the three groups, was roughly the same and remain weak (<2) at 30m. In the surrounding ocean, the increases with depth vary with groups the deep Proc; were 11 times more fluorescent as the surface one, the deep Syn; were 4 times more and the deep Peuk. 6 times more. These increases occurred under the mixed layers in the nitrate rich layers. In the mixed layers from the surface to 60m, the increase is weak (<2). The increase of fluorescence with depth of *Prochlorococcus* (normalised to surface fluorescence) was more important than the increase of fluorescence of the other groups. For the increase of fluorescence to be at a maximum, then the barrier layer need to be well developed. In the homogeneous well mixed layers from the surface to the bottom in the lagoon and in the upper layers (from the surface to 60m) ,the increase of fluorescence (normalised to surface values) remain weak (<2). In the external stations outside the barrier reef, the increase of fluorescence below the nitracline is high. This is particularly true for the Proc. and is likely due to 2 main reasons. The first one is the ability of *Prochlorococcus* to photoaclimate to low light intensities as reported by Olson *et al.* (1990c), with high increase of divinyl chlorophyll *a* and *b* per cell (Goericke and Repeta 1993, Moore et al., 1995, Partensky et al.; in press). The second one, occurred only at the oceanic stations below 60m, the presence of different genetic strains in the field, with dimly fluorescent strains in upper well lit layers and brightly fluorescent strains in the poorly illuminated layers as suggested by Figure 6 (St-Ext., 60m AST078) and proved by DNA analysis near Hawaii (Campbell and Vaulot 1993).

3.3.2 Group fluorescence

In the lagoon the Fluorescence of Syn. and Proc. were large, the two groups reaching a maximum at 30m. In the surrounding ocean, the 3 groups were highly fluorescent. The Syn. were the larger group from the surface to 40 m then decreased drastically downward. Proc. and Peuk. were present throughout the whole water column. their maximum extended from to 80-90m (Fig. 7).

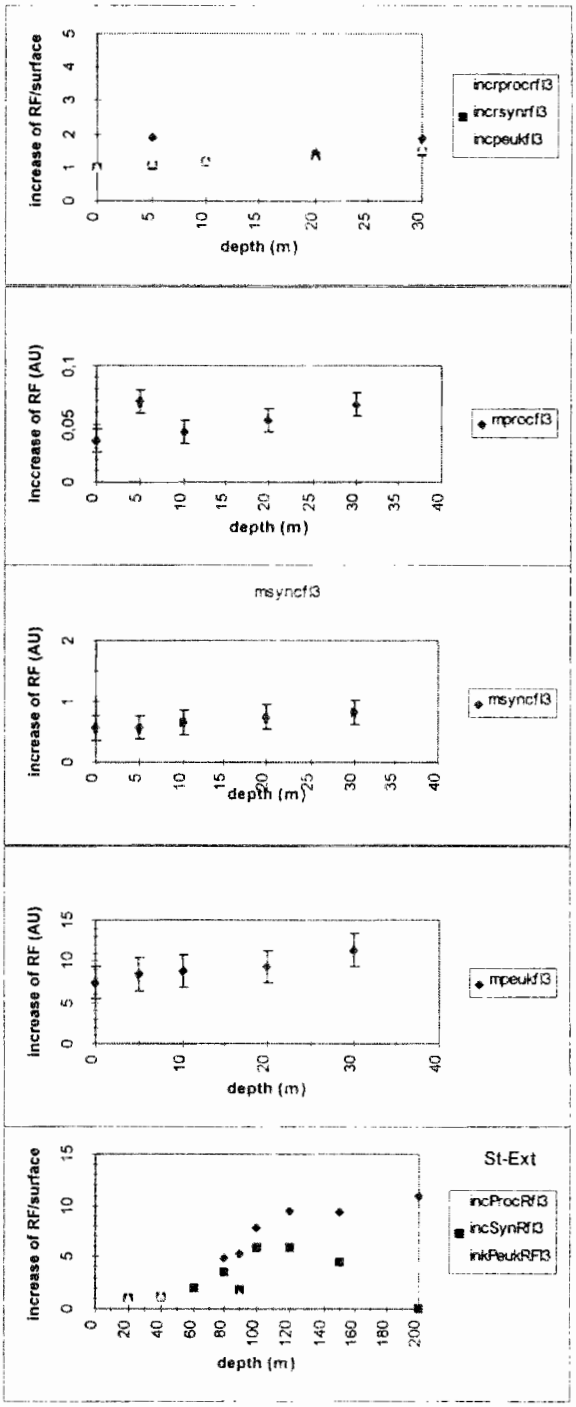

Figure 6: Increase in mean cellular red-fluorescence for *Prochlorococcus* (♦), *Synechococcus* (■), and Picoeukaryotes (V) as a function of % surface irradiance. Fluorescence values are normalized to surface values as defined by 100% irradiance. arbitrary units (AU).

3.4 Carbon biomass of phytoplankton estimated by conversion factor

At the typical station (St-4, 17 April), the Syn. are the dominant group throughout the whole water column. The respective percentage of integrated biomass for the Proc., Syn., and the Peuk. are respectively 10%, 61%, 29%.

At the oceanic station, the dominant group are the Peuk. The maximum carbon biomass occurred at the nitracline (60m), where the Peuk. reached their maximum. The Syn. disappeared below 60m. The respective contributions of the Proc., Syn., and the Peuk. 29%, 22% and 43% (Fig. 8).

3.5 Integrated values of cellular abundance, cellular in vivo fluorescence and Carbon biomass

To summarise our results we present Table-1, the integrated values percentages of cell abundance, *in vivo* fluorescence and carbon biomass. In the lagoon, the Syn. dominated all the integrated values: cell number, *in vivo* fluorescence and carbon biomass. In the surrounding Ocean no group clearly dominated the integrated values. The Proc. dominated in integrated cell numbers. The Syn. dominated in integrated fluorescence. The Peuk. dominated in integrated carbon biomass.

In the closed atoll of Takapoto Syn. was only slightly higher than Peuk.(Table 2) but in the two lagoons the dominance of Syn. is clearly a characteristic of inner waters. The percentage of Proc. at Takapoto lagoon was the half of the one from Fiji, and is a likely a consequence of the low exchange between the surrounding ocean and the lagoon. It could be also due to an apparent incompatibility to have great abundance of Proc. and Syn. in the same water for unknown reason (Blanchot and Rodier, *in press*; Partensky *et al.*, *in press*). So, to our knowledge the station 21 is atypical with 1.5 10^5 Proc. ml^{-1} and 2.010^5 Syn. ml^{-1}

In the surrounding ocean the dominance of Proc. was much higher around Takapoto than around Great Astrolabe Reef. This is the consequence of the non depleted waters in Fiji and also the exchange of water between lagoon and ocean.

3.6 Short term variability of cell abundance

The polynomial curved (x^3) fitted at the best the variations of abundance are presented Figure 10. There was a significant correlation between the tendency curves and the data ($p<0.001$, for Syn.) and ($P<0.01$, for Peuk.). The minimum of cell abundance occurred at noon for the Syn. and ad midnight for the Peuk. Inversely the maximum abundance of Syn. occurred ad midnight ant the maximum of Peuk. at noon. This preliminary results suggest that the cell cycle of Syn. and Peuk. are inverse (Fig. 9). The division of Syn. occurred after the sunset and are completed at midnight. Inversely the division of Peuk. occurred after the sunrise and is completed at noon. The division of Syn. agree

with the observed division of Proc. at 0°-150°W (Vaulot et al., 1995). To my knowledge little is know o the division of Peuk. in the tropical areas.

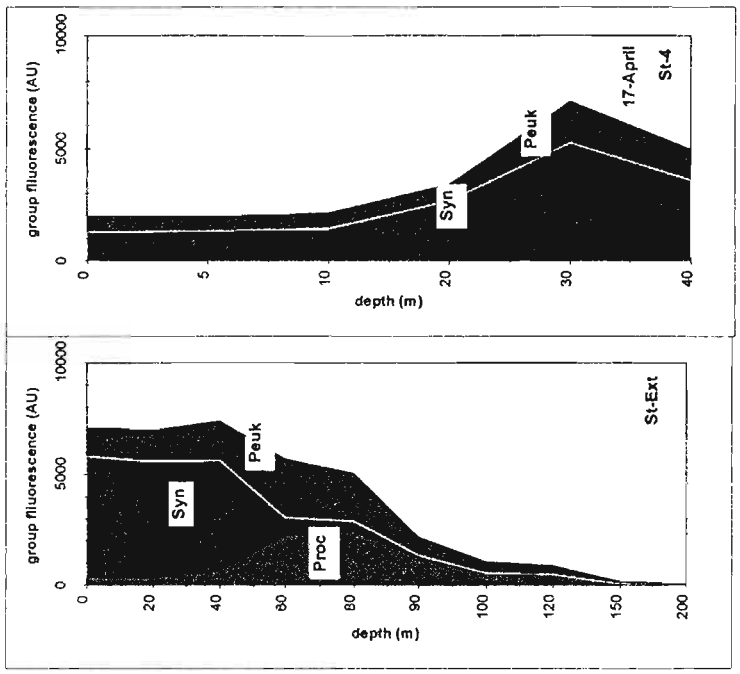

Figure 7: Total red-fluorescence in arbitrary units (AU) for *Prochlorococcus* (Pro.), *Synechococcus* (Syn.) and picoeukaryotes (Picoeuk.), overlaid by Chl *a* concentration. The red fluorescences of each population are calculated from mean cellular fluorescences weighted by cell concentrations. Same stations as for Fig.3.

Table 1 INTEGRATED VALUES in the lagoon (10 stations) and at the external stations

from surface to bottom	LAGOON		
integrated percentages	Prochlorococcus	Synechococcus	Picoeukaryotes
cell abundance	32 ± 11%,	66 ±11 %	1 ± 2%
fluorescence in vivo	3 ± 2%	69 ± 6%	28 ± 6%.
Integrated Carbon biomass	13±4%	46±11%	41±9%
from surface to 200m		OCEAN	
cell abundance	69%	29%	2 %
fluorescence in vivo	22%	46%	32%.
Integrated Carbon biomass	27%	19%	54%

Table 2 INTEGRATED VALUES in the lagoon of TAKAPOTO (145°20' W, 14°30'S), 9 stations) and at the external stations

from surface to bottom	LAGOON		
integrated percentages	Prochlorococcus	Synechococcus	Picoeukaryotes
cell abundance	17 ±8%	81±2	2±0%
fluorescence in vivo	2±1%	57±19%	41±19%.
Integrated Carbon biomass	7 ± 1%	55±3%	38±3%
from surface to 200m		OCEAN	
cell abundance	98%	1%	1 %
fluorescence in vivo	42%	5%	53%.
Integrated Carbon biomass	60%	1%	39%

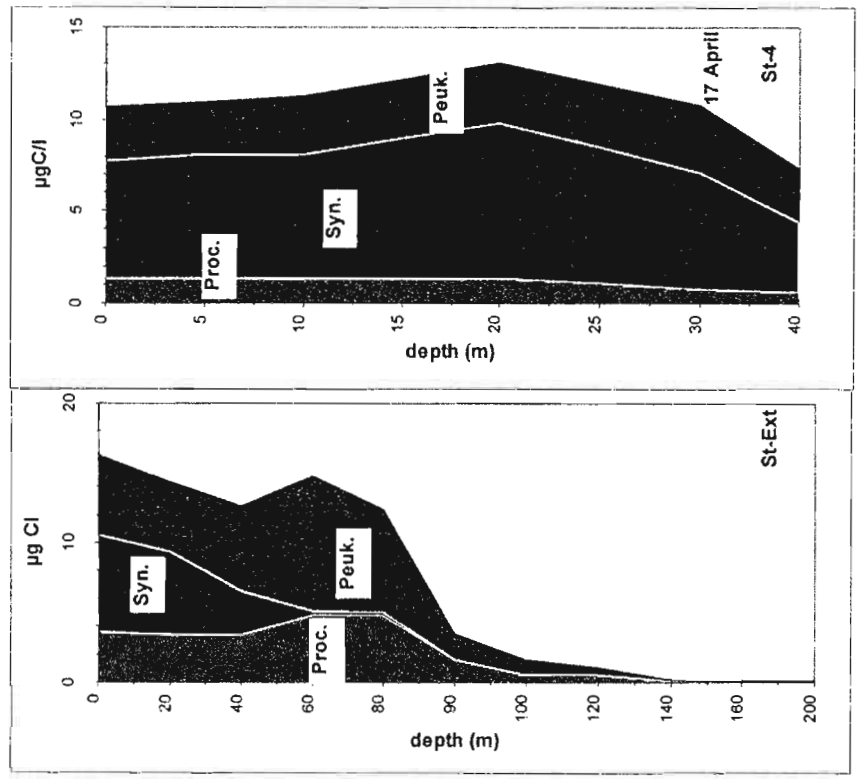

Figure 8: Carbon biomass (µgC l^{-1}) for Proc., Syn. and Peuk., The carbon biomasses are calculated from cell concentrations and conversion factors (see text).

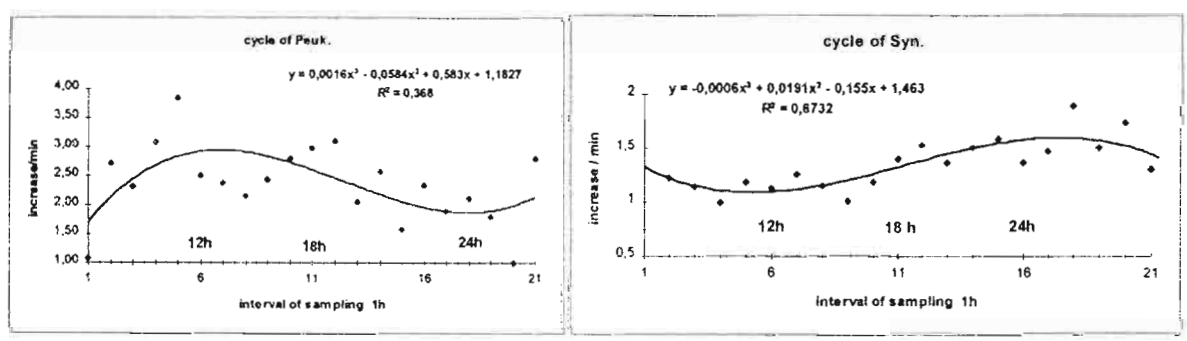

Figure 9: Short term variability of Syn. and Peuk. abundance St-5, 10m 27 April.

4. Conclusion

The community structures of the lagoon and of the surrounding ocean are different. In lagoonal environments the community is largely dominated by the *Synechococcus* (66% of cell abundance and 46% of Carbon biomass) and in the surrounding ocean there is no clear dominance of a group over the other.

In offshore ocean, in a very oligotrophic zone, the Proc. dominance is huge (96% of cell abundance and 78% of Carbon biomass ; 14°S-165°E). (Blanchot et Rodier in press). Contrary to the closed atoll of Takapoto (14°30S-145°20'W), the dominance of Syn. is large (78% of cell abundance and 53% of Carbon biomass); (Charpy and Blanchot, 1996). As our results are intermediate between those reported from offshore oligotrophic ocean, and as the lagoon of the Great Astrolabe Reef is open by several deep passages (Figure 1), we conclude that active exchanges occur between the lagoon and the surrounding ocean.

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