Synechococcus and *Prochlorococcus* dominance estimated by flow cytometry in Tuamotu Atoll lagoons

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INTRODUCTION

In two coral reef lagoons of the Tuamotu Archipelago, small phytoplankton cells identified as prokaryotic *Synechococcus* sp. (Blanchot *et al.,* 1989; Charpy *et al.,* 1992) dominate larger cells both in terms of biomass and primary production (Charpy, 1996). The use of a flow cytometer revealed small populations of *Prochlorococcus* in the lagoon waters of Takapoto atoll (Charpy & Blanchot, 1996, 1998). Due to high geomorphological diversity of atolls the extrapolation of the existing scientific knowledge on two atoll lagoons to the others seems to be hazardous. Here, we present results of a flow cytometric survey of picoplankton in 11 Tuamotu atoll lagoons and surrounding waters. We first examine the distribution pattern of picoplankton populations, then we research the relationships between atoll geomorphology and picoplankton community structure.

DATA

Chlorophyll based dominance of picoplankton groups

A classification by size with cell number, carbon biomass, chlorophyll biomass and primary productivity was established according to Li's method (1995). Assuming that red fluorescence is a proxy for chlorophyll *a*, we

estimated the picophytoplankton chl *a* from counts of red fluorescent particles (RF):

$$\text{chl } a = \sum_{i=1}^{i=3} n_i \times f_i \times \psi_i \quad \text{(Eq. 1)}$$

where i refers to the 3 recognizable groups (i.e. *Prochlorococcus*, *Synechococcus* and picoeukaryotes); n = cell concentration; f = mean red fluorescence per cell cell; ψ = fg chl *a* per relative unit of red fluorescence. As in November 1995 and March 1996, cytometric measurements were performed on fixed samples, Equation 1 cannot be used. Indeed, the picoeukaryotes were poorly preserved and as a result their RF were likely affected by the fixation. Assuming that the < 1 µm fraction consists primarily of prokaryotic cells (Charpy & Blanchot, 1996), we estimated the chlorophyll in the < 1 µm fraction using :

$$\text{chl } a < 1\ \mu\text{m} \sum_{i=1}^{i=2} n_i \times f_i \times \psi'_i$$

where ψ' = fg chl *a* < 1 µm per relative unit of RF. The values of ψ_i and ψ'_i were estimated by regression using measured values of chl *a*, chl *a* < 1 µm, n_i and f_i.

In Takapoto atoll lagoon, the distribution of picoplankton was heterogeneous in the water column, but values of ψ_i varied with depth. The fit of data for individual layers was extremely good for total chl *a* (R^2 = 97.5 and $p < 0.01$ in both layers) (Fig. 1).

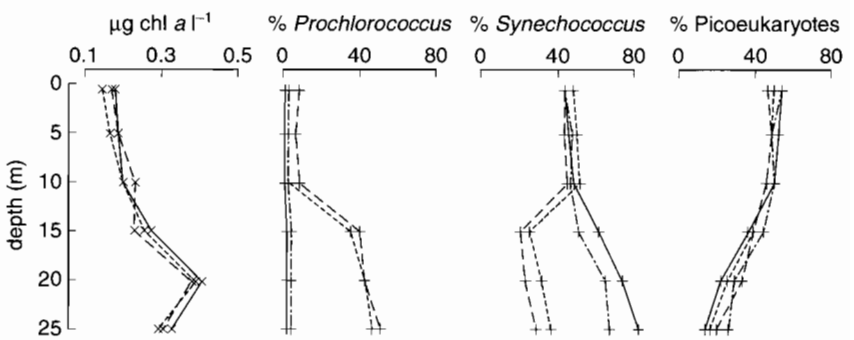

Figure 1. Example of depth profile of (×——×) measured chl *a*, (×– – –×) fitted values of $\sum_{i=1}^{i=3} n_i \times f_i \times \psi_i$, (×— — —×) fitted values of $\sum_{i=1}^{i=2} n_i \times f_i \times \psi'_i$ + chl *a* > 1 µm, (+——+) percentage of red fluorescence, (+– – –+) percentage contribution to chl *a*, (+— — — —+) percentage contribution to picoplankton carbon and (+— —+) percentage contribution to carbon assimilation rate in Takapoto lagoon (November 1994)

For the other atolls except Reka-Reka, the fits of chl $a < 1$ µm versus prokaryotic RF were also good ($p < 0.01$). The contribution of prokaryotic groups to total chl *a* were estimated by multiplying their contribution to the chl *a* in the < 1 µm size fraction by the proportion of chl *a* in < 1 µm size fraction. The contribution of picoeukaryotes to total chl *a* was estimated as the percentage of chl *a* > 1 µm.

Carbon based dominance

We estimated picophytoplankton biomass as carbon using cell volumes and carbon biomass to biovolume ratios taken from the literature (Verity *et al.*, 1992). We use forward light scatter (FSC) measured in vivo in Takapoto to estimate the cell sizes of the 3 picoplankton groups assuming that they have a spherical shape and similar refractive indexes. The relation between FSC and the size of spherical particles in the size range of picophytoplankton can be written as :

$$\frac{FSC_{cell}}{FSC_{beads}} = \left(\frac{Diameter_{cell}}{Diameter_{beads}}\right)^{x}$$

(Morel, 1991). Assuming that, in a given biotope (lagoon, ocean) the exponent (x) has the same value for the 3 picoplankton groups, (x) was estimated from the average *Synechococcus* cell diameter (0.8 µm) measured at Takapoto atoll lagoon with an optical microscope. We found x = 3.94 in lagoonal waters and x = 4.34 in the upper 100 m of oceanic waters. The carbon content of cells was estimated using the volume to carbon conversion factors of Verity *et al.* (1992). During our study in Takapoto, picoplankton size and carbon content peaked at the beginning of the afternoon for prokaryotes and at the end of the afternoon for eukaryotes. Since all other samples were collected in the morning, the C biomass of the three groups were estimated using the average cellular C contents: 53 fg C cell^{-1} for *Prochlorococcus*, 180 fg C cell^{-1} for *Synechococcus* and 4970 fg C cell^{-1} for picoeukaryotes. For surface ocean waters, we used cellular biomass values calculated for surface ocean waters surrounding Takapoto: 53 fg C cell^{-1} for *Prochlorococcus*, 191 fg cell^{-1} for *Synechococcus* and 2568 fg C cell^{-1} for picoeukaryotes.

Estimating the contribution of the picoplankton groups to primary production

The contribution of prokaryotic cells to total production can be estimated as the percentage of carbon assimilation rate attributable to the < 1 µm size fraction. The assimilation numbers of the three class size fractions (< 1 µm, 1-3 µm and > 3 µm) were similar (Charpy, 1996). Therefore, we assume that the contributions of *Prochlorococcus* and *Synechococcus* to < 1 µm chl *a* reflects their contributions to carbon assimilation rate in the < 1 µm fraction and we estimate their contributions to primary production by multiplying their contribution to chl $a < 1$ µm by the proportion of carbon assimilation rate < 1 µm. The contribution of picoeukaryotes was estimated as the percentage of carbon assimilation rate > 1 µm.

Comparison between different estimations of the picoplankton group dominance

The dominance of the different picoplankton groups varied with the method used to estimate their relative importance (Fig. 1). Picoplankton group dominance estimated by flow-cytometric analysis i.e., percentages of RF and percentages of picoplankton C (via size/FSC estimations) are similar and values estimated by their contribution to chl *a* were similar to values estimated by their contribution to primary production. *Prochlorococcus* dominance is strongly underestimated with RF and C contributions whereas it was the opposite for *Synechococcus*. This is likely due to an underestimation of *Prochlorococcus* in near surface waters due to a lack of sensitivity of the FACScan.

Relation between picoplankton biomass and community structure, and atoll geomorphology

In spite of the large variations in structure between atolls, some general trends can be noticed (Fig. 2). Prokaryotic plankton generally dominated the lagoonal phytoplankton community. Some exceptions were encountered at Taiaro and Tekokota atolls. In most cases, dominant group was *Synechococcus* but, when the lagoons are deep (≥ 30m) growth of *Prochlorococcus* appears to be promoted. It is particularly noticeable in the Kauehi (45 m) and Marokau (30 m) lagoons. The high salinity of the lagoon of Taiaro (> 40 PSU) could explain the large dominance of picoeukaryotes observed. However, biotic factors such as grazing also affect the picoplankton biomass and community structure. There are a variety of macro-invertebrates that feed on ultra-plankton (Jörgensen *et al.,* 1984; Vacelet & Boury-Esnault, 1995). In the coral reef lagoons the sponges have been reported to be a significant sink for plankton (Reiswig, 1971). Indeed, in coral reef water sponges significantly decreased concentrations of *Prochlorococcus* and *Synechococcus* while increasing concentrations of autotrophic picoeukaryotes (Pile, 1997). The occurrence of sponges and other benthic filter feeders depends of the presence of hard substrate like patch reefs or fringing reefs. At the end, human activity could have an impact on the community structure, especially in the atoll of Takapoto where intensive sea farming of *Pinctada margaritifera* affect the phytoplankton biomass (Vacelet *et al.,* 1996; Charpy *et al.,* 1997).

Comparison between lagoon and open ocean waters

With the exception of Tekokota atoll, volume specific picoplankton biomass was 2 to 10 times higher in atoll lagoons than in surrounding ocean surface waters. *Synechococcus* biomass dominates the picoplankton community structure in atoll lagoons and *Prochlorococcus* in ocean waters. This dominance of *Prochlorococcus* is a common feature in subtropical areas and tropical areas in the Pacific Ocean (Campbell *et al.,* 1997; Blanchot & Rodier, 1996). This switch in dominance from *Prochlorococcus* to *Synechococcus* in lagoons may be due to 3 factors: 1) a photoinhibition of *Prochlorococcus* in

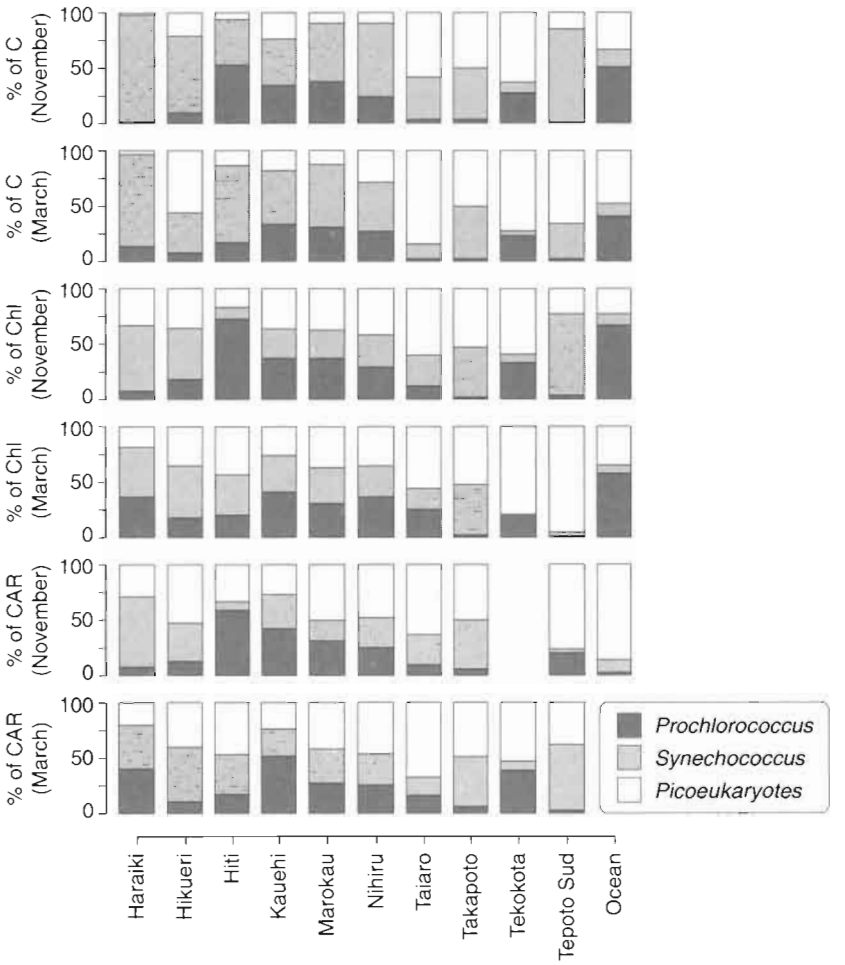

Figure 2. Contribution of *Prochlorococcus*, *Synechococcus* and picoeukaryotes to picoplankton biomass in 10 atoll lagoons and in surrounding oceanic waters.

shallow lagoons; 2) difference in nutrient (micronutrient) availability; 3) difference in selective grazing. Differences in nutrient concentrations between lagoon and ocean were observed in Tuamotu Archipelago. Indeed, in Takapoto and Tikehau, phosphate and silicate concentrations were lower in the lagoons than in the surrounding ocean water (Charpy-Roubaud *et al.*, 1990; Charpy, 1996). It was argued at that time that grazing by benthic macroinvertebrates, organisms which are absent in ocean waters, could significantly affect the picoplankton abundance and community structure. In addition, frequent blooms of different groups of zooplankton in lagoon can generate quantitative and qualitative differences in the control of phytoplankton.

Another hypothesis to explain the differences between oceanic and lagoonal picoplankton community structure is viral infection. Indeed, Blanchot & Rodier (1996) consider that viral infection could be responsible for the control of the abundance of prokaryotic phytoplankton and coastal waters are more suitable than ocean waters for cyanophage infection and growth.

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