SUPPLEMENTARY MATERIAL

"Contribution of picoplankton to the total particulate organic carbon (POC) concentration in the eastern South Pacific"

1 Methodology

1.1 Picoplankton analyses

Prochlorococcus, *Synechococcus* and picophytoeukaryotes populations were differentiated using flow cytometry based on their forward (FSC) and side (SSC) scattering signals, as well as on their fluorescence signals (Fig. A). Natural red fluorescence (FL3) from divinyl and monovinyl chlorophyll *a* was used to differentiate *Prochlorococcus* and picophytoeukaryotes, respectively (Figs. A1 & A2). *Synechococcus* was differentiated from the orange natural fluorescence of their phycoerithrin (Figs. A3 & A4), whereas bacterioplankton was counted based on the SYBR-Green II yellow-green fluorescence die added to their DNA (not shown).

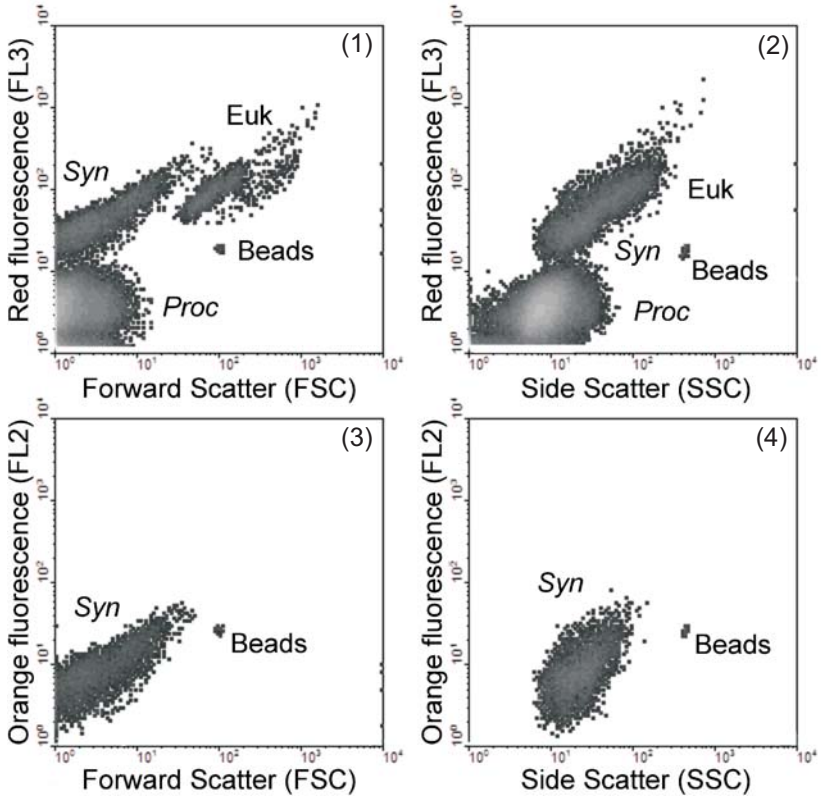

Fig. A. Examples of cytograms *Prochlorococcus* (*Proc*), *Synechococcus* (*Syn*), picophytoeukaryotes (Euk) and the reference beads (Beads) can be differentiated based on their Forward (FSC) and Side Scatter (SSC) signals and on their (mono or divinyl) chlorophyll *a* red fluorescence signal. *Synechococcus* can also be differentiated based on their phycoerithrin orange fluorescence (3 and 4). Note that whereas when using FSC only part of the cyanobacteria populations are visible (1 and 3), when using SSC the entire populations can be differentiated (2 and 4).

Mean forward scatter (FSC) cytometric signals for *Prochlorococcus*, *Synechococcus* and picophytoeukaryotes were obtained by projecting the targeted populations using Cytowin and calculating the arithmetical mean of the FSC distributions without considering the signal's outliers at both ends of the 256 channels distributions acquired with Cell Quest Pro. For the reference beads, because their concentration was too low to allow curve fitting with Cytowin, FSC were obtained by fitting a Gaussian curve to their projected distribution using MATLAB. To provide a consistent scattering measurement for the whole cruise, picophytoplankton signals were normalized to the corresponding reference beads and expressed in relative units (r.u.).

Because the flow cytometer scattering parameters were optimized to observe picophytoeukaryotes rather than cyanobacteria, we have mean FSC signals for almost every sample for the former but only in a few cases for the latter. In other words, the entire FSC distributions for *Prochlorococcus* and *Synechococcus* could only be retrieved for some samples, whereas in most of them the left-most end of these distributions was missing (see Figs. A1 & A3). In some cases, even more than half of the distributions were missing, so that it was not possible to estimate mean FSC signals. For this reason we took the average of the available data points as the mean FSC signal for *Prochlorococcus* and *Synechococcus* along the transect. The missing data on FSC distributions for cyanobacteria did not however induce any errors in cell counting, because these populations were entirely visible when plotted in terms of SSC and FL3 (*Prochlorococcus*) or FL2 (*Synechococcus*), as shown in Figs. A2 & A4.

In order to establish a direct relationship between FSC and size, mean cell sizes for picophytoplankton groups isolated in situ (Table A) and from culture (Table B) were determined using a Multisizer 3 Coulter Counter (Beckman Coulter). In the case of cyanobacteria, their size distribution was assumed to be normally distributed and the peak of this distribution was chosen to be representative of mean cell size. For picophytoeukaryotes, the whole Coulter's size distribution was used to calculate the arithmetical mean for cell size (Fig. B1).

Mean cell sizes for large phytoplankton (>3 µm) were either estimated using Coulter Counter's data as indicated for picophytoeukaryotes (Fig. B1) or using the HIAC particle counter's data. The HIAC was used when large phytoplankton peaks could not be detected with the Coulter and mean cells sizes were estimated as follows. First, the

data collected with the HIAC were represented in the form of volume distribution of particles standardized to 1 µm (µm^3 ml^{-1} per 1 µm), because small peaks are easier to identify using this representation (Fig. B2). In the example shown in Fig. B2 a large peak, assumed to correspond to a phytoplankton population, can clearly be seen around 4.5 and 5 µm. The average size of this population was calculated as the arithmetic mean of all data included within the identified peak, between its beginning and end, above the approximate location of the typical logarithmic baseline observed in the volume distribution of particles (Fig. B2). Those data points were then added to obtain the approximate cell abundance needed to estimate large phytoplankton-specific attenuation coefficients.

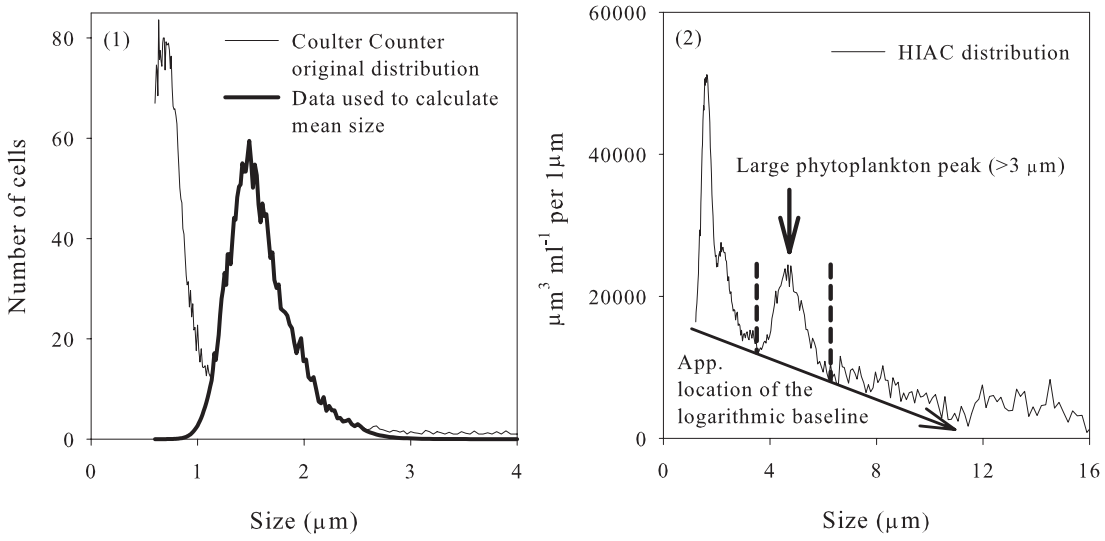

Fig. B. Example of the Coulter Counter's particle size distribution for a picophytoeukaryotes population isolated *in situ* using fast cell sorting. Both the original size distribution (light line) and the data used to calculate the arithmetic mean of the identified picophytoeukaryotes population (dark line) are shown (1). Example of volume distribution of particles in terms of µm^3 ml^{-1} per 1 µm obtained using a HIAC particle counter. A peak assumed to correspond to a large phytoplankton group (>3 µm) is observed around 5 µm. Vertical dashed lines indicate the beginning and end of the identified peak and the diagonal arrow shows the approximate (App.) location of the logarithmic base line for the volume distribution of particles. Only the data within these limits was considered to calculate the average size for this group, as its arithmetic mean. The number of particles within the same limits was taken as cell abundance for the identified phytoplankton group (2).

In order to establish an empirical relationship between FSC and intracellular carbon content, different phytoplankton populations from culture were used (Table B). The number of phytoplankton and bacterioplankton cells per ml in each culture was determined before filtering using flow cytometry. A known volume of each culture was then filtered onto Whatman GF/F filters (25 mm in diameter and 0.7 µm in porosity) in

triplicate. Flow cytometry samples were also taken after filtration, from all filtrates, in order to determine the number of phytoplankton and bacterioplankton cells passing through the filters. The filters were first dried at 60ºC during 24hrs. To eliminate any trace of inorganic carbon, the filters were fumigated with clorhydric acid (HCl) during 6 to 8hrs, after which they were dried again during 6 to 8hrs. Each filter was carefully put in a big tin capsule, paying attention not to leave any air bubbles on the inside. The filter-containing capsules were finally analyzed using a Carbon-Hydrogen-Nitrogen (CHN) autoanalyser (Thermo Finnigan, Flash EA 1112). The same procedure was followed for blank (control) filters. Carbon contents were estimated based on a calibration curve performed using Acetanilida. Bacterioplankton contamination on the filters was estimated from cell abundance by using a carbon conversion factor of 30 fgC $cell^{-1}$, established for large coastal bacterioplankton cells (Fukuda et al., 1998). Using this conversion factor contamination was always < 5-10% and was therefore considered to be negligible.

Surface and sub-surface abundances for weakly fluorescent *Prochlorococcus* populations were estimated from divinyl-chlorophyll *a* concentrations (dv-chl*a*, specific only to this group) by assuming an intracellular pigment content of 0.23 fg $cell^{-1}$. This value, obtained from the available surface data (see Fig. 2 in the article), matches the lowest value registered by Blanchot & Rodier (1996) and Partensky et al. (1999). At MAR, on the other hand, the ~3-fold higher dv-chla_i (0.6-0.8 fg $cell^{-1}$) may be due to (1) the presence of low-light (LL) adapted ecotypes (e.g., Partensky et al., 1999; (2) a deeper mixed layer (Bouman et al., 2006); or (3) a greater availability of nutrients. Divinyl chlorophyll *b* to dv-chl*a* ratios < 0.5 (not shown), suggests, however, that this population is dominated by high-light adapted (HL) ecotypes along the whole transect (Partensky et al., 1999 and references therein). Mixing, on the other hand, seems to be important in determining *Prochlorococcus* dv-chla_i at this station as indicated by the low ratio of the sum of photoprotective accessory pigments to the sum of both photoprotective and photosynthetic accessory pigments (D), an index for light history of the cells, because D and dv-chl_i are negatively related (Bouman et al., 2006 and references therein). MAR *Prochlorococcus* population is therefore clearly not representative of the rest of the transect and that is why we did not consider it when estimating mean dv-chla_i for samples above ~5% of surface light.

Table A. Picohytoplanktonic groups isolated and measured onboard.

Sorted group	Mean cell sizes measured onboard (µm)
Prochlorococcus	0.59
Synechococcus	0.75; 0.79; 0.84; 0.87
Picophytoeukaryotes	1.47; 1.53; 1.69; 1.74; 2.15

Table B. Phytoplanktonic groups from culture used to established relationships between FSC and size and FSC and intracellular carbon content. In this table we present the detailed values in fgC cell^{-1} obtained by dividing the carbon concentration in each filter (measured in mg) by the number of cells retained in that filter.

Culture Genus (Class)	Mean cell size (µm)	Mean intracellular carbon content (fgC cell^{-1})
Chlamydomonas (Chlorophyceae)	-	41109
Pelagomonas (Pelagophyceae)	1.67	1690
Ostreococcus (Prasinophyceae)	1.60	905
Tetraselmis (Prasinophyceae)	-	110874
Emiliana (Prymnesiophyceae)	-	18377
Phaeocystis (Prymnesiophyceae)	-	17700
Pycnococcus (Prasinophyceae)	-	2116
Synechococcus (Cyanophyceae)	1.07	229
Prorocentrum (Dynophyceae)	-	187075
Rhodomonas (Chryptophyceae)	-	59038
Micromonas (Prasinophyceae)	1.48	-
Dunalliela (Chlorophyceae)	-	189070
Minutocellus? (Cyanophyceae)	-	14753
Synechococcus (Cyanophyceae)	1.27	301

1.2 Picoplankton contributions to c_p, a proxy for POC

Group-specific attenuation coefficients were integrated from the surface down to 1.5 Ze (water column, $c_{0 \text{ to } 1.5 \text{ Ze}}$) and from the surface to 50 m (surface layer, $c_{0 \text{ to } 50 \text{ m}}$) to estimate their contribution to integrated c_p. In the case of MAR and EGY, c_{large} was only

available for the second and first two sampling days, respectively, whereas bacterioplankton abundance was not available for the first UPW day. For this reason, c_p-related results presented here correspond to a 2-days mean for EGY and UPW and to the second sampling day for MAR. The lack of bacterioplankton abundance data for the last station before UPW also prevented us from estimating c_p.

2 Results

2.1 Picophytoeukaryotes mean cell size

Picophytoeukarytoes mean cell size tended to decrease towards the coast, with the smallest cells being observed at UPX (Fig. C1). In terms of mean cell volume relative to the percentage of surface irradiance (Fig. C2) this group followed the same trend. Note the 0.22 µm (0.0056 µm^3) difference in mean cells size (volume) between the two neighbor stations MAR and HNL. Same thing for UPW and UPX, note the difference in size and volume for the two coastal upwelling stations. The differences summarized in Fig. C highlight the importance of considering picophytoeukaryotes cell size and volume variability when estimating their contribution to beam particle attenuation coefficient and therefore to particulate organic carbon in the ocean.

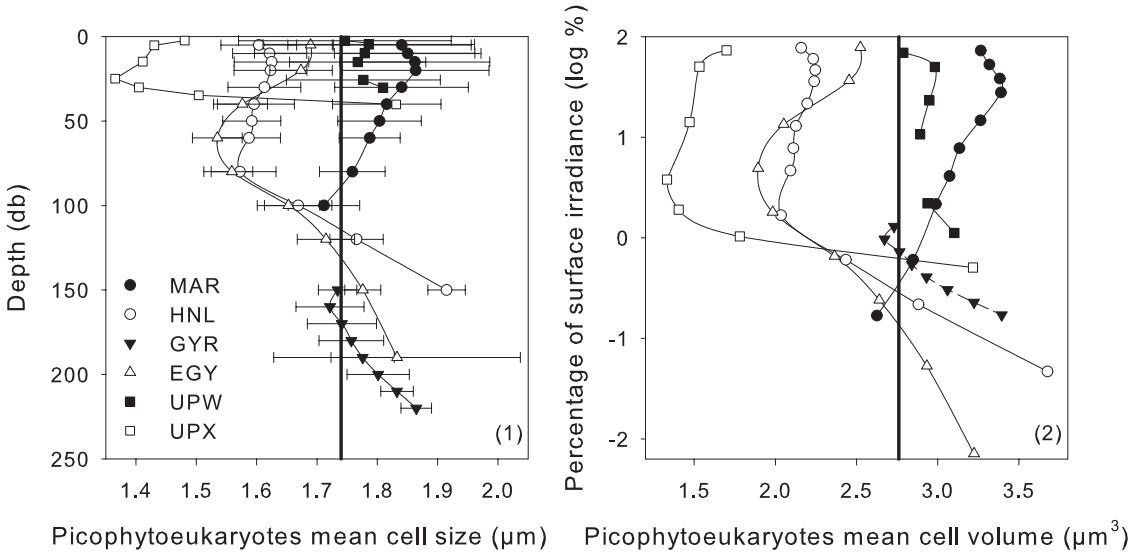

Fig. C. Mean and standard deviation (horizontal bars) of picophytoeukaryotes cell size as a function of depth (a) and mean volume as a function of percentage of surface irradiance (b) at MAR, HNL, GYR, EGY, UPW and UPX. Vertical black lines indicate mean size (a) and volume (b) for the whole transect.

Literature cited

Blanchot, J. and Rodier, M.: Picophytoplankton abundance and biomass in the western Tropical Pacific Ocean during the 1992 El Nino year: Results from flow cytometry. Deep-Sea Res, 43, 877-896, 1996.

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