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# Nutrients limitation of primary productivity in the Southeast Pacific (BIOSOPE cruise)

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## Abstract

Iron is an essential nutrient involved in a variety of biological processes in the ocean, including photosynthesis, respiration and nitrogen fixation. Atmospheric deposition of aerosols is recognized as the main source of iron for the surface ocean. In high nutrient, low chlorophyll areas, it is now clearly established that iron limits phytoplankton productivity but its biogeochemical role in low nutrient, low chlorophyll environments has been poorly studied. We investigated this question in the unexplored southeast Pacific, arguably the most oligotrophic area of the global ocean. Situated far from any continental aerosol source, the atmospheric iron flux to this province is amongst the lowest of the world ocean. Here we report that, despite low dissolved iron concentrations (~0.1 nmol $l^{-1}$) measured across the whole gyre (3 stations situated in the center, the western and the eastern edge), photosynthesis and primary productivity are only limited by iron availability at the border of the gyre, but not in the center. The seasonal stability of the gyre has apparently allowed for the development of populations acclimated to these extreme oligotrophic conditions. Moreover, despite clear evidence of nitrogen limitation in the central gyre, we were unable to measure nitrogen fixation in our experiments, even after iron and/or phosphate additions, and cyanobacterial *nifH* gene abundances were extremely low compared to the North Pacific Gyre. The South Pacific gyre is therefore unique with respect to the physiological status of its phytoplankton populations.

## 1 Introduction

The production of organic matter in the sea is sustained by a continuous supply of essential macro- (C, N, P) and micronutrients (metals, vitamins). The minimum threshold concentration needed is different for each essential element and the nutrients requirements also vary among different phytoplanktonic species. According to the Liebig's law, organic matter production is controlled by the element that is available in the



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lowest concentration relative to the needs for growth. This simple view is now replaced by the realization that multiple resources simultaneously limit phytoplankton growth in some parts of the ocean (Arrigo, 2005). Global environmental forcing, including human-induced climate change, could potentially modify the nutrient delivery processes to the ocean, leading to fundamental changes in the diversity and functioning of the marine food web. It is thus fundamental to understand which nutrients control primary productivity in the open ocean if we want to be able to predict the biogeochemical consequences of the global change. By representing 60% of the global ocean's area, the subtropical open-ocean ecosystems are the largest coherent biomes on our planet, and the biogeochemical processes they support have global consequences (Karl, 2002). The development of permanent time series stations in the North tropical Atlantic and Pacific over the past two decades have led to a revolution in our thinking about the mechanisms and controls of nutrient dynamics in these remote environments and there are growing evidences that they play a central role in the global carbon cycle (Emerson et al., 1997). These environments provide ideal ecological niche for the development of nitrogen-fixing organisms (e.g. Karl et al., 2002). In the North subtropical and tropical Atlantic and Pacific oceans, it has been estimated that $N_2$ fixation is equivalent to 50–180% of the flux of $NO_3$ into the euphotic zone (Karl et al., 1997; Capone et al., 2005), demonstrating that a large part of new primary productivity is fuelled by $N_2$ fixation, rather than $NO_3$ diffusing from deeper layer into the euphotic zone. $N_2$ fixation requires the iron-rich nitrogenase complex, and so $N_2$-fixing organisms have high iron (Fe) requirements compared to phytoplankton growing on ammonium (Raven, 1988; Kustka et al., 2003). Nitrogen fixation is thus controlled in these oceanic gyres by Fe availability, as well as phosphate, which can (co)limit the process (Sanudo-Wilhelmy et al., 2001; Mills et al., 2004). However, all of the studies dedicated to the nutrient control of primary productivity in general and $N_2$ fixation in particular have focused so far on the northern hemisphere and there are extremely few data available on the southern hemisphere. The South Pacific gyre, which is the largest oceanic gyre of the global ocean, had been particularly undersampled (Claustre et al., this issue). This

unique environment appears from satellite imagery and ocean colour to have the lowest chlorophyll *a* (Chl *a*) concentrations of the global ocean and thus represents an end-member of oceanic hyperoligotrophic conditions (Claustre and Maritorena, 2003). Contrary to the oceanic gyres situated in the Northern hemisphere, the South Pacific Gyre is far removed from any continental source (anthropogenic and natural desert aerosols) and receives amongst the lowest atmospheric Fe flux in the world (Wagener et al., 2007[1]). Consequently, the phytoplankton community as a whole, and particularly nitrogen-fixing organisms, have been suggested to be Fe-limited (Falkowski et al., 1998; Berman-Frank et al., 2001; Moore et al., 2002), but direct experiment were lacking.

The BIOSOPE cruise provided the first spatially extensive experiment in the Southeast Pacific. We conducted nutrient addition bioassays, designed to investigate which nutrient (N, P and/or Fe) controls primary productivity, photosynthetic efficiency and nitrogen fixation along a trophic gradient in the Southeast Pacific. A complementary paper (Van Wambeke et al., 2007[2]) examines the factors that control heterotrophic bacterial growth in the same area.

## 2 Material and methods

This research was carried out onboard the R/V Atalante in October-November 2004. The experiments were performed at three stations (Fig. 1a) located in the western edge (station HNL, 9° S 136° W), in the center (station GYR, 26° S 114° W) and in the southeastern edge of the gyre (station EGY, 34° S 92° W).

[1] Wagener, T., Guieu, C., Losno, R., Bonnet, S., and Mahowald, N.: Revisiting Atmospheric dust export to the South Hemisphere Ocean, Global Biogoechem. Cy., under revision, 2007.

[2] Van Wambeke, F., Bonnet, S., Moutin, T., Raimbault, P., Alarçon, G., and Guieu, C.: Factors limiting heterotrophic prokaryotic production in the Southern Pacific Ocean, Biogeosciences Discuss., to be submitted, 2007.

All experimental setups were performed under strict trace metal clean conditions inside a clean container. Seawater was collected at 30 m depth using a trace metal-clean Teflon pump system and was dispensed into acid-washed 4.5-liter transparent polycarbonate bottles. Under a laminar flow hood, nutrients or dust were added either alone or and in combination. The final concentrations were 1 $\mu$mol l$^{-1}$ $NH_4^+$ + 2 $\mu$mol l$^{-1}$ $NO_3^-$, 0.3 $\mu$mol l$^{-1}$ $NaH_2PO_4$, 2 nmol l$^{-1}$ $FeCl_3$ and 0.25 mg l$^{-1}$ of dust. Despite that Saharan event was unlikely to occur in the Southeast Pacific, the dust used in this experiment was the Saharan soils collected and characterized by Guieu et al. (2002) in order to allow a comparison with earlier efforts (Mills et al., 2004; Blain et al., 2004, Bonnet et al., 2005). Each fertilization was performed in triplicate. The bottles were immediately capped with parafilm, sealed with PVC tape, and incubated in an on-deck incubator with circulating surface seawater at appropriated irradiance (50% ambient light level). For each experimental treatment, triplicates were sacrificed at two selected time points during the course of the experiment ($T_1$=24 h; $T_2$=48 h) to minimize the risk of contamination. Subsamples were used for variable fluorescence, Chl *a* concentrations, epifluorescence microscopy counts and flow cytometry. For primary productivity and nitrogen fixation measurements, parallel incubations were run in triplicate for 4 h and 24 h, respectively. Treatment means were compared using a one-way ANOVA and a Fisher PLSD means comparison test.

### 2.1 Nutrient analysis

Nutrients concentrations have been analysed using a Technicon Autoanalyser II (Treguer and Le Corre, 1975). For low nitrate concentrations, the method described in Raimbault et al. (1990) have been used.

### 2.2 Dissolved iron (DFe) concentrations

DFe concentrations were measured on 19 vertical profiles (0–400 m) along the 8000 km transect. They were analyzed by Flow Injection Analysis with online preconcentration

and chemiluminescence detection (FIA-CL) (adapted from Obata et al., 1993). The mean blank, calculated from daily determinations, equaled 69±18 pmol $l^{-1}$ ($n$=19) and the detection limit was 54 pmol $l^{-1}$. Each sample was analyzed in triplicate. When at least two of the three runs agreed within expected reproducibility (10%), the average of the two or three concordant runs was taken as a correct concentration. If the concentration obtained deviated too much from the profile continuum expectations and seemed to be contaminated (as samples were collected in triplicate),one of the other sampled bottles was then analyzed (in triplicate) (see Blain et al. (2007) for more details on the methodology).

### 2.3 Nitrogen fixation

1 ml of $^{15}N_2$ gas (99% $^{15}N_2$ EURISOTOP) was introduced to each 0.6 l polycarbonate bottle through a Teflon-lined butyl rubber septum using a gas-tight syringe following the protocol from Montoya et al. (1996). After 24 h of incubation, the samples were filtered under low vacuum (100 mm Hg) through a precombusted (24 h at 450°C) 25-mm GF/F filter and dried at 60°C. Filters were stored in a desiccator until analysed. Determination of $^{15}N$ enrichments was performed with an Integra-CN PDZ EUROPA mass spectrometer. We have considered a background natural abundance, determined on 8 unlabelled samples, of 0.367±0.007% for N. Only excess enrichments higher than two times the standard deviation (0.014% for N) were considered as significant. As we worked with low levels of particulate N, we have calibrated the spectrometer in the same conditions of particulate N and the quality of the analysis was tested with standard molecules. Based on the lowest nitrogen level determined by our mass spectrometer (0.2 $\mu$moles), the detection limit for dinitrogen fixation was 0.3 $\mu$moles $l^{-1}$. Dinitrogen fixation rates (rN in nmoles N $l^{-1}$ $t^{-1}$) were computed from an equation based on final particulate nitrogen (Dugdale and Wilkerson, 1986).

## 2.4 Primary productivity

Primary productivity was quantified according to the experimental protocol detailed in Moutin and Raimbault (2002).

## 2.5 Flow cytometry

Cytometric analyses for picophytoplankton were performed on fresh samples with a FACSCalibur (Becton Dickinson) flow cytometer. Populations were differentiated based on their scattering and fluorescence signals (Marie et al., 2000). Samples were acquired for 3 min at ~80 $\mu$l min$^{-1}$ (~12 to 100×10$^3$ cells) using Cell Quest Pro software and data were analysed using Cytowin (see http://www.sb-roscoff.fr/Phyto/index.php?option=com_content&task=view&id=72&Itemid=123). Forward scatter (FSC) and chlorophyll *a* fluorescence (FL3) cytometric signals were normalized to reference beads (Fluoresbrite® YG Microspheres, Calibration Grade 1.00 $\mu$m, Polysciences, Inc) and then used as indicators of mean cell size and intracellular chlorophyll content, respectively (e.g. Campbell and Vaulot 1993). Significant changes in mean FSC and FL3 after incubation under the different treatments (48 h) was evaluated through ANOVA analyses.

## 2.6 Epifluorescence microscopy counts

Counts were performed with a Olympus BX51 epifluorescence microscope. Water samples (100 ml) were fixed with glutaraldehyde (0.25% final concentration) and filtered through 0.8 $\mu$m pore size filters. Samples were stained with 4'6-diamidino-2-phenylindole (DAPI, 5 $\mu$g ml$^{-1}$ final concentration). Eukaryotes were identified and counted by standard epifluorescence microscopy (Porter and Feig, 1980).



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## 2.7 Variable fluorescence

Chlorophyll variable fluorescence of phytoplankton was measured using the custom-built benchtop Fluorescence Induction and Relaxation (FIRe) system (Gorbunov and Falkowski, 2005). The excitation light was provided by 4 blue light-emitting diodes, LEDs, (central wavelength 450 nm, 30 nm bandwidth, with the peak optical power density of 2 W $cm^{-2}$). The variable fluorescence sequences were processed to calculate minimum ($F_o$) and maximum ($F_m$) fluorescence (measured in the dark), the quantum efficiency of PSII ($F_v/F_m$), the effective absorption cross-section of PSII ($\sigma_{PSII}$), and the rates of electron transport in PSII ($\tau$) according to Kolber et al. (1998). Measurements were made on dark-adapted samples (30 min). The background fluorescence signal (blank) was measured using filtered seawater (through 0.2 $\mu$m filter) and was subtracted from the measured variable fluorescence.

## 2.8 Abundance of nitrogen fixers

Water samples were filtered through 3 $\mu$m pore size filters (GE Osmonics) and subsequently through 0.2 $\mu$m pore size Supor filters (PALL corp.). Both filters were processed to determine the $N_2$-fixing microorganisms in the >3 $\mu$m and <3 $\mu$m size fractions. DNA was extracted from filters (Church et al., 2005) with the addition of a bead-beating step (2 m at what setting) prior to the lysis step. The *nifH* gene was amplified with nested PCR primers (*nifH1, nifH2, nifH3 and nifH4*) (Church et al., 2005). The amplification products were cloned into pGEM®-T vectors (Promega). Plasmid DNA was isolated with Montage kits (Millipore) and the cloned inserts were sequenced at the University of California-Berkeley Sequencing facility. Quantitative PCR for Group A and B unicellular cyanobacterial *nifH* was performed as described in Church et al. (2005).

### 2.9 Determination of pigments

2.8 l of seawater were filtered onto GF/F filters and immediately stored in liquid nitrogen then at –80°C until analysis on land which was performed according to the procedure described in Ras et al. (2007)[3]. Pigment grouping into pigment-base size classes was performed according to Uitz et al. (2006).

## 3 Results

### 3.1 Initial nutrient concentrations and phytoplankton composition prior to experiment ($T_0$)

The initial nutrient concentrations and phytoplanktonic species composition for these bioassay experiments are given in Table 1. Fe vertical profiles indicated low (below $0.134 \pm 0.05\,\mathrm{nmol\,l^{-1}}$) and relatively constant DFe concentrations from the surface to 400 m throughout the entire transect (station MAR throughout station EGY, $n$=110), except in the Chilean coastal upwelling zone (Fig. 1b, see also Blain et al., 2007). Surface DFe concentrations at the three stations studied were $0.14 \pm 0.02\,\mathrm{nmol\,l^{-1}}$, $0.10 \pm 0.01\,\mathrm{nmol\,l^{-1}}$ and $0.10 \pm 0.01\,\mathrm{nmol\,l^{-1}}$, respectively for HNL, GYR and EGY (Table 1). In contrast, macronutrients and Chl *a* concentrations differed markedly among stations, with Chl *a* concentrations being $0.029 \pm 0.01\,\mathrm{mg\,m^{-3}}$ in nitrate-depleted waters of GYR and $0.103 \pm 0.02$ and $0.110 \pm 0.01\,\mathrm{mg\,m^{-3}}$ respectively at EGY and HNL, where nitrate concentrations were higher ($0.02 \pm 0.02$ and $1.66 \pm 0.11\,\mu\mathrm{mol\,l^{-1}}$; Table 1). Waters were phosphate-replete along the whole transect as concentrations were always above $0.11\,\mu\mathrm{mol\,l^{-1}}$.

The phytoplankton community structure was dominated by picophytoplankton at the three stations, where it represented 58%, 49% and 47% of the total Chl *a* (Ta-

[3] Ras, J., Uitz, J., and Claustre, H.: Spatial variability of phytoplankton pigment distribution in the Southeast Pacific, Biogeosciences Discuss., to be submitted, 2007.

ble 1), respectively at HNL, GYR and EGY. Cyanobacteria, mainly belonging to the *Prochlorococcus* genera, dominated picophytoplancton. Phytoplankton pigment distribution along the transect is described in Ras et al. (2007)[3].

### 3.2 Biological response during the incubation experiments

For all the parameters measured in this experiments, the stimulation by nutrients was considered to be significant when the ANOVA comparison of distribution of triplicate treatments gave values of $p<0.05$. The significant responses (different from the control) are indicated by arrows above the histograms on Fig. 2.

#### 3.2.1 Physiological parameters (Photosynthetic quantum efficiency of photosystem II)

The western part of the gyre (station HNL) was characterized by low $F_v/F_m$ (0.16±0.01) at T0 (Table 1), indicating an apparently low yield of photosynthesis. Fe was found to be the nutrient that controls photosynthetic efficiency at that station, as indicated by the increase of 65% of $F_v/F_m$ after an Fe addition ($p<0.05$; Fig. 2). The station located at the eastern side of the gyre (station EGY) exhibited medium $F_v/F_m$ values at T0 (0.30±0.02) (Table 1) that increased significantly ($p<0.05$) after Fe addition, but in a lower proportion compared to station HNL (30%; Fig. 2). In contrast, the center of the South Pacific Gyre was characterized by high $F_v/F_m$ at T0 (0.51±0.03) (Table 1). This value did not increase after Fe, N, P or dust addition ($p>0.05$, Fig. 2). The addition of macronutrients without Fe had a positive effect at station HNL, but did not have any effect at station EGY, where only an Fe addition (alone or in combination) resulted in an increase of $F_v/F_m$. The dust treatment also had a positive effect on $F_v/F_m$ at the station EGY.

### 3.2.2 Rate measurements

Primary productivity. At station HNL, only the addition of Fe did result in a significant increase of primary productivity (+50%, $p<0.05$), whereas at station GYR, only the treatments having nitrogen (+N, +Fe and N, +Fe, N and P) resulted in a positive response (+45%, $p<0.05$). At station EGY, both the treatments having a source of Fe or N resulted in a positive increase (+25%). The addition of both Fe and N at the same time resulted in a higher response (+50%), indicating a clear Fe and N co-limitation. At stations GYR and EGY, the addition of P together with Fe and N did not result in a significantly higher response than the addition of Fe and N alone, indicating that P is not limiting.

Nitrogen fixation. $^{15}N_2$ assimilation consistently remained below the detection limit at the three stations in our incubation experiments, indicating absence of nitrogen fixation, even after Fe, Fe and P and dust additions (Table 2).

### 3.2.3 Abundance of nitrogen fixers

Water samples from 13 stations situated between HNL and EGY (Fig. 1a) were examined for presence of $N_2$-fixing microorganisms by amplification of the *nifH* gene. After amplification, cloning and sequencing the nitrogenase genes, our results indicate the absence of the filamentous cyanobacteria *Trichodesmium,* or any large (3–7 $\mu$m) unicellular putative nitrogen fixing cyanobacteria (Group B). The results suggest the presence of extremely low numbers of Group A cyanobacterial phylotypes (less than 200 copies $l^{-1}$ at two stations, Table 2).

### 3.2.4 Cells numbers (flow cytometry, microscopy)

Epifluorescence microscopy confirmed the absence of the two phylotypes *Trichodesmium* and unicellular from Group B at any station and treatment, which is in agreement with molecular biology data. Concerning non nitrogen-fixing organisms,

the addition of Fe or Fe and/or macronutrients at stations HNL resulted in a significant ($p$<0.05) increase in *Synechococcus* and picoeucaryotes abundances (Table 3). At station EGY, all the treatments containing nitrogen had a positive effect on pico-eukaryotes; the N treatment also induced a significant increase in *Prochlorococcus* and *Synechococcus* abundances. At station GYR, only the +N and +Fe and N treatments induced a significant increase in pico-eucaryotes abundances. The other treatments did not have any effect on this group, nor on *Synechococcus.* However, larger *Synechococcus* fluorescence (FL3) and forward light scatter (FSC) cytometric signals indicated at station GYR an increase in relative cell size and intracellular chlorophyll *a* content after addition of N, Fe and N and All ($p$<0.05 for FSC and FL3, Table 4). The presence of larger cyanobacteria (between 1 and 3 um) was later confirmed by epifluorescence microscopy counts.

## 4 Discussion

### 4.1 The role of Fe

The data indicate that Fe is the nutrient that controls photosynthetic efficiency and primary productivity outside the gyre, at station HNL. These results are in accordance with the patterns found in other HNLC waters (e.g. Boyd et al., 2000). Around Marquesas, Behrenfeld and Kolber (1999) also found similar low values of $F_v/F_m$ with pronounced decrease at night; as for our experiments, the addition of Fe eliminated the nocturnal decrease and increased $F_v/F_m$ values. In the central gyre (GYR), the high $F_v/F_m$ value (0.51±0.03) was however unexpected due to the extremely low ambient dissolved Fe concentrations. This value is close to the maximum value observed in the ocean (Falkowski et al., 2004) and did not increase after Fe or dust addition. Behrenfeld et al. (2006) found the same pattern (high $F_v/F_m$, absence of nocturnal decrease) in the North Tropical Pacific, but the ambient dissolved Fe concentrations there are two to seven times higher (Boyle et al., 2005) than in the South Pacific Gyre, where

we did our experiments. Our data also indicate that the addition of Fe did not change Chl *a* concentrations or primary productivity ($p$>0.05), indicating that -contrary to the HNL station- the photoautotrophic communities were not Fe-limited in the gyre. This suggests that the natural assemblage is acclimated to Fe deprivation.

Flow cytometer measurements identified *Prochlorococcus* (20 000 cells $ml^{-1}$) as a prominent component of the prokaryote-dominated phytoplankton assemblage at station GYR, whereas in terms of carbon biomass, picophytoeukaryotes dominated (0.89 mg $m^{-3}$, i.e. 2.6-fold higher than *Prochlorococcus*). Although *Synechococcus* abundance was similar to the latter (1400 cells $ml^{-1}$), their contribution to the phytoplanktonic carbon biomass was negligible (0.06 mg $m^{-3}$). To maintain high carbon fixation rates in such a low Fe environment, the organisms must have developed ecophysiological strategies to survive the shortage of Fe, including Fe scavenging systems (Geider and la Roche, 1994), efficient Fe transport systems over the plasma membrane (Katoh et al., 2001) or gene regulation systems consisting in rearrangements of photosynthetic apparatus (Sandström et al., 2002).

In summary, although DFe concentrations were identical at the three stations, our data clearly show contrasting physiological responses to Fe additions. Cultures experiments conducted under Fe limited conditions exhibit either low (~0.1) or high (~0.5) $F_v/F_m$ depending on whether growth is balanced or unbalanced (Price, 2005). The high $F_v/F_m$ values measured in the center of the gyre (GYR) are a clear indication that the phytoplankton assemblages are well acclimated to the stable environmental conditions of low N and low Fe. In contrast, station HNL (situated in the southern limit of the equatorial upwelling and embedded in the westward flowing South Equatorial Current) and station EGY (corresponding to a transition zone between the salty Eastern South Pacific Central Waters and the waters influenced by fresher Subantarctic Surface Waters (Emery and Meincke, 1986) are relatively less steady state environments, with low $F_v/F_m$ values and increased Fo possibly due to the presence of specific Fe-stress pigment-protein complexes (Behrenfeld et al., 2006). This might also suggest an unbalanced growth (Parkhill et al., 2001) in environment with occasional spikes of

nutrients.

It is interesting to note that a dust addition did not cause any increase of primary productivity at station EGY, while an Fe addition resulted in a positive response. This absence of response can be interpreted by the fact that the dissolution of Fe we measured from the dust in our experiment is only 0.1%, which is ten times lower than the dissolution found with the same amount of the same dust in the Mediterranean waters by Bonnet and Guieu (2004). This difference can be interpreted by the difference in organic ligands concentrations between the Pacific and the Mediterranean waters (Bonnet, 2005; Mendez et al., 2007[4]).

### 4.2 From Fe to nitrogen limitation

Dissolved Fe concentrations were low and constant along the three stations studied, but our data clearly show a progression from Fe towards nitrogen limitation as we progress from the edge (station HNL) to the center of the gyre (Station GYR). The station EGY, situated on the southeastern edge of the gyre, constitutes a transition station, where primary productivity is Fe and N co-limited. In the center of the gyre, at station GYR, nitrogen is the nutrient that controls primary productivity. The addition of a nitrogen source resulted in an increase of picophytoeukaryote's abundance and an increase in relative cell size and intracellular Chl *a* content of *Synechococcus* ($p<0.05$). It is interesting to note that bacterial production is also directly enhanced after a nitrogen addition (by a factor of 9 after 48 h, see Van Wambeke et al., 2007[2]).

Perhaps the most intriguing part of this study is the absence of nitrogen fixation in our experiments, even after dust, Fe and/or P additions. These results would suggest that neither P nor Fe limit nitrogen fixation in the tested waters à 30 m-depth. It has to be noted that Raimbault et al. (2007)[5] measured very low nitrogen fixation rates at

[4] Mendez, J., Guieu, C., and Adkins, J.: Atmospheric input of Manganese and Iron to the ocean: Seawater dissolution experiments with Saharan and North American dusts, Mar. Chem., under revision, 2007.

[5] Raimbault, P. and Garcia, N.: Carbon and nitrogen uptake in the South Pacific Ocean:

30 m in the central gyre (stations GYR), revealing the weakness of the process at this depth. However, these authors measured significant nitrogen fixation rates in subsurface waters (~1 nmol $l^{-1}$ $d^{-1}$), which is in accordance with the molecular data (Table 2), indicating the presence of nitrogen fixing organisms from the Group A (184 copies $l^{-1}$) at 5 m depth in the gyre. These numbers are however extremely low compared to those of the North Pacific Gyre (ALOHA station in December, 10 000 to 100 000 copies $l^{-1}$). The absence of *Trichodesmium* and Groupe B phylotypes also contrasts with amplification from oligotrophic waters of the tropical North Pacific Ocean, where cyanobacterial *nifH* genes from these two groups are abundant and easily amplified, even during the winter season (see Table 2; Church et al., 2005). In this area, nitrogen fixation rates are high throughout the water column (Dore et al., 2002) and provide a major source of newly fixed nitrogen to the euphotic zone, which sustains up to 50% of new primary production (Karl et al., 1997) and drives the system towards P- and/or Fe-limitation (Karl et al., 1997; Sohm et al., 2007[6]). The scarcity of nitrogen fixing organisms in the South Pacific Gyre may be one of the origin of the non-depleted phosphate stocks (always above 0.11 $\mu$mol $l^{-1}$; Moutin et al., 2007), as well as the N controlled status of the phytoplankton and bacterial communities. However, the other potential sources of nitrogen for the South Pacific Gyre are very reduced and nitrogen fixation may nonetheless represent the main source of new nitrogen for the system (Raimbault et al., 2007[5]): the vertical flux of nitrate from below the thermocline is extremely low compared to other gyres (10 to 12 times lower than the one measured in the North Atlantic gyre, Capone et al., 2005), and potential atmospheric deposition of nitrogen is almost absent according to the aerosols measurements performed in this area by Wagener et al. (2007)[1].

Evidence for efficient dinitrogen fixation and regenerated production leading to large accumulation of dissolved organic matter in nitrogen-depleted waters, Biogeosciences Discuss., to be submitted, 2007.

[6] Sohm, J., Krauk, J., Mahaffey, C., and Capone, D. G.: Diagnostics of phosphorus stress in the cyanobacterium *Trichodesmium* reveal the northwest Atlantic is more severely P limited than the tropical Pacific, Limnol. Oceanogr., submitted, 2007.

## 5 Conclusions

This paper attempts a comprehensive analysis of the nutrient factors that control primary productivity in the Southeast Pacific. We describe here a clear gradient in the nutrient control of phytoplanktonic communities, from an Fe-controlled system (on the edge of the gyre) towards a nitrogen-controlled system (in the center of the gyre). By combining physiological data, rate processes measurements and molecular approaches, we describe for the first time this unique nutritional status of the phytoplankton populations in the gyre. Autotrophic communities are indeed adapted to living under extremely low Fe levels; these results add a new perspective on life in extreme environments and give a new perspective to so-called HNLC areas of the world ocean. In addition, we show for the first time that nitrogen fixing cyanobacteria are scarce in the South Pacific Gyre. Due to the extremely low Fe-rich dust inputs at the surface waters of the gyre, these waters constitute a "low iron" environment (Blain et al., 2007); we hypothesize here that it must not represent a favourable environment for common photoautotrophic nitrogen fixing organisms (e.g. *Trichodesmium*), as they have elevated Fe quotas relative to non-diazotrophic phytoplankton (Kustka et al., 2003). However, the factors controlling nitrogen fixation are still poorly understood and further studies are needed to understand the distribution of these organisms and their biogeochemical impact in the ocean.

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**Table 1.** Initial conditions for bioassay experiments. % pico-, nano- and microphytoplankton correspond to the biomass proportion of total Chl *a* associated with each size class of phytoplankton calculated as described in Uitz et al. (2006).

| | Experiment 1 HNL | Experiment 2 GYR | Experiment 3 EGY |
|---|---|---|---|
| Latitude | 9°04′ S | 26°04′ S | 31°89′ S |
| Longitude | 136°97′ W | 114°02′ W | 91°39′ W |
| Chl *a* ($mg\,m^{-3}$) | 0.11±0.05 | 0.029±0.01 | 0.103±0.02 |
| $NO_3$ ($\mu mol\,l^{-1}$) | 1.66±0.11 | <0.003 | 0.02±0.02 |
| $PO_4$ ($\mu mol\,l^{-1}$) | 0.30±0.01 | 0.11±0.02 | 0.14±0.06 |
| $SiO_2$ ($\mu mol\,l^{-1}$) | 1.31±0.12 | 0.98±0.09 | 1.21±0.17 |
| Fe ($nmol\,l^{-1}$) | 0.14±0.02 | 0.10±0.01 | 0.10±0.01 |
| $F_v/F_m$ | 0.16±0.01 | 0.51±0.03 | 0.30±0.01 |
| Prim. Prod ($mg^{14}C\,mg\,Chl\,a^{-1}\,d^{-1}$) | 79±2 | 61±3 | 93±8 |
| % pico- | 58 | 49 | 47 |
| % nano- | 31 | 48 | 43 |
| % micro- | 11 | 13 | 10 |



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**Table 2.** Abundance of diazotrophs (gene copies per liter) determined by QPCR for samples collected at 13 stations between HNL and EGY (ND: Non Detectable). The depth at which the *nif* gene was detectable is indicated between parentheses.

| | <3 $\mu$m | >3 $\mu$m | | |
|---|---|---|---|---|
| Station | Group A | Group B | Trichodesmium | Chaetoceros/Calothrix |
| HNL | 27 (5 m) | ND | ND | ND |
| 1 | ND | ND | ND | ND |
| 2 | ND | ND | ND | 30 (70 m) |
| 3 | ND | ND | ND | ND |
| 4 | ND | ND | ND | ND |
| 6 | – | ND | ND | ND |
| 7 | ND | ND | ND | ND |
| GYR | 184 (5 m) | ND | ND | ND |
| 12 | – | ND | ND | ND |
| 13 | ND | ND | ND | ND |
| 14 | ND | – | – | – |
| 15 | ND | ND | ND | ND |
| EGY | ND | ND | ND | ND |
| ALOHA | 10 000–100 000[a] | 1000[a] | 1000–10 000[a] | – |



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**Table 3.** Evolution of the abundances of *Prochlorococcus*, *Synechococcus* and picophytoeukaryotes after 48 h of incubation at the three stations studied, for each treatment. The standard deviation (SD) is calculated on the triplicates. At station GYR, *Prochlorococcus* fluorescence was too dim to allow us to detect changes in either the abundance or cytometric signals.

| HNL | | | | | | |
|---|---|---|---|---|---|---|
| Sample | Proc/ml | SD Proc | Syn/ml | SD Syn | Euks/ml | SD Euk |
| Control | 264134 | 17091 | 30096 | 677 | 10140 | 371 |
| Fe | 116928 | 1858 | 47740* | 1256 | 15502* | 1039 |
| NPSi | 420368* | 43936 | 60892* | 4158 | 23309* | 1056 |
| FeNPSi | 142137 | 12420 | 61178* | 1726 | 18243* | 1399 |
| GYR | | | | | | |
| Sample | Proc/ml | SD Proc | Syn/ml | SD Syn | Euks/ml | SD Euk |
| Control | nd | nd | 1704 | 272 | 572 | 109 |
| Fe | nd | nd | 1873 | 176 | 519 | 37 |
| N | nd | nd | 1659 | 119 | 775* | 144 |
| FeN | nd | nd | 1492 | 79 | 916* | 106 |
| All | nd | nd | 1546 | 55 | 679 | 113 |
| Dust | nd | nd | 2013 | 129 | 625 | 33 |
| EGY | | | | | | |
| Sample | Proc/ml | SD Proc | Syn/ml | SD Syn | Euks/ml | SD Euk |
| Control | 158798 | 6284 | 18744 | 512 | 6422 | 162 |
| Fe | 102588 | 3761 | 15964 | 795 | 5724 | 141 |
| N | 253317* | 19927 | 20521* | 900 | 8642* | 590 |
| FeN | 129819 | 12825 | 18130 | 669 | 7960* | 238 |
| All | 127753 | 7424 | 17401 | 454 | 8694* | 186 |
| Dust | 138279 | nd | 17937 | 481 | 8288* | 312 |

**Table 4.** Cytometric signals obtained at station GYR for each treatment. Significant changes in mean FSC and FL3 after incubation under the different treatments (48 h) was evaluated using a one-way ANOVA. Significant data ($p<0.05$) are labelled with an asterisk. Abbreviations: Proc (*Prochlorococcus*), Syn (*Synechococcus*), Euk (picophyto-eukaryotes), SSC (side scattered light intensity), FSC (Forward scattered light intensity), FL3 (chlorophyll *a* fluorescence).

| | Fe | N | Fe and N | All | Dust |
|---|---|---|---|---|---|
| SSC Proc. | 0.065* | 0.013* | 0.024* | 0.034* | 0.016* |
| FSC Syn. | 0.470 | 0.012* | 0.006* | 0.009* | 0.858 |
| SSC Syn. | 0.792 | 0.805 | 0.374 | 0.444 | 0.400 |
| FL3 Syn. | 0.230 | 0.021* | 0.020* | 0.018* | 0.708 |
| FSC Euk. | 0.646 | 0.011* | 0.259 | 0.221 | 0.883 |
| SSC Euk. | 0.663 | 0.002* | 0.124 | 0.048* | 1.000 |
| FL3 Euk. | 0.096 | 0.032* | 0.117 | 0.01* | 0.595 |

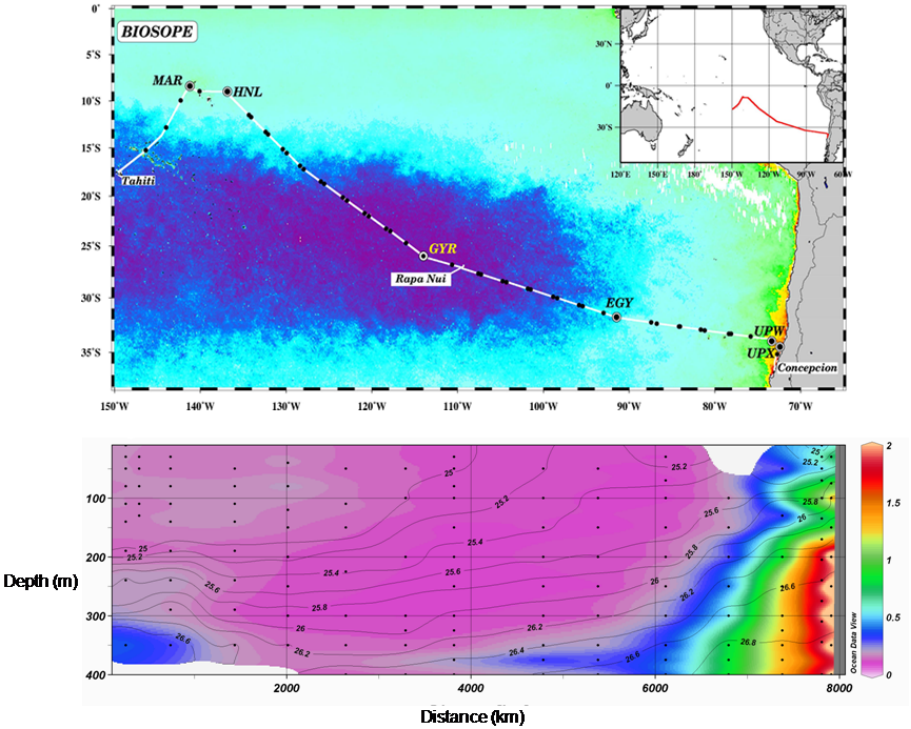


**Fig. 1. (a)** Transect of the BIOSOPE cruise from the Marquesas Islands to Chile superimposed on a SeaWiFS surface Chl *a* composite image (November–December 2004), and location of the short (numbers) and long-stations of the cruise (MAR, HNL, GYR, EGY, UPW, UPX). This study reports results of bioassay experiments performed at stations HNL, GYR and EGY. **(b)** Dissolved iron concentrations (0–400 m) (nmol $l^{-1}$) along the BIOSOPE transect from Marquesas Islands (left) to Chile (right).



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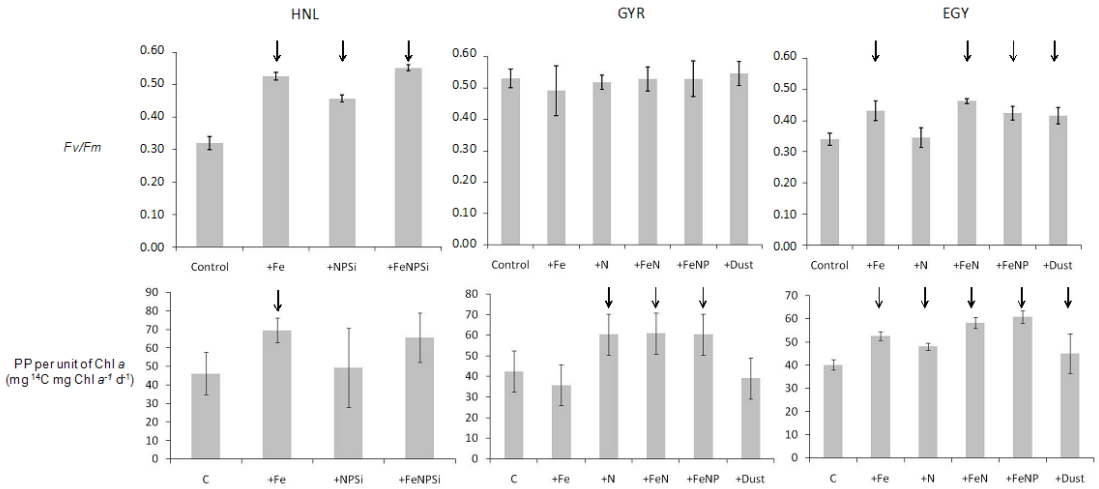


**Fig. 2.** Effect of nutrient additions during bioassay experiments performed at the three stations (HNL, GYR, EGY) **(a)** Photochemical efficiency of photosystem II ($F_v/F_m$) after 24 h incubation, **(b)** Primary production per unit chlorophyll *a* (mg $^{14}$C mg Chl $a^{-1}$ d$^{-1}$) after 48 h of incubation. $F_v/F_m$ and carbon fixation were measured from separate triplicate bottles, such that nine bottles were incubated for each nutrient treatment. The error bars represent the standard deviation from triplicate incubations. Treatment means were compared using a one-way ANOVA and a Fisher PLSD means comparison test. Means that are significantly different from the control ($p<0.05$) are labelled with an arrow.



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