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Calcite production by coccolithophores in the south east Pacific Ocean

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Abstract. BIOSOPE cruise covered an oceanographic transect through the centre of the South Pacific Gyre (SPG) from the Marquesas archipelago to the Peru-Chile upwelling (PCU). Water samples from 6 depths in the euphotic zone were collected at 20 stations. The concentrations of suspended calcite particles, coccolithophores cells and detached coccoliths were estimated together with size and weight using an automatic polarizing microscope, a digital camera, and a collection of softwares performing morphometry and pattern recognition. Some of these softwares are new and described here for the first time. The coccolithophores standing stocks were usually low and reached maxima west of the PCU. The coccoliths of *Emiliania huxleyi*, *Gephyrocapsa* spp. and *Crenalithus* spp. (Order Isochrysidales) represented more than 30% of all the suspended calcite particles detected in the size range 0.1–46 μm (22% of PIC in term of calcite weight). These species grew preferentially in the Chlorophyll maximum zone. In the SPG their maximum cell concentrations were recorded between depth of 150 and 200 m, which is unusually deep for these taxa. The weight of coccoliths and coccospheres were correlated to their size. Large and heavy coccoliths and coccospheres were found in regions with relatively high fertility in the Marquesas Island and in the PCU. Small and light coccoliths and coccospheres were found west of the PCU. This distribution is strongly related to ocean chemistry in particular to alkalinity and to carbonate ions concentration. The biotic (coccolithophores production) influence on calcification is mainly driven at the local scale (depth) whereas the abiotic (carbonate chemistry) plays its most important role at the regional (horizontal) level. Here 94% of the variability of coccolith and coccosphere weight can be explained by a change in 7 environmental variables.

1 Introduction

Coccolithophores represent an important group of unicellular algae. They are abundant both at high latitudes, where they form large blooms detectable by satellites (Brown and Yoder, 1994; Balch et al., 2007), and at low latitudes in oligotrophic (e.g. Okada and McIntyre, 1979; Winter et al., 1994) and upwelling (e.g. Winter et al., 1994; Giraudeau and Bailley, 1995) areas. They are responsible for about half of the total oceanic carbonate production (Milliman, 1993). Carbonate precipitation, settling (including ballasting aggregates containing organic matter), burial, and dissolution are key processes of the oceanic carbon cycle (e.g. Archer et al., 2000). Yet, despite their major role in the CO₂ cycle, many aspects of calcite production by coccolithophores are not well constrained. In particular the environmental impact on the secretion of coccoliths are poorly understood because direct field observations are scarce (Balch and Kilpatrick, 1996). Several laboratory and mesocosms experiments have shown a decrease in the production of calcium carbonate by coccolithophores when atmospheric CO₂ increases (e.g. Riebesell et al., 2000; Engel et al., 2005). This is because an increase of CO₂ in the atmosphere causes a decrease of oceanic water pH, with potentially dramatic consequences for oceanic calcifiers (Feely et al., 2004; Orr et al., 2005). It is therefore critical to determine how coccolithophores are calcifying in today’s Ocean.

The South Pacific Gyre (SPG) is the most oligotrophic and one of the least sampled ocean areas (Claustre and Maritorena, 2003), particular for coccolithophores. The primary objective of BIOSOPE (Biogeochemistry and Optics South Pacific Experiment) was to study the South Pacific Gyre along a transect through the central part of the SPG to the Peru-Chile Upwelling (PCU). The Isochrysidales represent, numerically, the most important order of coccolithophores. We document, along this transect, variations of



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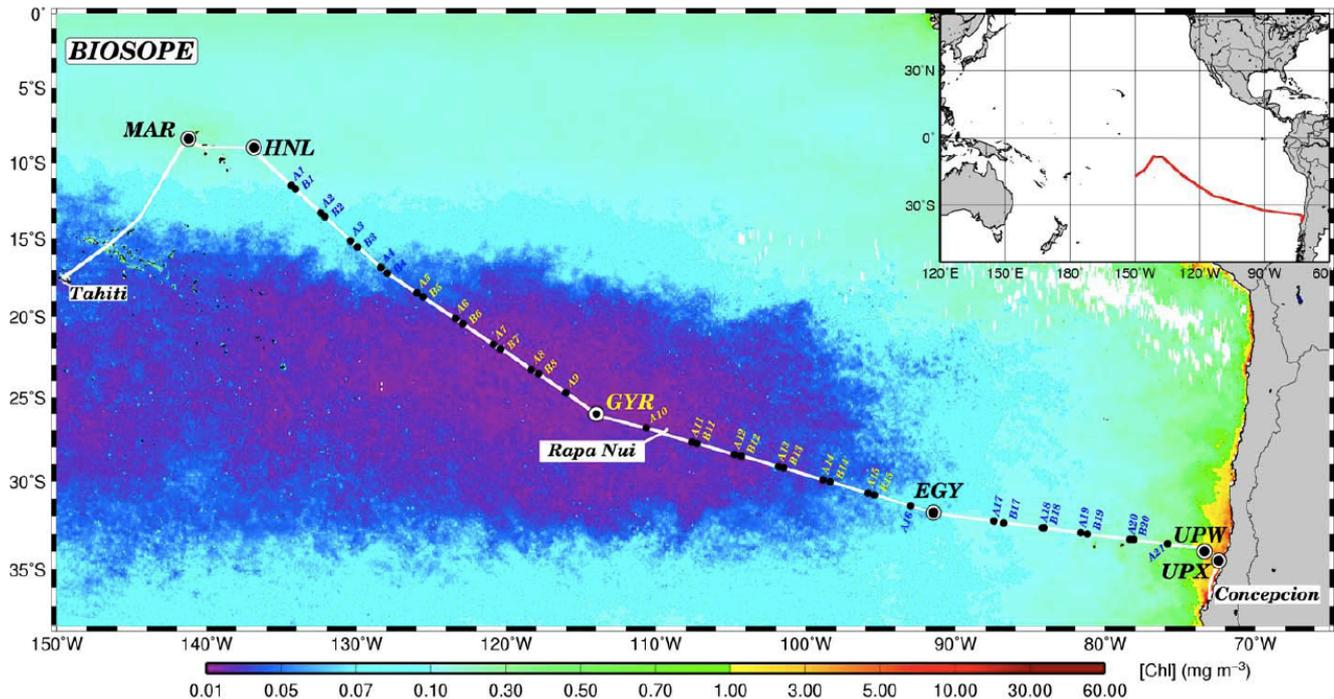


Fig. 1. Location map showing the transect and stations where water samples were taken at 6 depths, superimposed on the SeaWiFS composite (November–December 2004) for surface Chlorophyll concentration. Inset: geographic location of the transect in the Pacific Ocean.

Isochrysidales standing stock, as well as the absolute abundance of detached coccoliths and other small suspended calcite particles. The calcite stock produced by Isochrysidales was estimated using recently developed methods based both on microscopy automation and the birefringence properties of calcite. The size and weight of the coccospheres and coccoliths, was investigated in order to describe how Isochrysidales were calcifying in contrasting natural trophic environments, in particular in relation to calcite saturation state.

2 1-Material and methods

2.1 Setting

The BIOSOPE cruise (26 October to 11 December 2004) was conducted on board RV Atalante and covered a transect of about 8000 km in the South East Pacific. This enabled us to sample a full oceanic spectrum from the most oligotrophic oceanic waters in the south Pacific gyre to the fertile coastal waters of Chile (Fig. 1). Two features may explain why the broad South Pacific Gyre possesses the lowest surface chlorophyll concentration estimated through satellite imagery ($0.019 \text{ mg Chl-}a \text{ m}^{-3}$). It has the largest pycnocline depth recorded in the world ocean hydrological database ($>200 \text{ m}$), and the flux of atmospheric dust (e.g., iron) is extremely low (Claustre et al., 2008). In contrast, the PCU sys-

tem and the Marquesas archipelago (sub-equatorial area) are bathed by nutrient richer waters.

The sea surface temperature and salinity recorded during the cruise varied between 13° and 28°C and from 34 to 36.5, respectively, with higher values toward the west and lower values toward the east.

2.2 Sampling

Twenty stations were sampled for biogeochemical parameters (Claustre et al., 2008). At every station, samples for coccolithophores were taken depending on the position of the Deep Chlorophyll Maximum (DCM). At most stations, water samples were taken at 6 water depths: one sample at 5 m (i.e., just below the surface), two samples between 5 m and the DCM, one sample at the DCM, and two samples below the DCM. The DCM is located near the pycnocline which is considered as one of most important ecological boundaries in the ocean (Longhurst, 1998). The sampling strategy thus enabled us to study coccolithophores above and below this significant ecotone. In most cases, four liters of seawater were filtered on a nitrate cellulose membrane (47 mm diameter) having a pore size of $0.45 \mu\text{m}$. The membranes were quickly dried and stored at room temperature. Once in the laboratory, a quarter of each membrane was mounted between slide and cover slip and fixed with Canada balsam, which rendered the

membrane optically transparent. Additionally a small fragment of the filter was examined using a Hitachi 3000N Scanning Electron Microscope (SEM).

At the last four stations of the transect (i.e. in the PCU) the filter membrane diameter was 23 mm. Four liters of water were still filtered so the amount of particles on these filters was extremely high, leading to the possibility that significant amounts of coccoliths were concealed by large particles. Thus absolute abundances could not be reliably estimated for these stations.

2.3 Coccolithophore determination and species counts

The composition of the coccolithophore assemblages was determined using SEM at magnification of about 6000x and a Polarizing Microscope (PM) Zeiss Axioscop at a magnification of 1000x. Species abundances were estimated by counting more than 300 coccospheres in counted field of views (70 on average). The number of field of view was never lower than 10 even when coccolithophores were abundant on the PM.

2.4 Grabbing frames

A Polarizing Microscope (LEICA DMRBE) with a 50X oil immersion objective was used for automatic scanning of the microscope preparations in cross-polarized light. Microscope stage motions and focus were automated. For each sample, a 2 Megapixel Spot Insight camera grabbed forty fields of view. Each frame is $240 \times 180 \mu\text{m}^2$ with a pixel area of $0.0225 \mu\text{m}^2$. The amount of light going through the sample was held constant.

2.5 Analyzing calcite particles:

We developed a new software using routine LabView (National Instruments) that automatically detects and measures all birefringent particles from grabbed frames, hereafter called “*Particle Analyser*”. It takes advantage of the fact that some crystals are birefringent (they are illuminated in cross-polarized light) whereas other crystals and the preparation background remain dark. There is a relation between the thickness and the brightness of crystals, which can after calibration be expressed as a transfer function (Beaufort, 2005). The *Particle Analyser* opens all the frames in a sample, counts the number of objects brighter than background, and measures their surface. We placed a lower threshold at 3 pixels ($0.07 \mu\text{m}^2$) to get rid off background noise; and set an upper threshold at 74000 pixels ($1683 \mu\text{m}^2$ equivalent to circular particles having a $46 \mu\text{m}$ diameter; e.g., a foraminifera). This upper-threshold is high enough to include all particles in the size range of nanoplankton, including aggregates. The number (N) of particles ml^{-1} is calculated by

$$N = N_t \cdot S_m / (N_f \cdot S_f \cdot V_f) \quad (1)$$

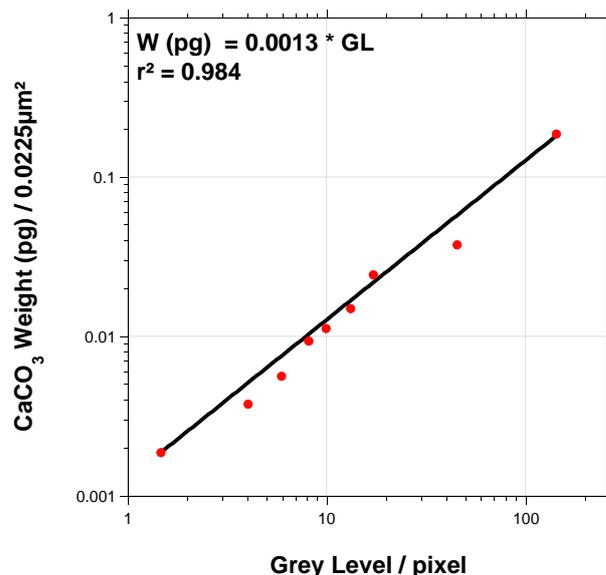


Fig. 2. Transfer function of Grey Levels into calcite weight. The x-axis represents the Grey Level value measure on average of one pixel. The y-axis gives the calcite weight (in pg) put onto the membrane per surface unit (here the area of one pixel). The line represents the best regression going through the origin.

where V_f is the volume filtered in milliliter, S_m is the surface area of the membrane, N_f is the number of the grabbed frames, S_f is the surface area of the grabbed frames, and N_t is the total number of particles analysed per sample.

The *Particle Analyser* automatically measures the “lightness” (L) of all frames as the sum of all grey levels (GL) pixel values. A transfer function was established following the protocol established in (Beaufort, 2005), but applied to samples prepared with cellulosic membranes instead of smear slides. In recalibrating we poured different (precisely weighted) amounts of pure calcite powder into known volumes of water. These suspensions were filtered on membranes of the same type as used for the BIOSOPE samples, and processed as described above. The relation between GL and weight on the membrane now serves as a transfer function (Fig. 2).

$$w = 0.0013 \cdot GL \quad (2)$$

where w is the weight in pg per pixel ($0.0225 \mu\text{m}^2$).

The calcite weight ml^{-1} (W) is calculated as following:

$$W = w \cdot N_p \cdot S_m / (N_f \cdot S_f \cdot V_f) \quad (3)$$

Where N_p is the number of pixel per frame ($=2 \cdot 10^6$). The values are given in pg ml^{-1} . Particulate Inorganic Carbon (PIC) is often given in $\text{mmol CaCO}_3 \text{m}^{-3}$. PIC values for the fraction $<46 \mu\text{m}$ ($\text{PIC}_{<46\mu}$) in this unit are obtained by dividing W by 10^5 .

Four different sources of error may be associated with this method:

1. The main source of error results from the patchiness of the distribution of particles on the membrane. Only a portion of the membrane is measured.
2. The error on the estimate of the light measure is extremely small and considered negligible.
3. High sediment loads on the membrane produce particle aggregation and is a potential source of error (underestimation of the weight) but for the open ocean waters investigated during BIOSOPE this problem did not occur.
4. Focus may be the cause of a small error on absolute values. Frames out of focus were excluded from the analysis.

The overall error associated with this method when dealing with carbonated sediments is estimated at ~12% (Beaufort, 2005), although no estimate regarding the measurements of PIC from water samples is available as yet.

2.6 Automated analysis of coccoliths and coccosphears: taxonomic recognition and size analysis

Coccoliths and coccosphears were automatically detected by SYRACO, a software developed in C++ at CEREGE (Dollfus and Beaufort, 1999; Beaufort and Dollfus, 2004). Based on Artificial Neural Networks (ANN) SYRACO is adapted to pattern recognition. In this study the ANN has been trained by the SYRACO learning algorithm, on a training set composed of two classes: (1) elliptical placoliths (essentially *Emiliania huxleyi*, *Gephyrocapsa oceanica* and other small placoliths in the genus *Gephyrocapsa* and *Crenalithus*; or *Reticulofenestra* for some authors), and (2) spherical coccosphears smaller than 10 μm in diameter bearing relatively birefringent, elliptical placoliths having a size of about 1/2 to 1/4 of the coccosphere. The great majority of taxa having these characteristics belongs to the Order of Isochrysidales. The training set is a monospecific sample from the Southern Indian Ocean with only coccosphears of *E. huxleyi*. However ANN has a large generalisation capability such that the coccosphere recognition used here is not species specific. Coccosphears from other orders (Syracosphaerales, Zygodiscales and Coccolithales) are generally not recognized by this ANN.

Because of the large generalisation capability of the ANN, a significant number of objects that resemble somewhat the targeted pattern are also included in the specific output frames. In the case of coccosphears, these foreign objects are “manually” erased from the frame. For the coccoliths, they are automatically eliminated from the analysis by a new software developed in LabView.

This software, hereafter called “*Coccolith Analyser*”, automatically measures coccoliths and coccosphears. It reads output frames where all the objects recognized as coccoliths are stored and analyses all of them. “*Coccolith Analyser*” is able to identify coccoliths based on 4 landmarks. If it determines that an object in the frame possesses these 4 landmarks, then it identifies it as a coccolith. If, on the contrary, it determines that an object does not possess these 4 landmarks, it discards the latter. With “*Coccolith Analyser*”, all objects incorrectly identified as coccoliths by SYRACO are eliminated. In the case of coccosphears, all the objects that were incorrectly identified as coccosphears by SYRACO were erased manually with “Adobe Photoshop” from the frames (we haven’t design yet a software capable to detect true coccosphere).

To check the reliability of SYRACO’s in estimating the abundance of coccosphears of Isochrysidales species in our samples, we compared SYRACO’s data with estimates based on direct counts of coccosphears using a polarizing microscope. We found that the two (automated and manual) estimates are highly correlated ($R=0.95$) (Fig. 3a). A slope of 2.2 could be interpreted as indicative that SYRACO misses about half of the coccosphears, but this is not the case. This slope reflects the fact that only few samples were taken at 4 stations (STB15 to STB18) located between 90 and 100° W an area where *E. huxleyi* coccosphears are extremely abundant and often contiguous. SYRACO is not capable of deciphering coccosphears in such groups. If the results from these 4 stations are excluded, the slope of the regression line is 0.98, indicating that the number of coccosphears of Isochrysidales species recognized by SYRACO is the same as recognized by direct human counts. Incidentally, this excellent fit confirms that only Isochrysidales are recognized by SYRACO (the slope is 1.75 when the entire (including not only the Isochrysidales but all the taxa) coccolithophore assemblage counted by human is used for comparison with SYRACO counts) (Fig. 3b). We estimated the abundance of coccoliths by directly counting the number of coccoliths contained in more than 10 fields of view ($=0.01 \text{ mm}^2$) in a total of 36 samples. Our estimates were then compared with those obtained by SYRACO. The estimates of coccolith abundance are significantly correlated for all stations (except STB15 to STB18), with a slope close to 1 and a $R>0.9$. For stations STB15 to STB18 the abundance of coccoliths was often $>800 \text{ ml}^{-1}$; aggregation of coccoliths prevents accurate counts of coccolith number in a frame by SYRACO. The abundance estimates reported in this paper are those determined by SYRACO for all samples except for those at Stations STB15 to STB18 for which manual counts were established. Our preference for SYRACO counts is that they are more representative of real values, counts being produced over a much larger area than is possible by humans. SYRACO counts are also more reproducible than human’s counts. It is important to note, however, that the patterns observed in BIOSOPE are the same whether established solely

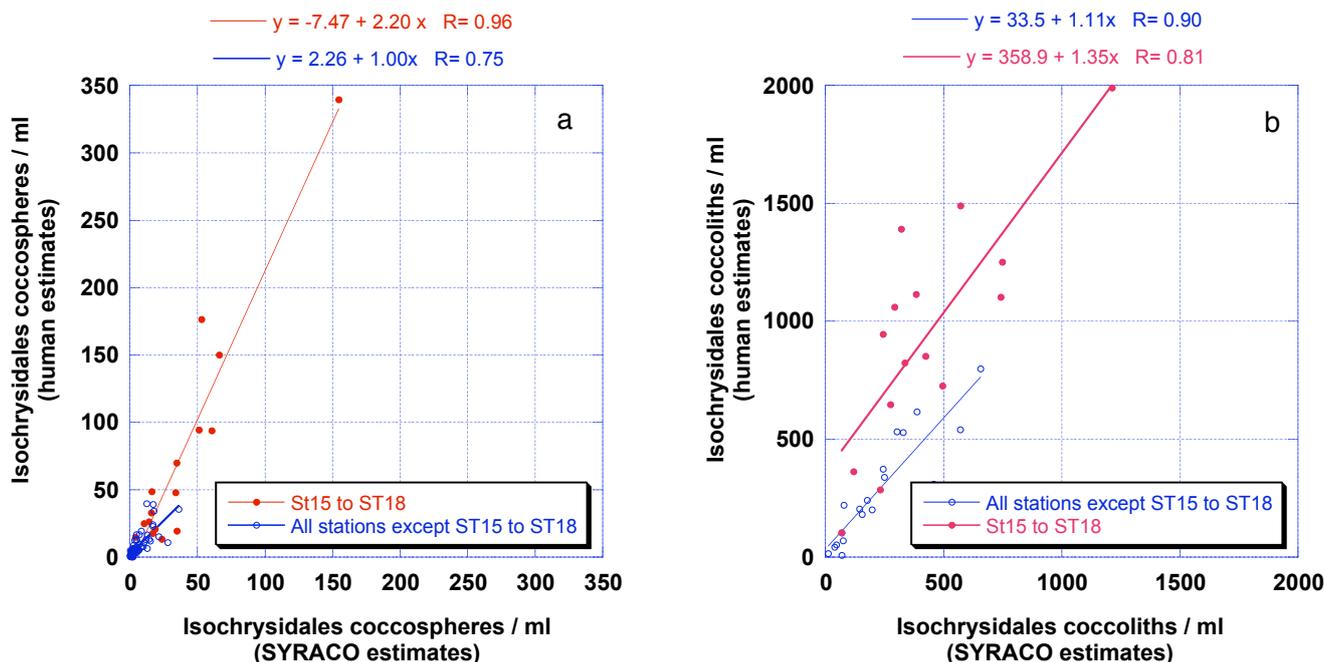


Fig. 3. Relation between “human” and “automated” (SYRACO) counts of Isochrysidales coccospheres (A) and coccoliths (B). In red are sample for 4 stations (STB15 to 18) where the density of Isochrysidales was too high (aggregation) for accurate automatic estimates. In blue are reported the other BIOSOPE stations.

from manual or automatic processes.

The *Coccolith Analyser* measures the grey level of objects, their diameter and surface, and tabulates the results. There is a bias of $0.6 \mu\text{m}$ in the measurement of the diameter of small, and dim objects, such as coccoliths. This is because we apply a minimum Grey Level threshold above background. This threshold erodes 2 pixels of the periphery of dim objects. Each pixel is $0.15 \mu\text{m}$, and 4 pixels are eroded in total when the long diameter is measured. Thus we added $0.6 \mu\text{m}$ to the measurement of coccolith length to compensate for this automatic loss. Another small bias in the measurements of the length of coccoliths of *E. huxleyi* exists, the distal shield being not detected in its entirety in cross-polarized light. Therefore the length of *E. huxleyi* presented here are slightly underestimated. We estimated this bias to a factor of 1.25 by comparing measures from the Coccolith analyzer and from SEM. The data presented here are uncorrected by this factor.

It should be noted that in the theoretical case of a pure *E. huxleyi* sample, the size distribution estimated by SYRACO would be narrower than that estimated with the *Calcite Analyser*. This is because SYRACO detects only well preserved, well-oriented and isolated coccoliths whereas the *Calcite Analyser* measures all particles, including aggregated, broken, out of focus and tilted coccoliths.

2.7 Chemical and physical parameters

The physical and chemical parameters used for comparison here were measured in situ during the cruise and are described in different papers in this volume. Temperature, salinity, fluorescence (Claustre et al., 2008) and backscattering (Twardowski et al., 2007) were measured precisely at location where water was collected for coccolithophores. Carbon chemistry was measured (Azouzi et al., 2007) at nearby locations but not exactly at the same water depth. We have selected the measurements available from the closest depths.

3 Results

3.1 Importance and composition of the Isochrysidales

Emiliania huxleyi and several species of the genera *Gephyrocapsa* and *Crenalithus* represent all the calcifying taxa of the marine Order Isochrysidales (de Vargas et al., 2007). SYRACO has been trained to recognize the Isochrysidales complex, which is therefore the focus of this paper. The specific composition of Isochrysidales varied significantly among BIOSOPE samples. Their variations have been studied in detail with both SEM and PM. East of Easter Island (about 110°W) the Isochrysidales dominated the coccolithophore community with relative abundance ranging from 60 to 100%. West of Easter Island the coccolithophore concentration diminished with the Isochrysidales representing

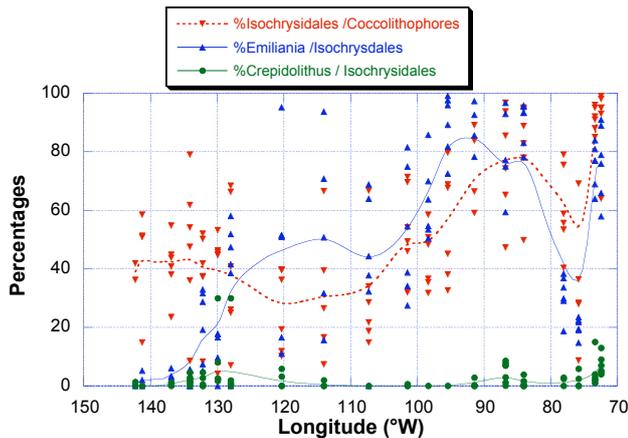


Fig. 4. Variations in the relative abundance of several coccolithophores taxa along the BIOSOPE transect. The data result from manual counts of coccospheres on the light microscope. The percentages of Isochrysidales in the coccolithophores are in red, the percentages of *E. huxleyi* in the Isochrysidales are in blue and the *Crepidolithus* (*Reticulofenestra* for some authors) are in green. The dots represent samples and the smooth lines show the stations means of these percentages.

40% on average of the coccolithophore community (Fig. 4). *Gephyrocapsa oceanica* dominated the Marquesas area. Between 130° W and 100° W the relative abundances of *Gephyrocapsa* and *Emiliania* were variable with a low number of *Emiliania*. From 100° W to the PCU, *Emiliania* dominated the communities. In some samples it constituted almost 100% of the coccolithophores. *Gephyrocapsa ericsonii* and *Crenalithus* always formed a minor part of the coccolithophore assemblages.

Because the version of SYRACO used in this study was trained to recognize only the coccoliths of the Order Isochrysidales, we relied on manual estimates for: 1) the percentages of the Isochrysidales coccospheres in the coccolithophores assemblages, and 2) the percentage of the various species in the Isochrysidales assemblages. To estimate the number of detached coccoliths of the different taxa, we combined the number of Isochrysidales coccoliths with our estimate of the coccolithophores taxonomic composition produced manually. Our first order assumption is that the number of detached coccoliths per coccosphere is the same in every species (which is not necessarily the case).

3.2 Spatial distribution of calcite particles

The concentrations of suspended calcite particles, and of detached coccoliths and coccospheres of the Isochrysidales Order showed very similar distribution patterns along the BIOSOPE transect (Fig. 5): maximum concentrations were found between 80° and 100° W, associated with the subtropical front (Claustre et al., 2008)

The concentration in coccospheres of Isochrysidales was generally low with an average of 11 ml⁻¹ but it reached a maximum of 350 ml⁻¹ in the eastern part of the transect, while the concentration of detached coccoliths ranged from 11 to ~2000 ml⁻¹ with an average of 250. The concentration in suspended calcite particles was on average 733 ml⁻¹, and the total weight of calcite per milliliter was 11 200 pg ml⁻¹ (or PIC_{<46μ}=0.11 mmol CaCO₃ m⁻³). The corrected total weight of the Isochrysidales detached coccoliths and coccospheres was on average 2224 pg ml⁻¹ (or 0.022 mmol CaCO₃ m⁻³), which represents 22% of the PIC_{<46μ}. Large aggregates that may be rich in coccoliths composed a large part of remaining 78%.

The spatial distributions of coccospheres, detached coccoliths, and suspended calcite particles showed higher concentrations between 85° W and 100° W in the upper 80 m of the water column.

The observed pattern of density distribution of calcite particles is confirmed by the study of backscattering properties (Twardowski et al., 2007). The backscattering ratio (i.e. the ratio of backscattering to scattering) depends on the size distribution of particle assemblages (high when dominated by small particles and reciprocally) and on refractive index (high for particles with high refractive index, like calcite). This ratio approximately scales with the number of suspended calcite particles and the PIC estimated by the “*Calcite Analyser*” more specifically, it exhibits the two prominent scatterers of coccoliths at the exact same position than shown in the present analysis, and confirms the relative “patchy” distribution of these biogenic particles.

3.3 Grain size distribution of suspended calcite particles, detached coccoliths and coccospheres

Ninety five percent of the 416 000 suspended calcite particles analyzed from the BIOSOPE samples had a surface area <20 μm² or a diameter <5 μm (in the 0.1–46 μm range). Their size distribution was unimodal and slightly skewed toward larger particles, with a mode at 3.2 μm² (Fig. 6a). The distributions of detached coccoliths and coccospheres was also unimodals with modes at 3.2 μm² and 40 μm², respectively (Fig. 6a). Interestingly, the mode of the suspended calcite particles was the same as that of the detached coccoliths of Isochrysidales species. The number of detached coccoliths (mostly *E. huxleyi*, a few *Gephyrocapsa* and rare *Crenalithus*) represented 1/3 of all suspended calcite particles. At locations where coccoliths and coccospheres were extremely abundant, for instance at 30 m at Station 18 which corresponded to an almost monospecific bloom of *E. huxleyi*, the distribution of calcite particles and of coccoliths/coccospheres were very similar (Fig. 6b).

The concentrations of coccoliths are significantly correlated with the concentrations of calcite particles detected by the *Calcite Analyser* (Fig. 7). The Isochrysidales taxa, and in particular *E. huxleyi*, are the most important contributor

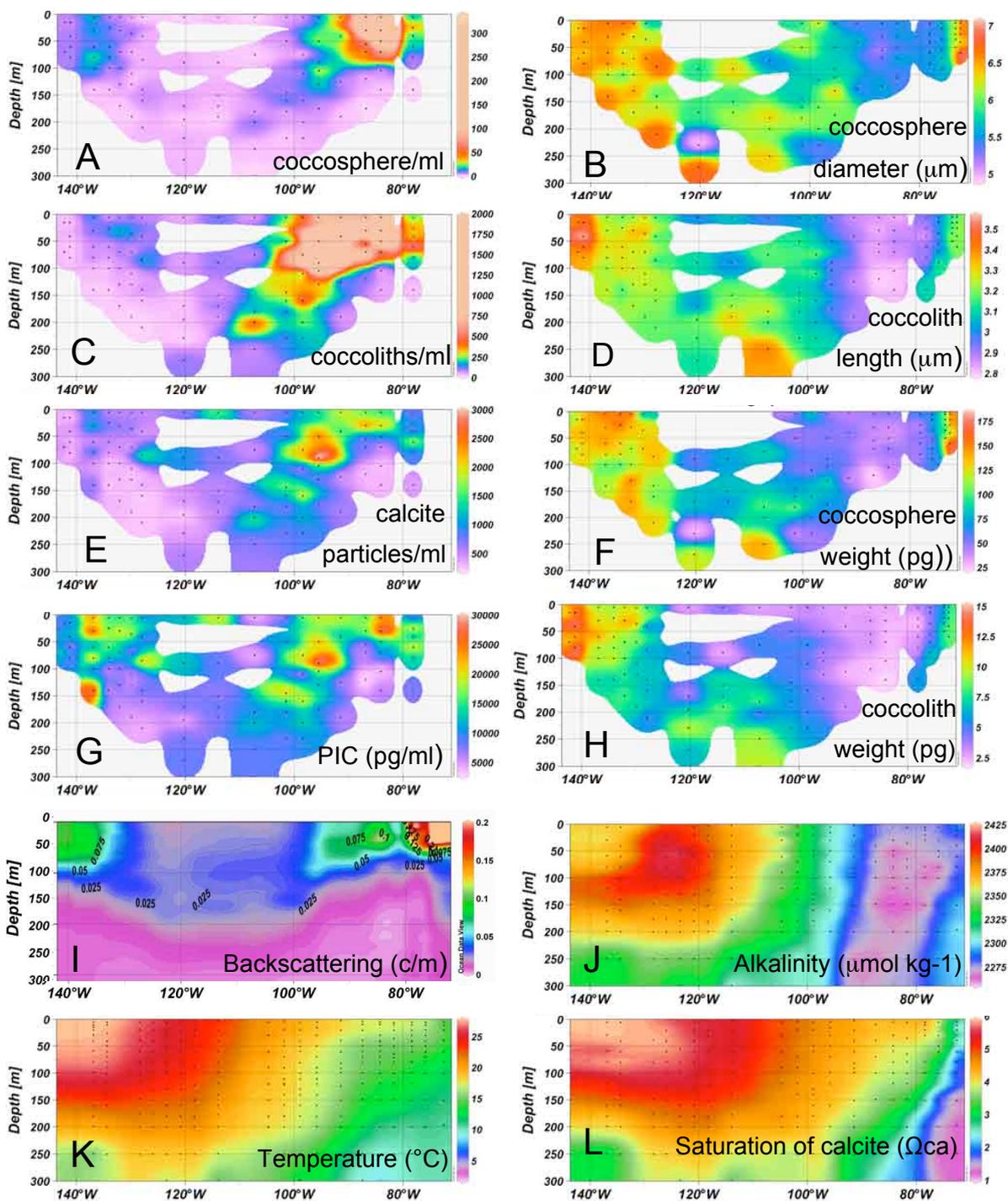


Fig. 5. Section along the BIOSOPE transect of (A) concentration in *Isochrysidales* coccospheres; (B) length of detached *Isochrysidales* coccoliths (μm); (C) concentration of detached *Isochrysidales* coccoliths; (D) Average diameter of *Isochrysidales* coccospheres (μm); (E); concentration in suspended calcite particles; (F) Average weight of detached *Isochrysidales* coccoliths (pg); (G) Total concentration of suspended calcite particles (PIC); (H) coccolith weight; (I) Attenuation coefficient (processed as described in Claustre et al., 2008) (m^{-1}); (J) Alkalinity ($\mu\text{mol kg}^{-1}$); (K); In situ temperature ($^{\circ}\text{C}$); (L) Saturation of Calcite (ΩCa).

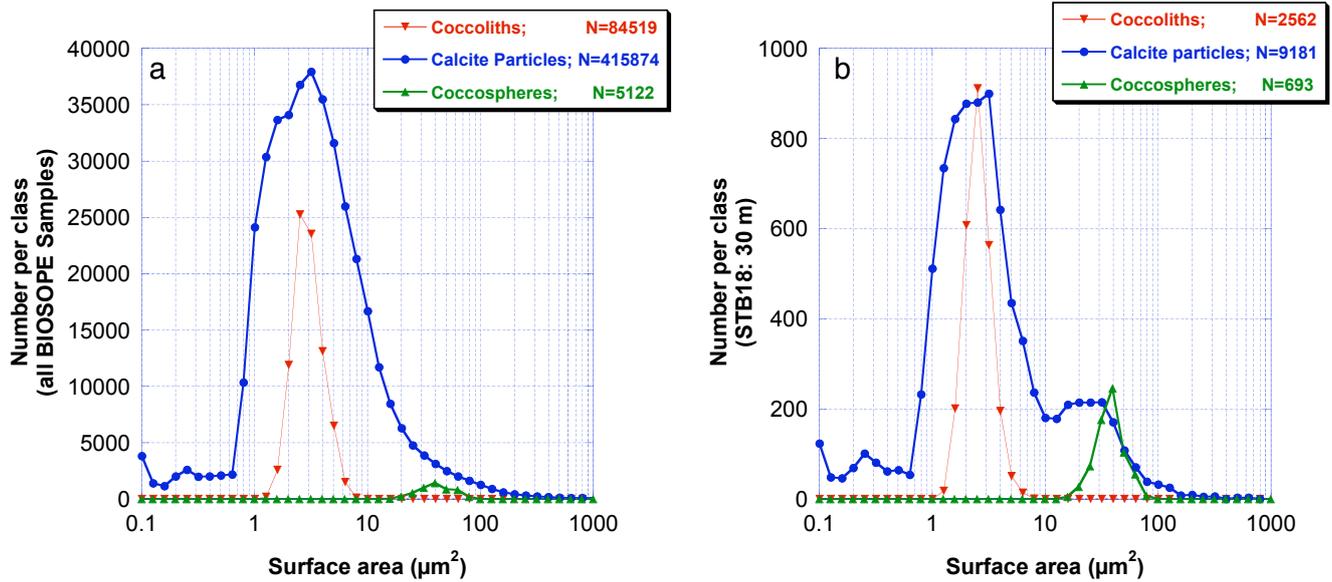


Fig. 6. Size Distribution of area of coccoliths (red), coccospheres (green) and calcite particles (blue) in all samples (A) and in sample taken at 30 m at Station 18 (B).

to this correlation. The non-*E. huxleyi* Isochrysidales and the non-Isochrysidales coccolithophores were less correlated with the carbonate particle density (Fig. 7). *Emiliania huxleyi* dominated the assemblages where the coccolith density and calcite particles were the highest, demonstrating that *E. huxleyi* were a major source of fine suspended calcite particles in the open ocean.

3.4 Size and weight distribution and number of coccoliths per coccosphere

The diameter and weight of the coccoliths and coccospheres show the same spatial distribution (Fig. 5). In general, both had higher values in the western part of the transect, decreased progressively toward the east until they reached their minima around 90° W, and slightly rose in the PCU (Fig. 8). There were significant correlations (Fig. 9) between the average length of coccoliths and the average diameter of coccospheres ($r=0.85$). The same was true for their weights ($r=0.88$). There were also significant correlations between the average of the weight and length of the coccoliths ($r=0.95$) and diameter of the coccosphere ($r=0.94$).

Assuming that the detached coccoliths have the same morphological characteristics as the attached coccoliths on the coccosphere, then the number of coccoliths per coccosphere was obtained by dividing the average weight of coccospheres by the average weight of the coccoliths. Doing so, we found an average of 15 coccoliths per coccospheres with standard deviation of 5. No clear pattern was found in the spatial distribution of that number.

3.5 Depth profiles

Morphometric and abundance data showed depth profiles which are similar to depth profiles of chlorophyll concentration. The maxima of abundance, weight, for both coccospheres and coccoliths were generally found associated with the chlorophyll maximum (Fig. 10). In consequence, the concentrations in coccolithophores and coccoliths, their weight and their size, were highest at shallow depth in the upwelling area, and deep in oligotrophic area. For example in the centre of the gyre, Isochrysidales taxa were most abundant between 150 and 200 m.

4 Discussion

4.1 Abundance distribution

The coccosphere stocks estimated in the South East Pacific were low, with a median value of 4000 cell l^{-1} . The lowest values were found at the centre of the South Pacific Gyre (GYR station). However in the centre of the gyre and at all stations, coccolithophores were continuously present down to the deepest sample (200–300 m) where sufficient light is remaining for the growth of some species (Claustre et al., 2008). The average Isochrysidales stock at Station GYR was 1250 cell per liter. This is equivalent to 375×10^6 cells m^{-2} in a 300 m deep water column; this value represents only the stock of marine Isochrysidales which represent a small coccolithophore fraction (1/3). These data imply that coccolithophore are in abundance even in the extreme oligotrophic environments that we investigated. The

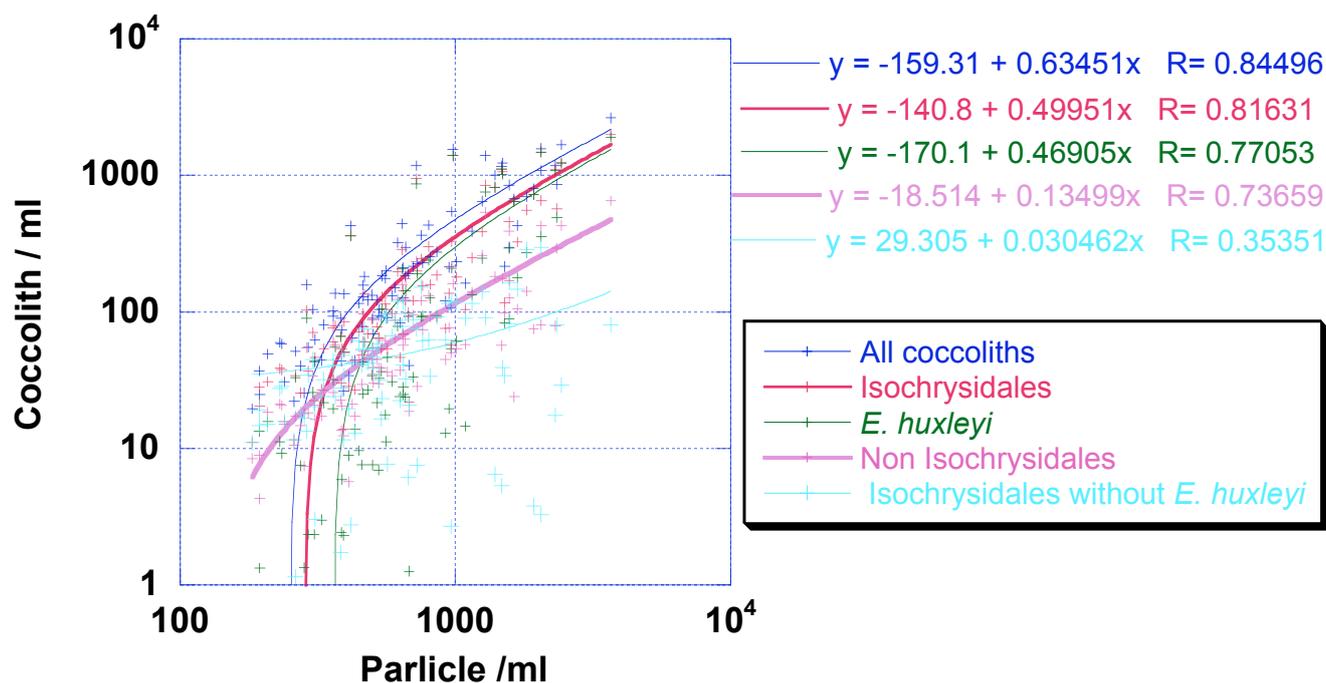


Fig. 7. Correlation between concentrations of suspended calcite particles and detached coccoliths of all coccolithophores taxa (deep blue), all Isochrysidales species (red), *E. huxleyi* (green), non-Isochrysidales coccolithophores taxa (purple), non-*E. huxleyi* Isochrysidales species (light blue). These numbers have been obtained by combining the concentration of Isochrysidales coccolith data (Fig. 5c) with the percentage of the different taxa estimated from coccosphere count shown in Fig. 4.

coccolithophore stocks estimated in this study are in the same range as previously reported for the more productive tropical Pacific, 1–240 cell ml⁻¹ (Hagino and Okada, 2006), 0–60 cell ml⁻¹ (Balch and Kilpatrick, 1996), 1–100 cell ml⁻¹ (Okada and Honjo, 1973; Ohkouchi et al., 1999), and 0–60 cell ml⁻¹ (Giraudeau and Beaufort, 2007). The highest cell density of *E. huxleyi* (240 cell ml⁻¹) in the South Equatorial Pacific was reported in the Peru Upwelling (~85° W–2° S) (Hagino and Okada, 2006). This is equivalent to what was found in BIOSOPE, where up to 350 cell ml⁻¹ were observed west of the PCU. The sample taken in the PCU on smaller membrane does not permit us to estimate the abundance of coccolithophores. One would nevertheless expect even higher abundance in this area because higher numbers of coccospheres of *E. huxleyi* are generally reported in the centre rather than outside of upwelling systems (Giraudeau and Bailley, 1995) where their abundance can reach several thousand of cell ml⁻¹ (Mitchell-Innes and Winter, 1987).

4.2 Deep production of marine Isochrysidales

Coccolithophores are growing at the boundary of their greatest depth habitat in the South Pacific Gyre. For example at Station STB11, *Florisphaera profunda* was found between

200 and 300 m. In the gyre, the maximum abundance of Isochrysidales occurred at about 120 m, i.e., deeper than usually found for coccoliths in oligotrophic area (e.g. Okada and Honjo, 1973; Okada and McIntyre, 1979). The fact that coccolithophores calcify at depth greater than 100 m represents a significant change from the proposed high-light niche of *E. huxleyi* (e.g. Nanninga and Tyrrell, 1996) and the observed restriction of calcification to shallower waters than silicification (Poulton et al., 2006). A possibility is that these coccospheres were not living cells but the sinking remains of coccolithophores that grew at shallower depths. Several lines of evidence argue against this: 1) the maximum abundances of coccospheres coincide with the Deep Chlorophyll Maxima (DCM) 2) the production in the upper photic zone is too low to fuel the coccosphere highest number at the DCM which is 3 times larger than at the surface. This is particularly true for *Florisphaera profunda*, which is found only below 200 m. 3) the community vertical structure is typical of oligotrophic area, 4) it is interesting to note that the DCM is not only the place of maximum abundance of Isochrysidales, but also an area in which they secrete heavier coccoliths and have larger cells. Why should a morphological change occur in dead assemblages during settling?

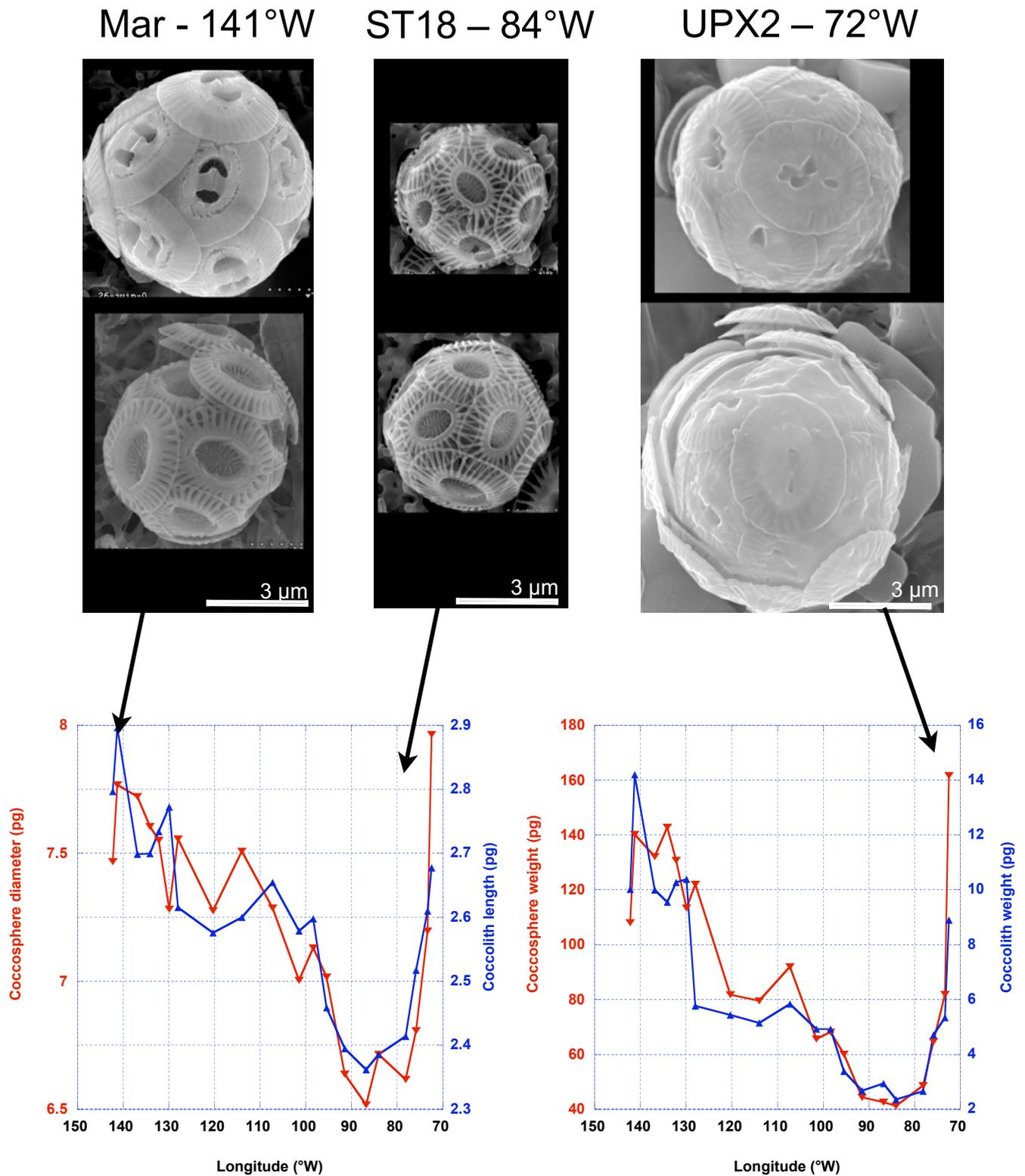


Fig. 8. Bottom left: Variability of *Isochrusidales* coccosphere diameter (red) and coccolith length (blue) averaged for every BIOSOPE station (average weighted by the concentration at each depth). Bottom right: variability of coccosphere (red) and coccolith (blue) weight averaged for each BIOSOPE station (average weighted by the concentration at each depth). Top: 6 SEM pictures (scale bars represents 3 μm: same scale in every picture) of typical *Isochrusidales* in 3 BIOSOPE stations.

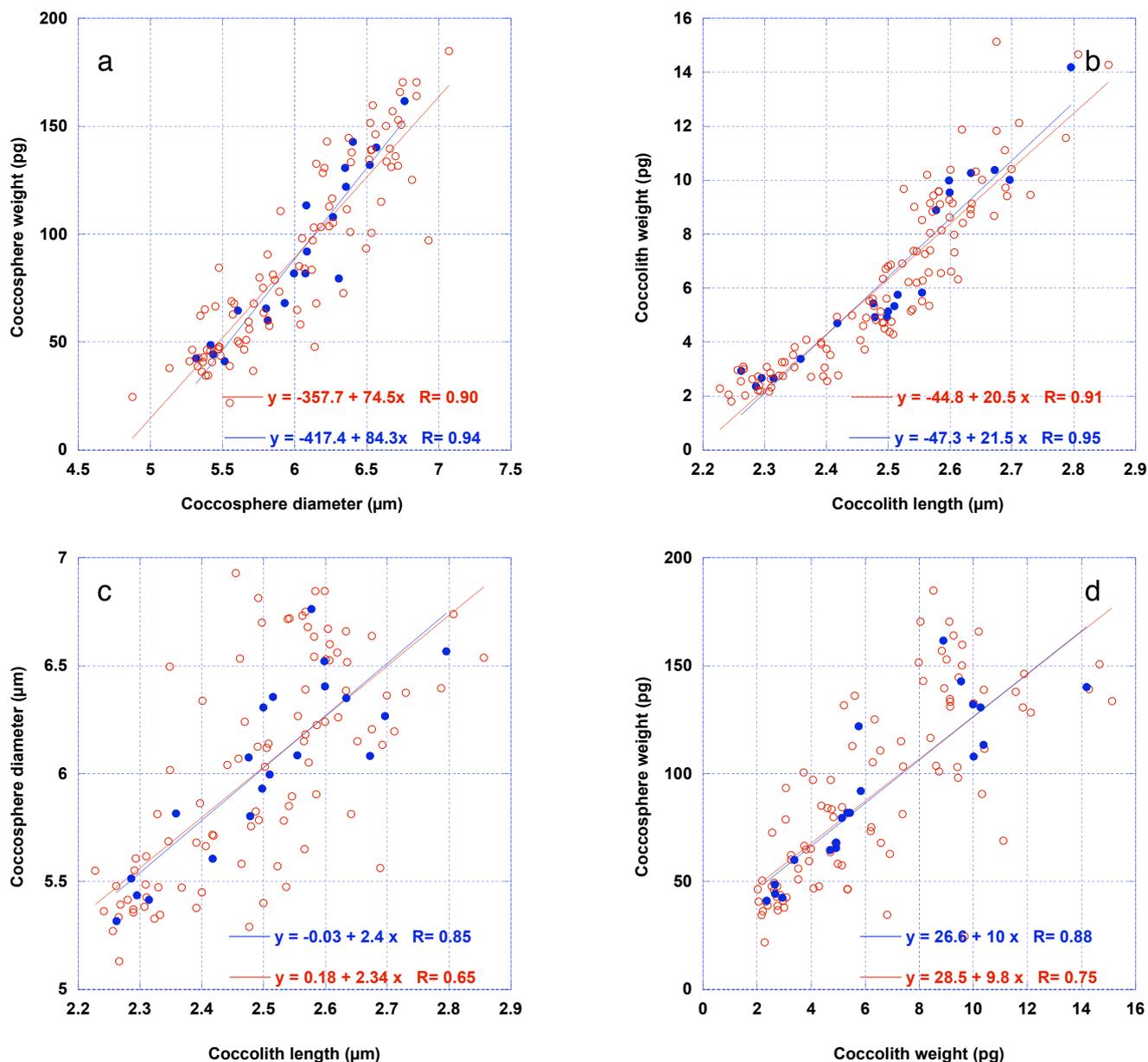


Fig. 9. Correlation between coccosphere diameter and weight (**A**), coccolith length and weight (**B**), coccolith length and coccosphere diameter (**C**) weight of coccolith and coccosphere (**D**) of *Isochrysidales* observed in BIOSOPE. Open red circles represent single BIOSOPE sample and the filled blue circles represent station average.

In conclusion, the system investigated can be considered as an endmember of oligotrophic systems with the deepest chlorophyll maximum and the clearest waters ever reported (Morel et al., 2007). The coccolithophores assemblage is typically adapted to these conditions with maximum cell density in general closely associated with the DCM. Furthermore it is very clear from pigment signature that below the chlorophyll maximum and up to depths of 250 and above, the dominant (sometimes the only) carotenoids is 19'-hexnoyloxyfucoxanthin, the marker of prymnesiophyceae

(Ras et al., 2008, in their Fig. 11). This observation must be integrated with the layer of high backscattering ratio (the calcite marker) that is recorded at ~ 240 m (Twardowski et al., 2007) at the GYR station.

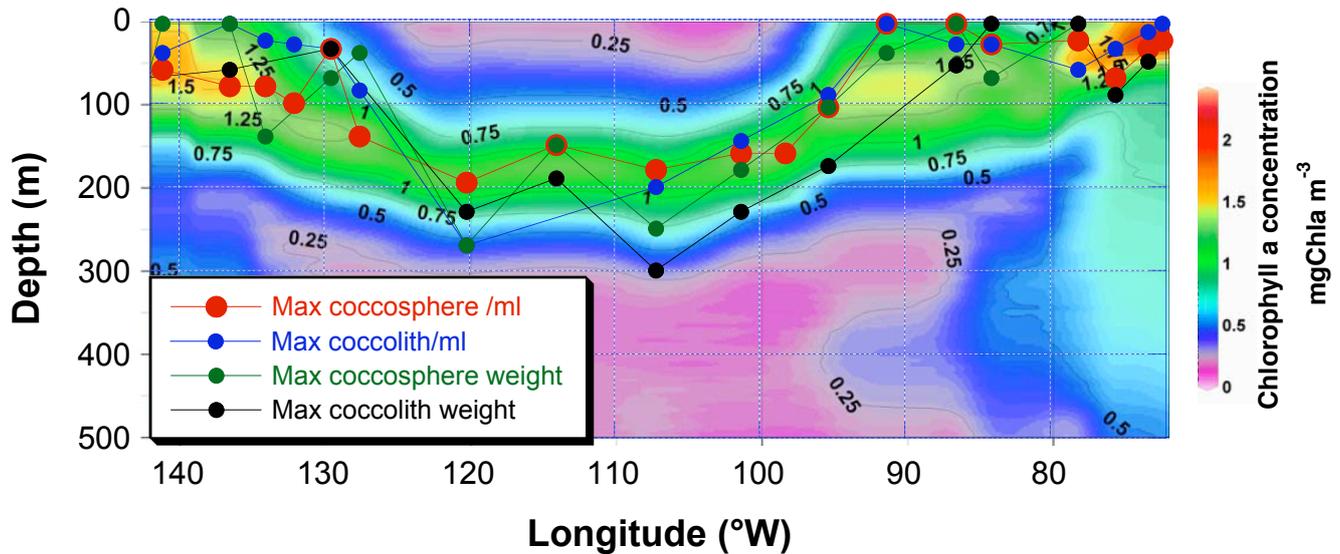


Fig. 10. Color section of the chlorophyll a concentration mg m^{-3} along the BIOSOPE transect with the position in depth where the maximum value at a given Station is observed for the Isochrysidales coccosphere density (red), detached coccolith density (blue), weight of coccosphere (green), weight of coccoliths (black).

4.3 Implication of deep production for alkenone paleothermometry

When the temperature difference between the water surface and the depth of maximum abundance of the Isochrysidales, is calculated, it appears that for 1/3 of the stations, the difference is greater than 2°C (Fig. 11). The Isochrysidales are the producers of alkenones used in paleoceanography as sea surface temperature (SST) proxy. Ohkouchi et al. (1999) described some discrepancies between SST estimates from North Pacific surface sediments and the observed SST at the same locations attributable to the fact that alkenones were produced in the DCM. Also, Conte et al. (2006) found some differences between the alkenone calibration curve based on surface sediments (Muller et al., 1998) and their calibration based on mixed-layer water measurements. But these differences were essentially recorded at high latitudes in absence of a DCM. Our results would indicate that it is may be excessive to infer SST from an alkenone record core taken below the South Pacific Gyre because alkenone would have been produced far below the surface (there are no suitable sediments to establish such a record in the Central Southern Pacific; Rea et al., 2006). But it has been shown that alkenones are produced exclusively in the mixed layer depth, and above the DCM in ALOHA Station in the oligotrophic North Pacific Gyre (Prahl et al., 2005). Either Station ALOHA is different from the South Pacific gyre (SPG) where *E. huxleyi* was abundant above DCM, or the secretion of alkenones by *E. huxleyi* is light dependent. In that case the deep production of Isochrysidales observed in SPG would not temper the SST reconstruction based on alkenones.

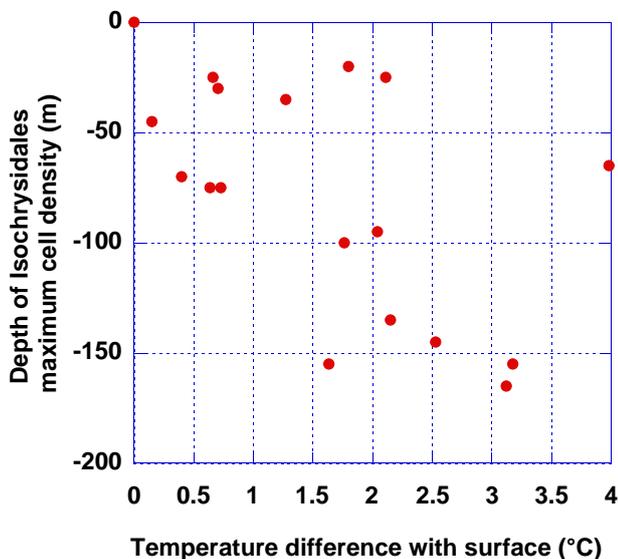


Fig. 11. Depth of Isochrysidales maximum density as a function of temperature difference between the depth of this maximum and surface.

4.4 *Emiliana huxleyi*: important calcite producer

The BIOSOPE PIC values are in the same range ($0.05\text{--}0.35\text{ mmol m}^{-3}$) as previously reported for the Equatorial Pacific (Balch and Kilpatrick, 1996) if we exclude one value from the latter study of 1.33 mmol m^{-3} in the open ocean upwelling. One important finding of the present study is a strong relation between the numbers of coccoliths of *E. huxleyi* and the number of suspended calcite particles (hence PIC) (Fig. 7). The “cloud” of highest abundance of coccolithophores (Fig. 5a,c) was dominated by *E. huxleyi* (Fig. 4); it also was the place of highest PIC and of significant enhancement of backscattering (Fig. 5g, i). *Emiliana* has been sometimes considered as one of the most important calcite producers (e.g. Westbroek et al., 1993); but it has been also suggested that it represents only an insignificant part of the oceanic calcite production (Paasche, 2002; Ziveri et al., 2007), because this species secreted one of the lightest coccoliths (Beaufort and Heussner, 1999; Young and Ziveri, 2000). The BIOSOPE transect covers the diversity of trophic conditions potentially observable in open ocean waters, we show that a large part of the fine calcite particles is attributable to Isochrysidales coccoliths. Calcification in the Tropical Pacific is very high, (similar the rate of photosynthesis) and the turnover times of calcite in the euphotic zone ranges from 3 to 10 days (Balch and Kilpatrick, 1996). These high turnover rates of calcite induce a high ballasting of organic matter by carbonate particles and a decrease of PIC possibly associated with a depletion of Ca^{++} ion in the euphotic zone (Balch et al., 2007). Because of the high abundance of detached coccoliths and of coccospheres, the ballasting due to *E. huxleyi* coccoliths must have been particularly efficient around $90^\circ\text{W}\text{--}30^\circ\text{S}$. Ballasting could also have an effect on the alkalinity of the surface water. It is interesting to note that the area of highest standing stock of coccoliths present also has particularly low alkalinity. An inverse relation exists between alkalinity and PIC (Fig. 12), which is indicative of the effect of calcification on ocean surface alkalinity.

It is also interesting to note that the highest coccolith density was found in a scatter at depth greater than 30 m, the depth limit of detection of coccoliths by satellite. Hitherto coccolith blooms detected by satellite are always in regions of shallow organic production (high latitudes, continental shelves, and upwelling zones) (Brown and Yoder, 1994; Balch et al., 2007), therefore, the calcite density of this scatter will be largely underestimated, if ever detected, by satellite imagery.

4.5 Weight and size relation between coccolith and coccosphere

An interesting aspect of this study were the fact that there is a close relationship ($r=0.65$) between the diameters of the Isochrysidales coccoliths and coccospheres (Fig. 8). A factor

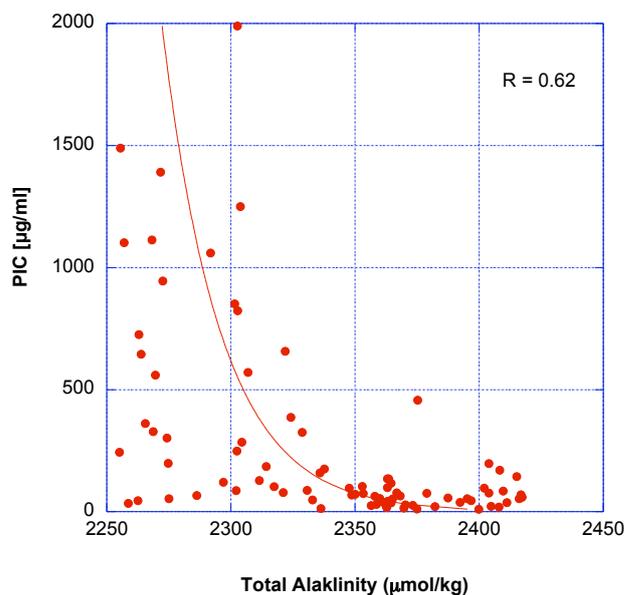


Fig. 12. Relation between the total weight of calcite particles and the total alkalinity.

of about ~ 2.4 (or ~ 1.9 taking into account the measurement bias for the coccolith length) can be used to estimate the diameter of a coccosphere from the length of a coccolith. Also the number of coccoliths per coccosphere is 15 on average without variations throughout the BIOSOPE transect. If the same relations were found elsewhere, these values would be useful in paleoceanographic studies for estimating the number and size of the cells of marine Isochrysidales from the number and length of their coccoliths.

4.6 Calcification, cell diameter and carbonate chemistry

The most calcified Isochrysidales are found in the high fertility zones of the Marquesas area and Peru-Chile Upwelling (PCU) (Figs. 5 and 8). Also we have shown that the maximal weight of both coccospheres and coccoliths are generally located in the DCM, which is close vicinity of the nitracline (Raimbault et al., 2008). These two observations may indicate that coccolithophores secrete more calcified coccoliths in higher fertility environments. This is in agreement with recent culturing and mesocosms experiments showing that *E. huxleyi* is more calcified in waters rich in *P* and *N* in batch cultures (Beaufort et al., 2007) or after addition of nutrients in mesocosms (Engel et al., 2005). However, these results contrast with those discussed in Paasche (2002). In BIOSOPE, the weights of the coccospheres and the coccoliths were weakly correlated with chlorophyll content and with stock of coccolith and coccosphere. Therefore other environmental factors than fertility or productivity might influence the calcification of coccolithophores.

The lightest coccoliths and coccospheres were recorded between 80 and 95° W, which is also exactly the place of the lowest alkalinity values in the upper 300 m of the BIOSOPE transect (Fig. 12). The concentration in carbonate ion was also particularly low in that area. These low values could be responsible, in part, for the low calcification of Isochrysidales cells. But this does not mean their growth was limited in such conditions because it is the place of highest concentrations of Isochrysidales coccoliths. In contrast, significant precipitation of calcite by *E. huxleyi*, revealed by highest PIC and coccolith concentrations, may have the effect of decreasing the dissolved carbonate concentration and the alkalinity of this area. Similarly, it has been recently suggested (Balch et al., 2007) that high PIC turnover such as recorded in the tropical Pacific, induces a depletion of calcium ion in the photic zone as a response of losses of PIC ballasted particles. We found a significant ($R=0.62$) reverse logarithmic correlation between the concentration of coccoliths and alkalinity. Significant linear positive correlation exists between alkalinity and the weight of coccolith and coccospheres ($R=0.84$ and $R=0.85$ respectively on the station averages or $R=0.59$ and $R=0.74$ respectively on all samples) (Fig. 13c and i). This highlights the likely fundamental importance of alkalinity on calcification. Except in the 3 upwelling stations, carbonate concentration and calcite saturation state appears to also share a strong influence on the coccolith and coccosphere weights (Fig. 13a, b, g and h). The partial pressure of CO₂ as well as pH does not appear to have significant impact on the degree of calcification of coccospheres and coccoliths ($R<0.03$) (Fig. 13d, e, j and k). This could be seen as contradicting with previous findings from culture and mesocosms experiments that predict a decrease of calcite production with increasing concentration of CO₂ (Riebesell et al., 2000; Engel et al., 2005). Recent culture experiments show that an increase of CO₂ may have a positive effect on calcification of *E. huxleyi* (Iglesias-Rodriguez et al., 2008) and other species (Langer et al., 2006). These experiments involve not only changes in CO₂ and pH, but also carbonate chemistry in very different ways depending on how the experiment was conducted. Our findings stress the importance of the entire carbonate system in the calcification of Isochrysidales.

The strong dependence of the concentration of carbonate ion on the coccolithophore calcification, has been recently illustrated by comparing Baltic and Black seas: the absence of coccoliths of *E. huxleyi* in the Baltic Sea result from low carbonate saturation states particularly during the winter (Tyrrell et al., 2008). In a comparison of numerical simulation and observed data from seasonal blooms in the Bering Sea, it has been shown that *E. huxleyi* production benefits greatly from an increase in the concentration of carbonate ion in the surface water resulting from the increase in phytoplankton production (Merico et al., 2006). These authors hypothesized that in a zone of seasonal blooms, *E. huxleyi* would calcify more after a spring bloom in response to the increase in carbonate ion concentration. This hypothesis

may explain why the heaviest coccospheres were observed in the eutrophic and mesotrophic areas of the BIOSOPE experiment. The least calcified Isochrysidales were found at the subtropical front in the highest coccosphere abundance zone of the BIOSOPE experiment. Because it is not a highly productive area, the production of coccoliths may have decreased the carbonate ion concentration, making calcification more difficult for *E. huxleyi*.

Multiple regression, applied to the weight of coccoliths or coccospheres with seven environmental parameters (total alkalinity, concentration of O₂, CO₃, and OH, temperature, salinity, and saturation of calcite (ΩCa)), shows high correlations: $R=0.97$ (0.75) and 0.97 (0.82) respectively for the station averages (all samples) (Fig. 13f and l). The fact that the correlations are relatively less significant for the entire sample set than for the station average results not only from noise but also from the fact that at every station the maximum calcification was found at the maximum of coccolithophore density in the DCM (Fig. 10 and discussion above). Therefore the depth profiles of weight do not follow the carbonate chemistry profiles. The biotic (coccolithophores production) influence on calcification exist mainly at local scale (depth) whereas the abiotic (carbonate chemistry) plays a more important role at regional (horizontal) scale where 94% of the variability of coccolith and coccosphere weight can be explained by the change in these 7 independent variables. An important finding is that the degree of calcification of Isochrysidales strongly depends on environmental parameters. This relation was found at the "order" level rather than at the species level, suggesting that the specific composition of Isochrysidales could be related to carbon chemistry of the water. For example the fact that *G. oceanica* dominates around the Marquesas area could be related to the higher alkalinity of this area that presents an advantage for this relatively highly calcified species.

5 Conclusions

In most of the BIOSOPE stations the coccolithophore standing stocks were low, except in the vicinity of Peru Chile Upwelling system (80 to 100° W), where the Isochrysidales were highly abundant at mid photic zone depth (up to 350 000 coccospheres per liter between 30 and 60 m). This low abundance has been also reported in other studies conducted in the Tropical Pacific. In the South Pacific Gyre coccolithophores are rare at the surface but grow over the entire 0–300 m water column. The Isochrysidales coccoliths compose a significant fraction of PIC. The area of highest coccolithophore standing stock (80 to 100° W) here corresponds to the place of lowest alkalinity. There is a close relationship between the diameter of the coccoliths and length of the coccospheres of Isochrysidales. The most calcified Isochrysidales were found in the Marquesas area and in the Peru-Chile Upwelling (PCU) and the least calcified were found west of

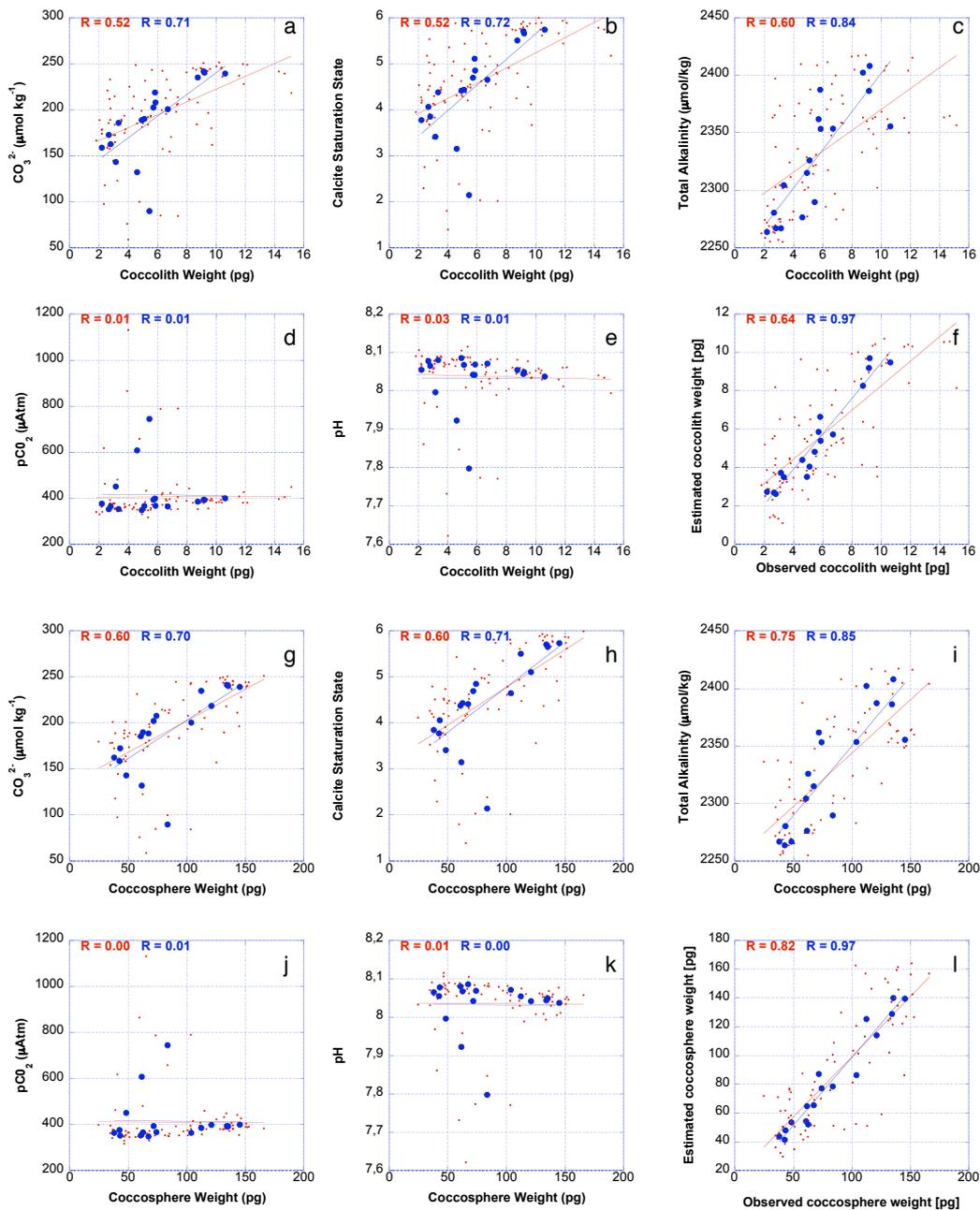


Fig. 13. Correlations between carbonate ion concentration (**a, g**), calcite saturation state (**b, h**), alkalinity (**c, i**), $p\text{CO}_2$ (**d, j**), pH (**e, k**) and the weight of Isochrysidales coccoliths (**a, b, c, d, e**) and coccospheres (**g, h, I, j, k**), and between observed and estimated weight of Isochrysidales coccoliths (**f**) and coccospheres (**l**). The entire suite of sample is represented in red, and the Station averages are represented in blue. The weights were estimated with the following equations:

$$\text{Coccolith weight} = 3.265 + (0.075 \cdot \text{Alkalinity}) - (0.040 \cdot [\text{O}_2]) - (0.380 \cdot \text{Temperature}) - (4.557 \cdot \text{Salinity}) + (0.207 \cdot [\text{CO}_3]) - 10.178 \cdot \Omega\text{Ca} + (2.268 \cdot [\text{OH}]) \quad (1)$$

$$\text{Coccosphere weight} = -1001.222 + (1.980 \cdot \text{Alkalinity}) + (0.078 \cdot [\text{O}_2]) + (4.448 \cdot \text{Temperature}) - (102.652 \cdot \text{Salinity}) - (0.952 \cdot [\text{CO}_3]) - (31.654 \cdot \Omega\text{Ca}) + (4.864 \cdot [\text{OH}]) \quad (2)$$

Those equations have been obtained by performing multiple regressions between these parameters and coccospheres and coccolith weight with the Station averages.

the PCU where the abundance of coccosphere is highest. The regional variability in the degree of calcification of Isochrysidales (weight of their coccoliths and coccospheres) strongly depends on environmental parameters (essentially the carbonate chemistry and the temperature). In area of high alkalinity the Isochrysidales secretes the heaviest coccospheres. The weight of Isochrysidales' coccoliths and coccospheres can be predicted at 94% from a set of 7 abiotic components.

Coccolithophores occur at great depths in the South Pacific Gyre: the maximum abundances of Isochrysidales were found between 150 and 170 m. The Deep Chlorophyll maximum is not only the place of maximum abundance of Isochrysidales, but also an area in which they secrete heavier coccoliths and have larger cells. Given the spatial extension of subtropical gyres, the role of coccolithophore in mediating particulate flux in these extreme oligotrophic waters may be significant when dealing with global ocean estimates.

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