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Effects of acidification and primary production on coccolith weight: Implications for carbonate transfer from the surface to the deep ocean

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[1] The coccolithophores are not only an important group of oceanic primary producers; they are also one of the main carbonate producers in the ocean. Because the calcium carbonate plates (coccoliths) secreted by these unicellular algae are so small and light (a few picograms each), they cannot be directly weighed, with the result that very little is known about the effect of primary production and dissolution on the coccoliths. Using a new method that allows a rapid estimate of the weight of discrete coccoliths, we analyzed the effect of dissolution. The results of two acidification experiments, using cultured and fossil coccoliths, indicate that acidification does not change significantly the weight and size of coccoliths. On the basis of these experiments, we identify four parameters for identifying the effects of dissolution on a coccolith assemblage. These parameters were applied in samples from two sediment trap time series taken at the same tropical Atlantic site (EUMELI), but separated by 2000 m water depth. There was no evidence of increased dissolution with water depth between 250 and 2500 m. This is indicative of the absence of dissolution of coccoliths between the photic zone and the lysocline. Other studies have identified supra-lysocline dissolution, and we propose that most of this takes place in the photic zone and possibly in the guts of grazers. Most of the coccolith weight changes observed over the duration of trap deployment were related to the occurrence of two coccolithophore blooms which resulted in significant increases in average coccolith weight of the dominant coccolithophore species during the blooms. Changes in growth rate and cell density appear to have a significant effect on the weight of coccoliths. To test this observation, we cultured *E. huxleyi* at different P and N concentrations. This test confirmed that coccoliths weigh more when coccolithophore production is highest. An important consequence for the carbon pump is that during higher primary production events the coccoliths will increase the ballasting of the aggregates in which they are carried to the seafloor, increasing the rate and amount of organic matter sequestered into the deep sea.

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1. Introduction

[2] The dissolved inorganic carbon sequestered in the world's ocean, mostly in the form of carbonate, represents 98% of the carbon stored in the ocean-atmosphere system. Variations in the size of this reservoir ultimately have strong incidence for atmospheric $p\text{CO}_2$ concentration. The biological production of calcium carbonate by pelagic organisms participates significantly to the export of carbonate from the ocean to the sediment. The coccolithophores, an important component of the phytoplankton community, are one of the main carbonate producers. They secrete minute calcareous plates called coccoliths, which, although extremely light (typically in the range of tens to hundreds of picograms per lith), are produced in very large quantities in most of the world oceans. The coccolithophores are most diverse in oligotrophic warm oceanic areas where they often dominate over other test-producing algal groups (e.g., diatoms and dinoflagellates). Coccolithophores may also be extremely abundant in colder coastal and shelf waters. In Norwegian fjords they form seasonal blooms in which cell concentrations can exceed 6 million cells per liter [Birkenes and Braarud, 1952]. Seasonal blooms may cover extremely large areas and can be detected by satellite [Ackleson *et al.*, 1988; Balch *et al.*, 1992; GREPMA, 1988; Holligan *et al.*, 1983, 1993]. For example, the North Atlantic seasonal bloom of spring 1991 extended over thousands of square kilometers south of Iceland [Balch *et al.*, 1992]. The production of large quantities of calcite by *Emiliania huxleyi*, the main bloom-forming coccolithophore species in modern oceans, is a source of CO_2 while the production of organic matter during blooms consumes CO_2 . The balance between production of carbonate and organic matter has been demonstrated to constitute a sink of CO_2 , at least under certain conditions [Buitenhuis *et al.*, 2001]. A certain amount of the calcite produced during blooms is exported to the seafloor. It has been generally accepted that the difference between calcite production and burial is the result of dissolution below a certain depth, called the *lysocline*,

where oceanic water becomes undersaturated in carbonate. The lysocline is usually located several kilometers below the sea surface [e.g., Berger, 1973]. Several lines of evidence, however, indicate significant supra-lysocline dissolution of calcite [Milliman *et al.*, 1999]: first, the flux of carbonate in about 100 sediment traps located at a depth of 1000 m was estimated to represent only 40% of the production of carbonate in surface waters; second, suspended CaCO_3 has been reported to decrease markedly in the upper 1000 m of the ocean; and third, the composition of coccolithophore communities in the photic zone differs markedly from coccolith assemblages at greater depths. Milliman *et al.* [1999] proposed two possible mechanisms to explain supra-lysocline dissolution. One is dissolution in the guts and feces of grazers; the second involves an increase in acidity caused by microbial degradation of organic matter during the settling of aggregates carrying coccoliths (i.e., marine snow).

[3] Milliman *et al.* [1999] recognized that more data are needed to confirm supra-lysocline dissolution. Their observations were based on analysis of quantitative parameters such as concentration of CaCO_3 and proportions between species. A different approach consisting of examination of coccoliths undergoing the sedimentary process is followed here to address the issue of supra-lysocline dissolution. Some studies have qualitatively described morphological changes of coccoliths due to dissolution, but this does not allow quantification of slight morphological changes linked to limited dissolution [Mitsuoka and Okada, 1989]. Several studies have shown that dissolution affects the weight of foraminifera; the greater the dissolution, the lighter their test [Broecker and Clark, 2001; Lohman, 1995; Rosenthal *et al.*, 2000]. Measuring the weight of discrete coccoliths at different depths in the water column would allow assessment of the intensity of dissolution during sedimentation. The problem is that coccoliths are too small to be individually weighed. Their shape and volume can be measured by electron microscopy and atomic force microscopy [Takahashi-Shimase and Nakashima, 2004], but these measurements are time consuming and require

Table 1. Description of the Culture Experiments Conducted With *E. huxleyi* Strain AC474

Experiment	Nitrate, μMol	Phosphate, μMol	Day	Cell Density, cell/mL
1	160	10	2	186000
1	160	10	2	136000
1	160	10	2	172000
1	160	2	2	222000
1	160	2	2	196000
1	160	2	2	154000
1	32	10	2	102000
1	32	10	2	144000
1	32	10	2	194000
2	32	10	5	1034000
2	32	10	5	1074000
2	80	10	7	2825000
2	80	10	7	2875000
2	160	2	7	1453188
2	160	2	7	1566510
2	160	5	7	2875000
2	160	5	7	2510000
2	160	10	7	3339999
2	160	10	7	3399660

access to sophisticated (and expensive) equipment and are therefore not adapted for routine studies. Here we use a recently described method that enables estimation of coccolith weight by means of their brightness in cross-polarized light. We first conducted two laboratory acidification experiments (one on cultured *E. huxleyi* and one with a multi-specific surface sediment sample) in order to evaluate the effect of decreasing pH on the dissolution of coccoliths of the 4 most abundant species present in low latitude sediments. We then compared field data to the laboratory data. We analyzed sediment trap samples collected at 250 m and 2000 m at the same location in the tropical Atlantic during the bloom season in order to determine the respective effects of production and dissolution during coccolith settling on the weight of coccoliths. An additional *E. huxleyi* culture experiment was performed to validate the conclusions of the sediment trap experiment.

2. Material and Methods

2.1. Laboratory *E. huxleyi* Experiments

2.1.1. *Emiliania huxleyi* Nutrient Limitation Culture Experiment

[4] *Emiliania huxleyi* strain AC474 (AlgoBank Culture Collection, University of Caen, France) was acclimatized for >10 generations and then

cultured for 7 days in artificial seawater medium containing K/2 (-Tris, -Si, -NH₄) supplements [Keller *et al.*, 1987] with variable concentrations of nitrate and phosphate, at 18°C with a light intensity of $\sim 150 \mu\text{E m}^{-2} \text{s}^{-1}$ and a photoperiod of 14L:10D. Samples were collected on the second day at the beginning of the exponential growth phase and at the end of the experiment during the stationary phase. Cell density, measured by hemocytometer counts, depended strongly on nutrient concentration and on the time at which the sample was collected (Table 1).

2.1.2. *Emiliania huxleyi* Dissolution Experiment

[5] The clonal *E. huxleyi* (heavily calcifying morphotype R) culture strain AC472 (AlgoBank Culture Collection) was grown in batch culture in 4l Erlenmeyer flasks containing 2l of filter-sterilized K/5 (-Tris, -Si) medium [Keller *et al.*, 1987] using natural seawater (salinity 35) collected off the Normandy coast (France) as the medium base. Prior to inoculation, the pH of the medium was adjusted to 8.2 by the addition of 1N NaOH. Cultures were grown at 18°C with daylight fluorescent lights providing a light intensity of $\sim 100 \mu\text{E m}^{-2} \text{s}^{-1}$ and a photoperiod of 14L:10D. The cultures were harvested by centrifugation (10 min at 3000 g) during early exponential growth phase when cell density was approximately $8 \times 10^5 \text{ cells mL}^{-1}$ (for this strain under these culture conditions the maximum cell density at the end of the growth phase was $\sim 4 \times 10^6 \text{ cells mL}^{-1}$). At harvesting, the pH of the culture medium had deviated by <0.1 from the original value of 8.2. After centrifugation the supernatant was removed and the pellets dried at 50°C for 2 hours.

[6] For the acidification experiments, a given weight of this coccolith powder (250 mg in the control (Exp1) and the second experiment (Exp2), 5 mg in the third experiment (Exp3)) was resuspended in 500 mL of artificial seawater (salinity 35, pH adjusted to 8.2 by the addition of 1N NaOH) in a 4 cm diameter measuring cylinder. This solution was bubbled from the bottom of the cylinder through a long pipette with either air (control experiment) or air enriched with different levels of CO₂. The pH was monitored continuously by means of a Radiometer PhC4000 electrode immersed in the solution in the cylinder. In the control experiment, samples were taken at t0 and after 2, 4 and 8 hours and over this time period pH remained stable (8.2 ± 0.03). During the CO₂

Table 2. Description of the Acidification Experiments Conducted With *E. huxleyi* Strain AC472

	Minutes	CO ₂ Enrich %	pH	Alk (Mean)	Omega Ca	HCO ₃ , $\mu\text{mol/kgSW}$
<i>Experiment 1 (Control)^a</i>						
t0	0	0	8.188	2528.5	6.39	1880.54
t1	90	0	8.228	2521	6.82	1829.34
t2	180	0	8.228	2560.5	6.93	1859.45
t3	270	0	8.197	2560	6.58	1894.89
t4	360	0	8.198	2584.5	6.66	1912.74
<i>Experiment 2 (Liths Dense)^b</i>						
t0	0	0.5	8.298	2560	7.77	1773.89
t1	30	0.5	8.2	2562	6.62	1893.03
t2	52	0.5	8	2586	4.67	2115.46
t3	90	0.5	7.8	2580	3.15	2262.49
t4	138	0.5	7.5	2585	1.68	2415.26
t5	176	0.5	7.2	2582	0.87	2494.20
t6	226	0.5	6.8	2638	0.36	2601.81
t7	294	5	6.4	3398	0.19	3380.31
t8	398	12.5	6	3993	0.09	3985.70
<i>Experiment 3 (Liths Not Dense)^c</i>						
t0	0	0.5	8.321	2718	8.58	1858.64
t1	24	0.5	8.2	2687.5	6.96	1990.14
t2	48	0.5	8	2692	4.86	2204.95
t3	96	0.5	7.8	2711	3.31	2379.84
t4	144	0.5	7.5	2701.5	1.76	2525.34
t5	204	0.5	7.2	2696.5	0.91	2605.44
t6	264	0.5	6.8	2758	0.38	2720.43
t7	350	1.5	6.4	2790.5	0.15	2775.61
t8	424	2.5	6	2870.5	0.06	2865.26

^aExperiment 1 (control): ~250 mg liths in 500 mL artificial water; t = 20°C, Sal = 35 PSU.

^bExperiment 2 (liths dense): ~250 mg liths in 500 mL artificial water; t = 20°C, Sal = 35 PSU.

^cExperiment 3 (liths not dense): ~5 mg liths in 500 mL artificial water; t = 20°C, Sal = 35 PSU.

enrichment experiments samples were taken at pH 8.2 (start of the experiment), 8.0, 7.8, 7.5, 7.2, 6.8, 6.4 and 6.0. At each sample point 25 mL of the solution were removed and filtered onto pre-weighed 0.2 μm pore size cellulose nitrate membranes which were subsequently dried at 50°C for 2 hours and reweighed. Filters were subsequently used for analysis of coccolith weight by automated light microscopy and of coccolith morphology by SEM (see below). The filtrate was used immediately for duplicate analyses of alkalinity by potentiometric titration. These experiments are summarized in Table 2.

2.2. Coccolith Assemblage From Surface Sediment

[7] Core MD97-2125 was collected during the IMAGES III cruise (1997) on Lord Howe ridge in the southwestern Pacific Ocean (23°S, 123°E) at 1675 m water depth. The core consisted of homogeneous Holocene and Pleistocene nannofossil oozes. The coccolith assemblage was well preserved (Figure 1, Photo 1) and diverse, comprising all of the typical dominant extant tropical species.

Coccoliths represented 75% of the sample and foraminifera 15% (Table 3). About 4 g of crushed sample was poured into 900 mL of filtered Mediterranean Seawater. The sample was dissolved by several additions of small quantities of HCl. The sample was constantly homogenized and maintained at low pH for two hours and then left for 13 days. Five sub-samples were collected at regular intervals over the first two hours in order to assess the state of dissolution (Table 3). Only 1.71 g of dry sediment was left at the end of the experiment, implying that more than half (57%) of the initial sample dissolved. Examination under a light microscope of smear slides prepared from the suspensions obtained at each step of the experiment showed that the absolute number of coccoliths decreased during the experiment, about 2/3 of the coccoliths no longer present by the end of the experiment.

2.3. Field Experiment: Sediment Traps

2.3.1. General Settings and Sediment Traps

[8] Carbonate production and sedimentation in the form of coccoliths was studied using sediment trap

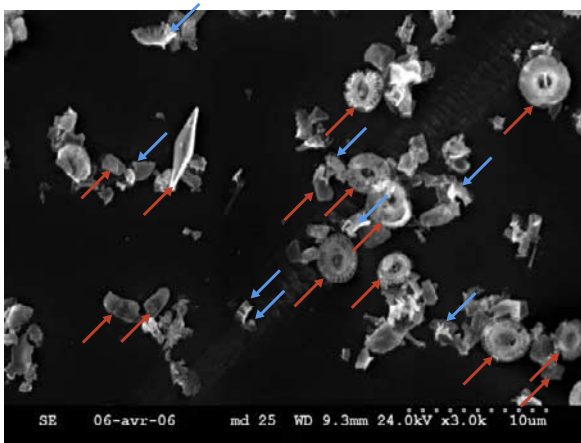


Photo 1 : Original Surface Sample MD97-2125

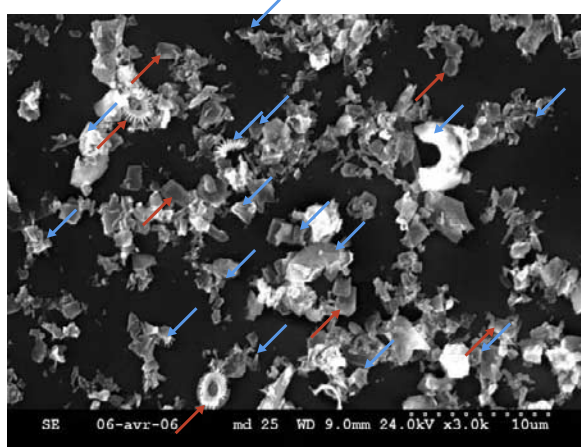


Photo 2: Surface Sample MD97-2125 at the end of the experiment

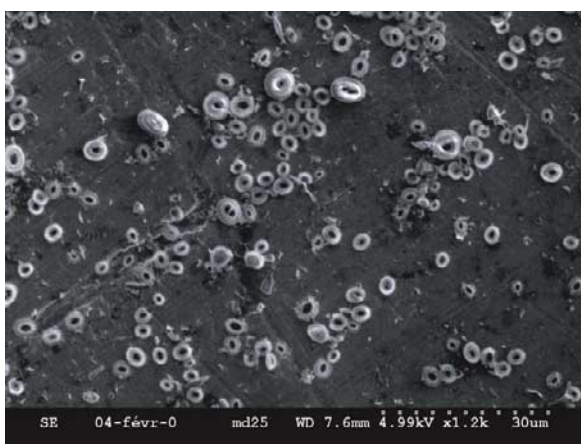


Photo 3 : Deep EUMELI trap Sample II2M6

Figure 1. Photo 1: SEM view of the intact surface sediment of core MD97-2125. Blue arrows in the bottom left and red arrows in the top right indicate fragments and entire coccoliths, respectively. Photo 2: SEM view of the surface sediment of core MD97-2125 at the end of the dissolution experiment (13 days). Blue arrows in the bottom left and red arrows in the top right indicate fragments and entire coccoliths, respectively. Photo 3: SEM view of sediments from sample II2M6 of the EUMELI experiment at 2500 m of water depth.

Table 3. Description of the Acidification Experiments Conducted With MD 97-2125 Core Top Sediments

Steps	Time Start, mn	Time End, mn	Duration, mn	Volume of HCl Added at End of Step, mL	pH Start	pH End	Subsample Volume, mL	Subsample Dried Weight, g
0	0	0	0	10	8.1	6.3		
1	0	51	51	10	6.3	7.5	20	0.0645
2	51	98	47	20	6.5	7.3	20	0.0596
3	98	113	15	50	6.1	6.9	20	0.0467
4	113	148	35	50	5.8	6.7	20	0.0289
5	148	180	32	0	6	6.5	20	0.0244
6	180	20000	19820		6.5	7.9		

Steps	Sample Dried Weight, g	Weight Loss, %	Carbonate Content, %	Fraction > 63 μm (100*WF/WT) ^a , %	Fragment/Foraminifera, %	Coccolith Abundance, billion/g	Mean Cocco. Weight, pg	Cocco. Relative Weight in Sed., %
0	4.05		96.3	13.5	6	73	10	75
6	1.71	57	91.4	6.3	97	27	19	52

^aWF, weight of fract. > 63 μm ; WT, total weight (about 0.20 g).

samples from the French-JGOFS program EUtroph MESotroph oLIgotroph (EUMELI). Sediment traps were deployed off the Mauritanian Cap Blanc upwelling cell at two mooring sites in the early 1990s. Two sediment traps were moored at 250 and 2500 m below sea level on the Cape Verde Terrace (18°8.0N, 21°8.0W), 400 km off the African coast. The seafloor at this location was at 3100 m water depth.

[9] In this area the trade winds blow between 20° and 26°N for most of the year causing almost constant upwelling along the shore [Mittelstaedt, 1991]. The integrated primary production measured four times during the experiment in the area of the traps was on average 350 gC m⁻² year⁻¹, which is similar to estimates from remote sensing [Morel, 1996]. At the surface, the North Equatorial Current (NEC) flows to the west. The trap at 250 m was located below the Canary current, in central Atlantic waters. The deep trap (at 2500 m) was located in North Atlantic Deep Water (1200 to 4000 m). Two water masses separated the two traps, the Antarctic Intermediate Water below and Mediterranean Water above [Pierre et al., 1993]. The two traps thus were clearly deployed in different water masses.

[10] Trap deployment and other results are described by Bory et al. [2001]. Settling particles were collected with 1 m² conical sediment traps (Technicap PPS5) equipped with a rotary with 24 cups. Time series samples were obtained at 10-day intervals between February 1991 and

November 1992. However, this study is restricted to the interval from February to May 1991 because during this time 2 events of relatively higher flux occurred [Bory et al., 2001]. Due to technical problems, only the first 5 cups of the shallow trap collected enough sediment and contained coccoliths. Also the current-meter placed in the vicinity of this trap showed that the speed of the horizontal current often exceeded 15 cm/s, a limit above which it is not possible to extract reliable information on the flux. Current speed was lower in the vicinity of the deep trap [Bory et al., 2001].

2.3.2. Production and Sedimentation

[11] The specific composition of the coccolithophore assemblages collected in the traps was similar to that in the surface sample of core MD97-2125 retrieved from the Pacific Ocean. The similarity is not surprising since coccolithophores are ubiquitous organisms. However, there were differences in the proportion between species. The assemblages of the oligotrophic South Pacific were dominated by *Florisphaera profunda*. In the upwelling zone off Cape Blanc, *E. huxleyi* dominated the assemblage.

[12] The flux in the traps during the 60 days selected for this study varied considerably, from 99 mg/m²/day at the beginning of the experiment to 681 mg/m²/day a month later. These high fluxes lasted for 40 days with two pulses of stronger fluxes from 4 to 14 March and 25 March to 4 April 1991. A second period of high flux occurred in

June 1991. During these periods, the flux of coccospheres and coccoliths of *E. huxleyi* increased in the same proportion [Bory *et al.*, 2001]. The relative abundance of *E. huxleyi* in the coccolithophore assemblages also increased from about 60% before or after, to 95% during these times. For the period of interest, the percentage of CaCO₃ at 2500 m varied, being around 50% on average, and ranging from 42 to 45% before and after, and from 56 to 62% during the “bloom” events (data from Bory *et al.* [2001]: %CaCO₃ = 100* PIC * 8.333/Flux, where PIC is the particulate inorganic carbon flux in mg/m²/day and Flux is the mass flux in mg/m²/day). We therefore attribute these changes in flux to blooms of *E. huxleyi*. Opal fluxes were highest between 4–14 March and 3–13 June, indicating that diatoms proliferated at the same time as *E. huxleyi*. The export opal flux was however about 10 times less than that of CaCO₃.

2.3.3. Sample Preparation

[13] When present, the skeletons of large zooplankton and nekton were removed by sieving the samples through a 1 mm nylon mesh. Homogenized samples were divided into sub-samples with a peristaltic automatic splitter [Heussner *et al.*, 1990]. Twenty microliters of wet sediment were smeared on a slide, dried and mounted with a cover slip using EUKIT. The coccolith flux data have been published elsewhere together with the method used to establish flux estimates [Bory *et al.*, 2001].

2.4. Coccolith Measurements

2.4.1. Scanning the Slides

[14] An optical microscope (LEICA DMRBE) with a 50× oil immersion objective was used for automatic scanning of slides in cross-polarized light. Microscope stage motion and focus were computer-controlled. For the sediment trap and sediment acidification experiment, a standard (756 × 582 pixels) black and white camera (MICAM2000) was used with a frame grabber (Domino). The camera grabs frames (images) of 126 × 97 μm² and a pixel represents an area of 0.0256 μm². For the culture experiments the field views were grabbed by a 2 Megapixel Spot Insight camera. The frames are 240 × 180 μm² with pixel area of 0.0225 μm². Light was precisely controlled and checked regularly (the intensity of light decreases progressively as the bulb ages).

2.4.2. Image Treatment and Coccolith Measurement

[15] The frames were processed by SYRACO which is a system for automatic taxonomic identification of coccoliths [Dollfus and Beaufort, 1999; Beaufort and Dollfus, 2004]. During routine work SYRACO recognizes approximately 96% of coccoliths belonging to 11 Pleistocene taxa. In this study, we focused more particularly on three groups of coccoliths: (1) *F. profunda*, (2) small placoliths (essentially *E. huxleyi* and small *Gephyrocapsa* such as *G. ericsoni* and *G. caribbeanica*), and (3) *G. oceanica* and *G. muelleriae*. The images of the specimens identified by SYRACO are saved in specific output files (SOF) of 100 specimens which can be cross-checked and subsequently used for morphometric measurements. The main weakness of SYRACO is potential misidentification of non-coccoliths as coccoliths. To correct this error, SYRACO was run a second time on the SOF [Beaufort and Dollfus, 2004]. This secondary automatic scanning eliminates most of the misidentifications for all species except *F. profunda*. About 23% of non-coccolith objects are confused with *F. profunda* and remain in the output frames. We thus eliminated misidentified coccoliths manually from the frames. Apart from this step, there is no human involvement in the collection of the data discussed below. The group of small placoliths was divided into two sub-groups, one consisting of placoliths with a large open central area (mainly *E. huxleyi*) and the other comprising more birefringent coccoliths with a smaller or closed central area. This latter subgroup includes small *Gephyrocapsa spp.* and heavily calcified *E. huxleyi*. The two subgroups are referred to as Small Light Placoliths (SLP) and Small Heavy Placoliths (SHP), respectively. In the case of the culture of *E. huxleyi* these distinctions were not necessary.

2.4.3. Coccolith Weighing

[16] It is possible to rapidly and accurately estimate the weight of coccoliths by taking advantage of the brightness of carbonate grains when viewed in cross-polarized light (birefringence) [Beaufort, 2005]. Calcite is one of the most birefringent minerals. The brightness and color of a birefringent object depend on its thickness and orientation. Therefore it is common for the nannopaleontologist to associate varying birefringence with differences in the thickness of coccoliths. This simple observation constitutes the basis upon which to measure the brightness of a frame grabbed by a black and

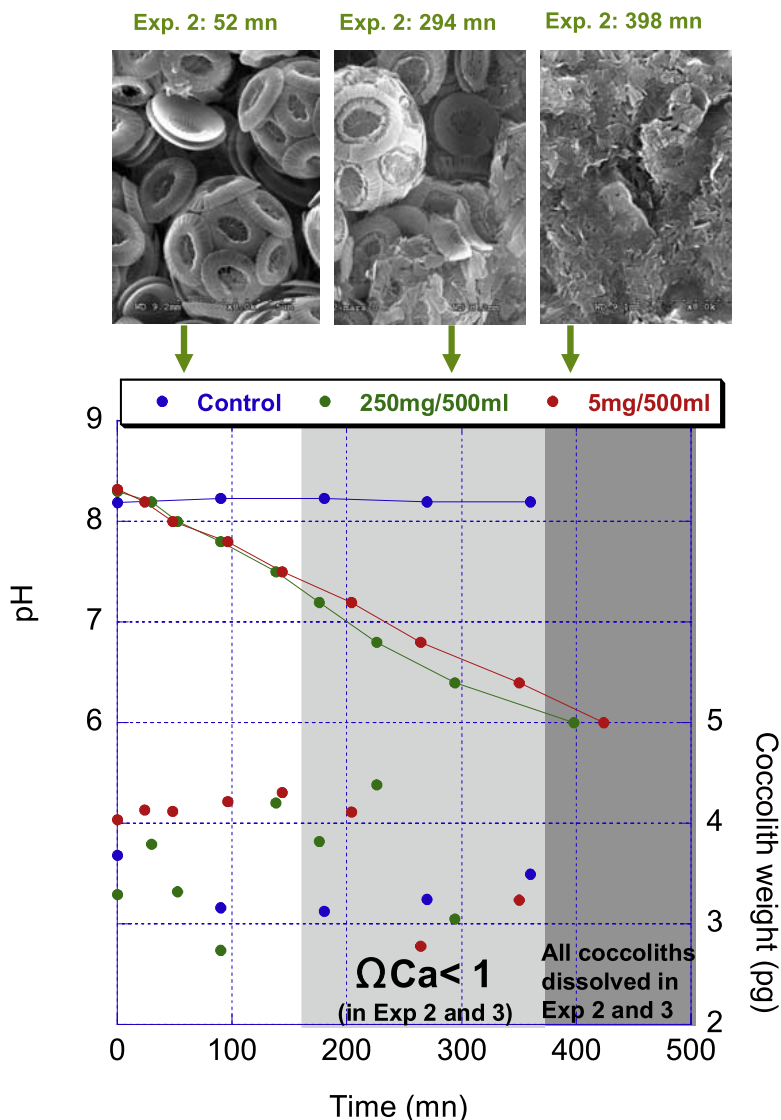


Figure 2. Evolution of the weight of coccoliths of *Emiliania huxleyi* during acidification experiments (Exp 1 (control) in blue, Exp 2 (high lith density: 500 mg/L) in green, Exp 3 (low lith density: 10 mg/L) in red). Top graph: pH. Bottom graph: coccolith weight (pg). The light shaded area corresponds to CaCO_3 saturation (ω) less than 1. The dark shaded area corresponds to the time when coccoliths were no longer present in Exp 2 and Exp 3. Top row: 3 SEM micrographs show the state of preservation at 3 steps of Exp 2: left at 52 min, center at 294 min, and right at 398 min.

white CCD camera placed on a light microscope equipped with a polarizer and an analyzer. The brightness of calcite is proportional to its thickness in the range of 0 to 1.5 μm . By measuring the total brightness of a coccolith as the sum of Grey Level (GL) of every component pixel it is possible to convert brightness into weight after proper calibration. With the light tuning and the magnification of the microscope, the sum of the GL of every pixel multiplied by a given factor (transfer function given below) gives an accurate estimate of the weight (in picograms of calcite) of a coccolith.

The error on the calibration is estimated to be about 12% (2 sigma of the distribution in the calibration). This error is due to the sample preparation method used for the calibration. This method is highly reproducible (± 0.13 pg).

[17] Each SOF taken by SYRACO consists of 10 rows of 10 specimens, corresponding to a mosaic image of 100 specimen frames (SF). Each SF is a square of 64×64 pixels, and each specimen is at its exact centre. A “NIH images” macro was developed that takes advantage of this disposition

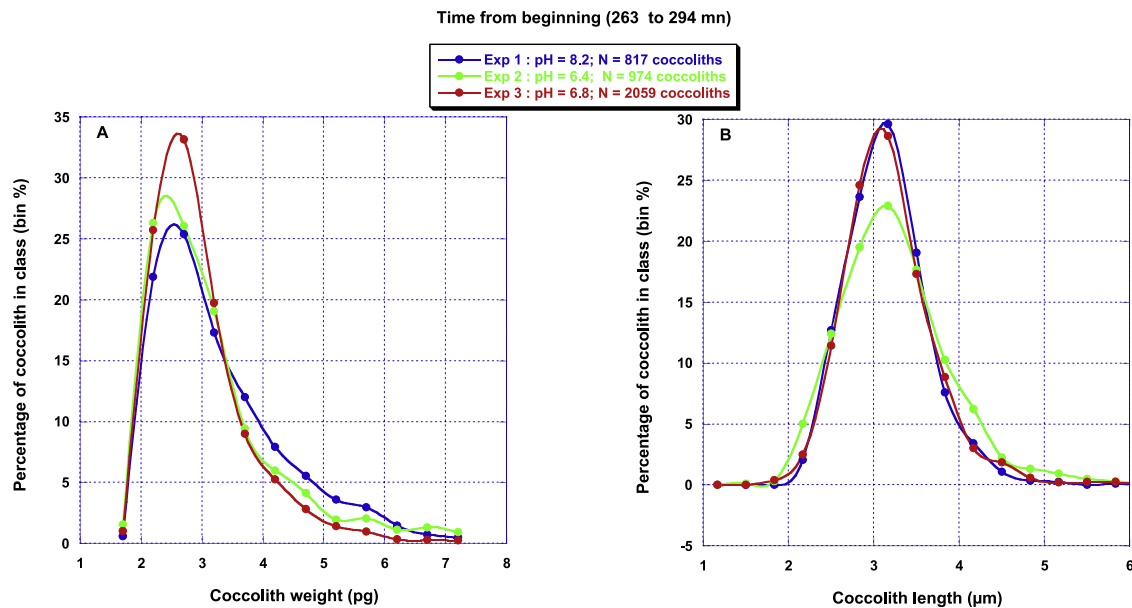


Figure 3. Comparison of the distribution of (a) weight and (b) length of coccoliths of *E. huxleyi* measured in the control (blue) and the two acidification experiments (green and red). The curves are cubic splines. The samples were taken at 270 mn (Exp 1), 294 mn (Exp 2), and 270 mn (Exp 3) after the beginning of the experiments. The pH values were 8.2 (Exp 1), 6.4 (Exp 2), and 6.8 (Exp 3).

in the SOF to automatically estimate the weight of coccoliths. Because the two cameras (MICAM and SPOT Insight) did not have the same sensitivity, and the samples prepared on membrane filters are darker than the smear slides, the transfer function from GL to pg [Beaufort, 2005] was different ($\text{pg} = 0.00054 * \text{GL}$ (for smear and MICAM) and $0.0013 * \text{GL}$ (for membrane and SPOT Insight)). The same macro also measures the length of the coccoliths.

2.4.4. Electron Microscopy

[18] Samples were observed on a Hitachi 3002 CN SEM.

3. Results

3.1. Dissolution of Coccoliths of a Cultured Strain of *E. huxleyi*

[19] Seawater chemistry was monitored in these experiments. Because only a small quantity of coccolith material was used, it was not possible to assess the absolute coccolith abundance through the experimental time-course. However, in the two acidification experiments, the degree of saturation with respect to CaCO_3 (Omega Ca) became lower than zero (undersaturation) after 140 min and all

coccoliths had been dissolved after 370 min (Figure 2). In the last samples before the complete disappearance of coccoliths, average coccolith weight was very similar in the control (Exp1) and in the two acidification experiments. SEM observations showed that at this stage many coccoliths were broken, and that minor (but clearly observable) etching occurred on the shields of the remaining coccoliths. The total amount of coccoliths was very different in the two experiments (500 mg of coccoliths per liter in Exp2, and 10 mg per liter in Exp3). By the time at which no more coccoliths were recognized, alkalinity had increased by $\sim 1400 \mu\text{mol/kg}$ (equivalent to 140 mg of CaCO_3) in Exp2 and by $\sim 150 \mu\text{mol/kg}$ in Exp3. This indicates that dissolution was not complete in Exp2, but was complete in Exp3. SEM observation revealed a large quantity of small elements of coccoliths in the last sample of Exp2 (Figure 2). Although carbonate dissolution was not complete, no intact coccoliths were present.

[20] The distributions of weight and size of the coccoliths in the three experiments (control and acidification) stayed relatively stable, as shown in Figure 3, where samples in late stages of the experiments are compared. This is an indication of the absence of preferential dissolution of cocco-

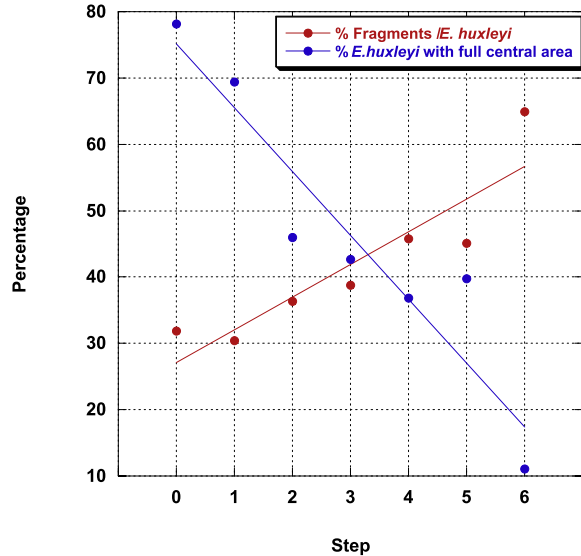


Figure 4. Evolution of the percentage of fragmented *E. huxleyi* coccoliths (open triangles) and *E. huxleyi* coccoliths without central grid (closed triangles) in a group of at least 150 *E. huxleyi* coccoliths counted with an SEM, in the different steps of the core top acidification experiment. The lines represent the regression between the sets of data ($R = 0.80$ and 0.89 for fragments and loss of central grid, respectively).

liths of particular size (e.g., smaller) or weight (lighter).

[21] Coccolith weight cannot be used as a dissolution proxy since it did not change significantly during these experiments. However, the number of fragments relative to the number of entire coccoliths of *E. huxleyi* appears to be a good indicator of the degree of dissolution in these experiments: no fragments were observed in samples which had an omega Ca > 1 and 100% fragments were present in the sample where coccoliths were no longer present. In intermediate samples, the amount of fragments increased as Omega Ca decreased.

3.2. Dissolution of a Fossilized Assemblage

[22] The objective of this second experiment was to assess changes in a mixed coccolith assemblage with increasing acidity. For this experiment we did not use a fresh coccolith assemblage since the amount of material required in order to be able to accurately weigh the sample was too high. A fossil assemblage was therefore used. The limitation is that this assemblage has presumably already undergone significant dissolution during sedimentation and burial. We chose a core top in which

preservation is pristine, showing well-preserved *E. huxleyi* with little fragmentation. If significant dissolution had occurred, we consider that the *E. huxleyi* coccoliths would not have been so well morphologically preserved.

3.2.1. Quantity of Dissolved Coccoliths

[23] A progressive decrease of the absolute abundance of coccoliths (number of coccoliths per gram of sediment) took place over the duration of the experiment. Initially there were about 73 billion coccoliths per gram of sediment. At the end of the experiment only approximately 27 billion coccoliths per gram of sediment were present. Because the bulk sediment weight decreased from 4.05 g to 1.71 g, about 84% of the coccoliths were dissolved during the experiment.

[24] During successive acid treatments, coccoliths were broken up into smaller particles whose number increased progressively through the experiment. Using the SEM, we counted about 170 specimens of *E. huxleyi* sorted into three classes: (1) coccoliths with the entire grid of the central area, (2) coccoliths without a grid in the central area, and (3) fragments of coccoliths. The evolution of the percentage of fragments versus entire coccoliths and of *E. huxleyi* without central grid versus those with central grid is plotted in Figure 4. The amount of coccolith fragments and coccoliths without central grids clearly increased during the experiment (Figure 1). In Photo 2 the number of coccolith fragments (black arrows) is larger than the number of unbroken coccoliths (white arrows). It is likely that most of the non-identified objects on this image are also small pieces (even single elements) of coccoliths. Some coccoliths, however, are still well preserved. Structures as delicate as the central grid and the distal shield of *E. huxleyi* with its T-shaped elements can be seen preserved.

[25] In both experiments, on the fossil assemblage and on cultured *E. huxleyi*, the amount of fragments increased with time. Fragmentation may also be important for understanding the origin of unidentified micro-particles of carbonate. A large part of these could be fragments of coccoliths.

3.2.2. Changes in Coccolith Weight by Taxa

[26] The weight of each coccolith species recognized by SYRACO was estimated. In general this parameter did not change significantly through the experiment (Figure 5). The significance of the

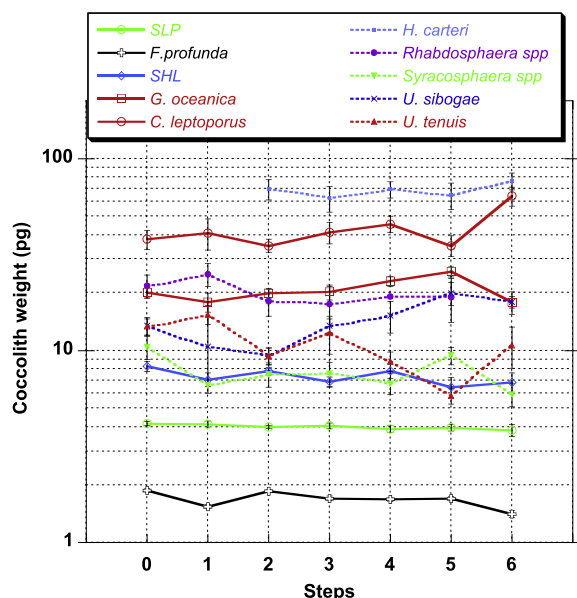


Figure 5. Evolution of the weight (log scale) of the 10 coccolith species or classes at the different steps of the core top acidification experiment. The dashed line is used for species with number of coccoliths per sample (step) inferior to 30 individuals and therefore showing large error bars.

change in mean weight was established by calculating a correlation coefficient between mean weight and time (or steps). Only the weight of the SLP showed a significant trend, with a slight (7%) decrease in weight through successive steps (the slope is significant at $r^2 > 0.83$). Although the observed decrease in weight is within the error bar of the analysis, it is not surprising that the weight of SLP should decrease as a result of successive acid attacks because *E. huxleyi* coccoliths have a delicate structure with the distal shield consisting of T-shaped elements that are easily broken (Figure 1, Photo 2). An SEM analysis showed that in the final sample (after 5 acid attacks) placoliths of *E. huxleyi* with broken distal shields and without their central grid dominated, whereas they were rare in the original sample. Statistically less significant, two other classes of small coccoliths (SHP and *F. profunda*) also decreased in weight. No clear trend was observed in the other group, either because it was not present (*G. oceanica*), or because there were not enough measurements (dashed lines in Figure 5). In the culture experiment with the highly calcified *E. huxleyi* Type R which has bulky T-shaped elements, this slight decrease in weight was not observed.

[27] It may seem counterintuitive that the mean weight of the different species did not change

significantly through successive acid attacks. If we take into account the fact that only unbroken coccoliths were measured, it becomes obvious that the main response to acidification was that coccoliths broke into pieces. Coccoliths that were still complete had not yet been affected by dissolution. This explains the increase in the number of coccolith fragments in the later stages of the experiment. The coccoliths left complete at each step of the experiment were those that did not suffer significantly from the acid attacks.

[28] It could be argued that this minor change in weight results from the fact that SYRACO has been trained to recognize only coccoliths in a limited range of shape and weight and has not been trained to recognize partially dissolved coccoliths. This is not the case, however, because the training of the artificial neural network was performed using a large set of coccolith assemblages representative of different environments [Beaufort and Dollfus, 2004]. Furthermore, the artificial neural network was designed to perform good generalization. This is well illustrated by the fact that in the dissolution experiment the average weight per sample of the SLPs ranged between 7.1 and 7.7 pg, whereas in the trap experiment the average weight ranged between 6.1 and 6.7 pg.

3.2.3. Changes in the Composition of Coccolith Assemblages

[29] The relative proportions between species did not change significantly during the successive acid

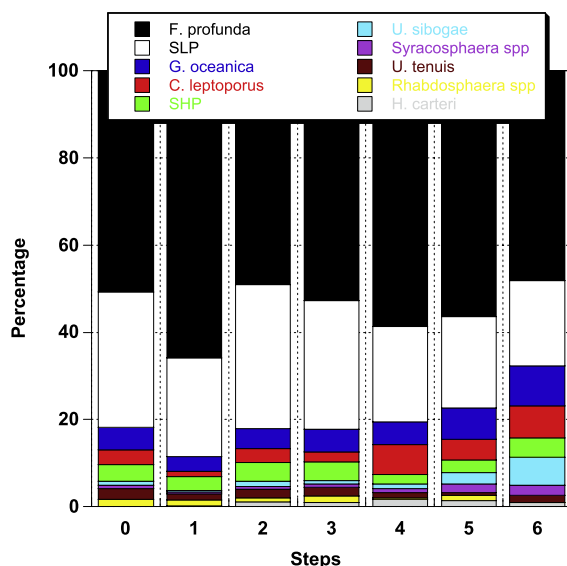


Figure 6. Relative abundance of the 10 most abundant species or classes of coccoliths at the different steps of the core top acidification experiment.

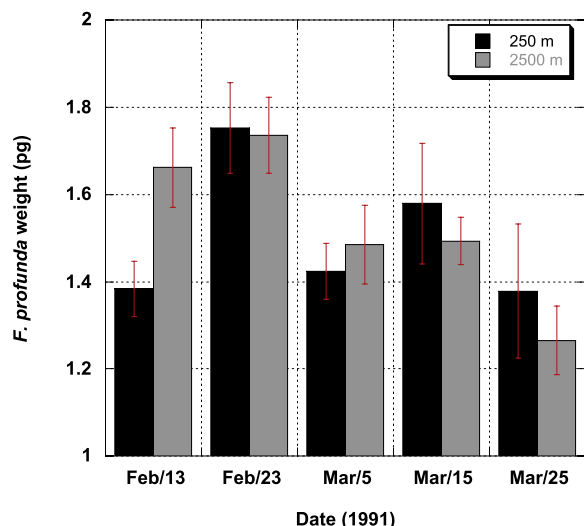


Figure 7. Depth (250 m and 2500 m) comparison of the evolution of the average weight of *F. profunda* over a 50 day period in the Mesotroph EUMELI traps.

attacks (Figure 6). However, notable changes affected the rhabdoliths (coccoliths with a long process), which were not found at the end of experiment and the relative abundances of *Umbilicosphaera sibogae* and *Calcidiscus leptoporus* whose frequency increased through the experiment. These changes were however relatively insignificant, which is surprising considering the fact that 88% of the coccoliths were dissolved. The composition of coccolith assemblages is known to vary significantly below the lysocline [Roth and Berger, 1979]. Placoliths (coccoliths composed of two plates, or shields, connected by a central cylinder, or collar), are among the most dissolution-resistant coccoliths. The genera *Emiliania*, *Gephyrocapsa*, *Calcidiscus*, and *Umbilicosphaera* produce placoliths. *Florisphaera profunda* liths are not placoliths [Roth and Berger, 1979], but seem resistant to dissolution since we have often observed this species from core tops taken at the level of the CCD in the Indian Ocean. Indeed, often it is the only taxon remaining together with *Gephyrocapsa* spp. in such extremely corrosive environments. Placoliths and *Florisphaera* were the dominant coccoliths in the present analysis. They were too resistant to dissolution to cause marked changes in the assemblage composition during the experiment. The abundance increase of *C. leptoporus* coccoliths may reflect the fact that this large placolith may be more resistant to dissolution than smaller ones. From several studies of surface sediments, *C. leptoporus* has been considered to be the

most resistant coccolith [Berger, 1973; Roth and Berger, 1979; Roth and Coulbourn, 1982].

3.2.4. Markers of Dissolution

[30] The two acidification experiments show that only a few markers are useful to detect dissolution in the trap experiment: (1) an SEM qualitative analysis revealed signs of dissolution on the edge of placoliths; (2) the percentage of well-preserved *E. huxleyi* coccoliths decreased in comparison to the number of fragmented *E. huxleyi* coccoliths and of *E. huxleyi* coccoliths without their central grid (SEM counts); (3) the average weight of small coccoliths decreased slightly; and (4) the percentage of coccoliths per weight of sediment diminished.

3.3. Comparison of the Sediment Traps at 250 and 2500 m

[31] Because only the first five cups of the shallow trap worked correctly, we have a record of only 50 days of synchronous sedimentation at 250 and 2500 m. The coccolith bloom was recorded in cups 3, 4 and 5 of each trap.

3.3.1. No Indication of Supra-lysocline Dissolution During Sedimentation

[32] 1. No qualitative morphological difference was seen between the two depths (Figure 1). The sediment, extremely rich in coccoliths, consisted essentially of placoliths of *E. huxleyi*. There was no trace of etching similar to that which occurred in the acidification experiment. This was true for coccoliths produced before and during the bloom of 4 to 14 March 1991. In other words, the qualitative analysis did not reveal any signs of dissolution.

[33] 2. In the deep trap, there were no fragments of coccoliths. The number of *E. huxleyi* coccoliths without a central grid was less than 20% in all samples.

[34] 3. The small *Florisphaera profunda* coccoliths did not show any significant difference in weight at the two depths, as predicted by the results of the acidification experiment performed on the fossil assemblage (Figure 7). Their weight changed through time, but in parallel at the two depths.

[35] 4. The relative abundance of coccoliths in the sediment varied in parallel fashion at both depths with values around 10 million coccoliths per milligram of sediment in the first 4 cups (both traps)

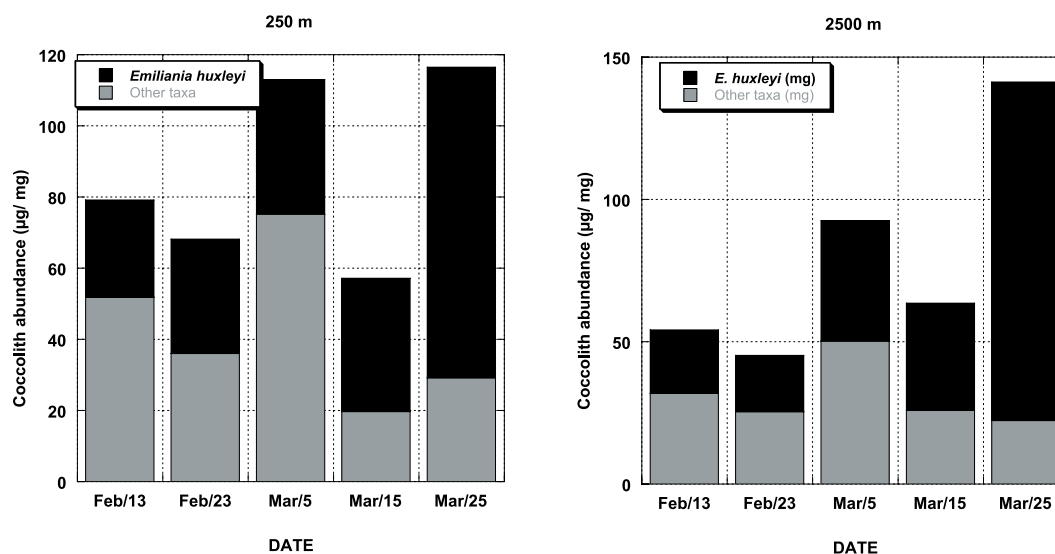


Figure 8. Comparison of the evolution of the relative weight abundance of coccoliths per milligram of sediment over a 50 day period at (left) 250 m and (right) 2500 m in the Mesotroph EUMELI traps. Grey bars, all coccoliths except small placoliths; black bars, small placoliths (essentially *E. huxleyi*). The first spring bloom event started on 5 March and ended in early April.

and a subsequent two-fold increase in the abundance in the fifth cup (both traps). By multiplying the average weight of taxa by the number of coccoliths of that taxa per milligram, we obtained the relative abundance of coccoliths measured in weight per milligram. This calculation was conducted for *E. huxleyi* (SHP and SLP together) and for the other coccolith taxa together (Figure 8). The relative load of the second pooled assemblage increased from about 50 $\mu\text{g}/\text{mg}$ before the bloom to about 100 $\mu\text{g}/\text{mg}$ during the bloom. Again a strikingly similar trend at the two depths was observed. This is the fourth indication of the absence of dissolution during the settling of particles at this site between 250 and 2500 m.

[36] The absence of dissolution between 250 and 2500 m is at first sight in contradiction with Milliman *et al.* [1999] who proposed that calcium carbonate is dissolved well above the chemical lysocline. These authors discussed several lines of evidence that would suggest that as much as 60–80% of the carbonates are dissolved in the upper 1000 m of the ocean. In fact they first discussed the rapid decrease of Ca concentration in the upper part of the water column. This decrease occurs in the upper 200 m [Sherrell *et al.*, 1998], which is above the shallowest EUMELI trap. Milliman *et al.* [1999] also used the change in species composition of coccolith assemblages recovered in a surface and a deep trap. In agree-

ment with these authors, the absence of holococcoliths (a fragile type of coccoliths) in the trap samples is remarkable. Holococcoliths are, however, abundant in the ocean. If holococcolith-bearing coccospheres were present in the EUMELI production zone, they were not present in the aggregates in which the heterococcoliths were found. This would suggest that dissolution of coccoliths takes place while aggregates form (e.g., in the guts of grazers) rather than during their sedimentation. This study shows that very little change occurs in the quality of the aggregates during sedimentation between 250 and 2500 m.

3.3.2. Abundance of Coccoliths and Effect on the Ballast of Aggregates

[37] *E. huxleyi* was the major contributor to CaCO_3 by weight during the bloom (Figure 8). The relative proportion by weight of *E. huxleyi* in the coccolith assemblage increased progressively (following that of the mass flux) and *E. huxleyi* liths became dominant during the bloom (Figure 9). The coccoliths of *E. huxleyi* are minute and although this species often dominates nannoplankton communities, in many cases *E. huxleyi* liths do not constitute the bulk of carbonate sedimentation [e.g., Beaufort and Heussner, 1999; Broerse, 2000] and consequently participate little in the ballasting of aggregates [Ziveri *et al.*, 2007]. Our results demonstrate that when *E. huxleyi* bloomed, the sheer abundance

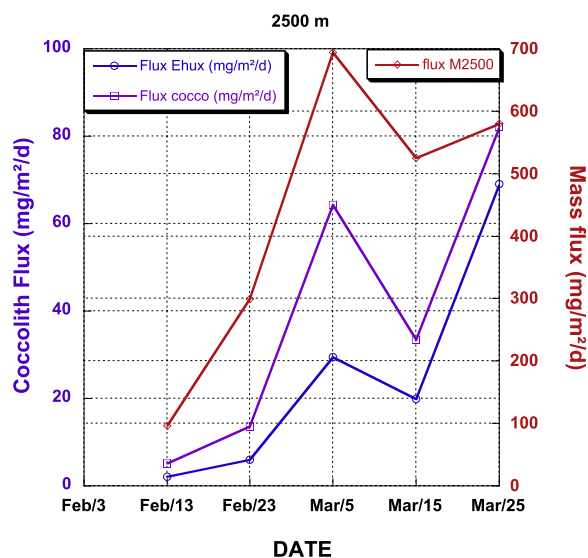


Figure 9. Record of the mass flux (red), weight flux of the entire coccolith assemblage (purple), and weight flux of small placoliths (e.g., *E. huxleyi*) (blue) over a 50 day period at 2500 m in the Mesotroph EUMELI traps.

of liths compensated for their minute size, and that they provided most of the ballast for aggregates. The contribution of carbonate to the mass flux increased from 43% (Cup 1) to 58% (Cup 5) during the first bloom [Bory et al., 2001], highlighting the importance of carbonate in ballasting. Coccoliths represented only 25% of total carbonate, but this is likely a considerable underestimation because it does not take into account coccoliths on coccospheres (the coccosphere/coccolith ratio increased during the bloom [Bory et al., 2001]) or coccoliths contained in aggregates that were not broken during preparation of the smear. Visual inspections by SEM (e.g., Figure 1) and light microscope indicated that coccoliths were almost the only contributor to overall carbonate in the bloom events.

[38] Bory et al. [2001] estimated settling rates by comparing the time lag between events in the EUMELI traps at 1000 m and 2500 m. During the bloom the sedimentation rate was extremely fast (500–750 m/day), but was more moderate outside these events (150–300 m/day) and was about 10 m/day in oligotrophic areas [Bory et al., 2001]. Transport at such high rates during blooms of *E. huxleyi* has been reported elsewhere (e.g., Beaufort and Heussner [1999] measured sedimentation rates exceeding 600 m/day during an *E. huxleyi* bloom in the Gulf of Biscay). The increase of settling rate during the bloom of *E. huxleyi*

reflects the ballasting effect of abundant coccoliths in aggregates [van der Wal et al., 1995].

3.4. Variability of the Weight of Coccoliths During Blooms

[39] Two groups of placoliths, *G. oceanica* and SLP (*E. huxleyi*), were sufficiently abundant to allow study of variability in their weight during spring 1991 off Mauritania (Figures 10 and 11). A variability of about 25% around the mean was observed in both groups. Maxima occurred during high flux events and minima during low flux. A good correlation exists between the record of mass flux and the weight of *G. oceanica* ($R = 0.80$) and to a lesser degree the weight of SLP. This indicates that during the two bloom events, marked by high flux, the coccoliths increased in weight. Alkenones were measured in the same trap samples. The UK'37 temperature estimates indicated a decrease of a few degrees Celsius of the sea surface temperature, suggesting that the blooms resulted from two upwelling events (Y. Ternois and M.-A. Sicre, unpublished results, 2006). The increase of coccolith weight could result from physiological adaptation to temperature or to increase of primary production through change of growth rates. A decrease in coccolith size (and therefore certainly weight) of *E. huxleyi* coccoliths has been correlated with an increase of temperature in culture [Blackwelder, 1971], in contrast to our results. Also, in the Bay of Biscay, heavily calcified coccoliths occur during summer when temperatures are highest [Beaufort and Heussner, 2001]. This is in complete contrast with what has been found in the California Basin, where *E. huxleyi* calcified more during the winter when temperatures are colder [Ziveri et al., 1995]. This absence of a global relationship between temperature and calcification (or size) of the coccoliths of *E. huxleyi* has also been noted in other studies [Samtleben and Bickert, 1990; Winter, 1985]. Degree of calcification may be related to other factors. In the California Basin [Ziveri et al., 1995] the fluxes of *E. huxleyi* are highest in the winter, whereas in the Gulf of Biscay blooms of *E. huxleyi* occur in the summer during the season of high production [Beaufort and Heussner, 2001]. An increase in coccolith weight was observed during blooms of *E. huxleyi* in a mesocosm experiment in a Norwegian fjord [Engel et al., 2005]. The blooms were induced by the addition of nutrients in 9 mesocosms subjected to different CO₂ concentrations. In all mesocosms, an *E. huxleyi* bloom occurred, and the coccolith weight increased by 25 to 50%. In our

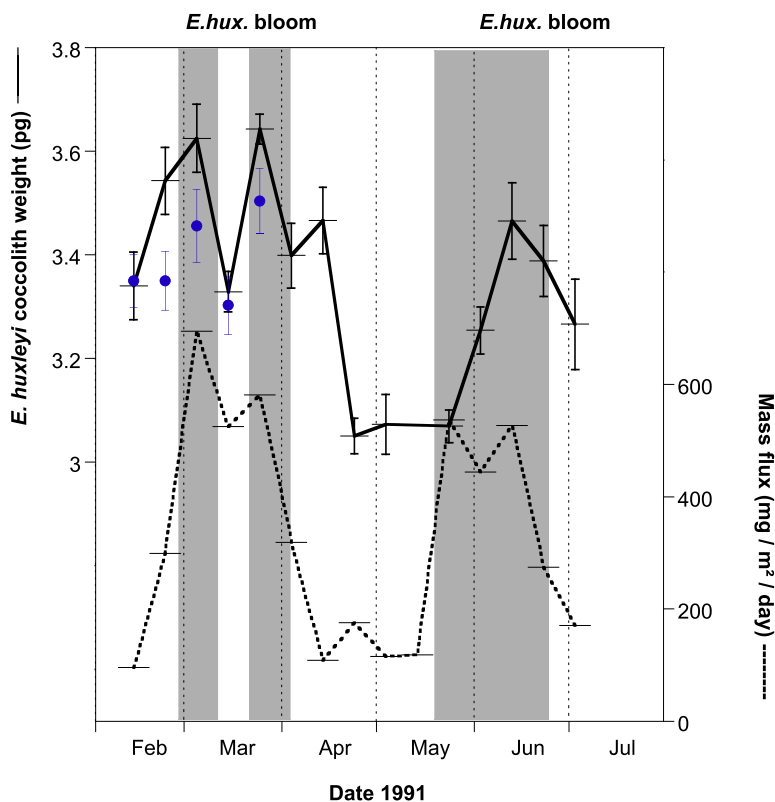


Figure 10. Five month record of the evolution of the weight of *E. huxleyi* (SLP) and of the mass flux at 2500 m in the Mesotroph EUMELI traps. The blue dots correspond to the weight of *E. huxleyi* at 250 m at the same location. The grey areas correspond to bloom periods.

study, the weight of coccoliths of *E. huxleyi* and *G. oceanica* increased during the two blooms. These data suggest that degree of calcification may be linked to primary production.

[40] Culture experiments using *E. huxleyi* have shown that calcite production per cell is positively correlated to growth rate [Stoll *et al.*, 2002]. For this species, the rate of calcification may be related to the rate of photosynthesis [Buitenhuis *et al.*, 1999; Paasche, 1968]. An increase in weight of the coccoliths produced during the two bloom events is therefore not surprising. It results from higher rates of calcification as a function of increased rates of growth and photosynthesis during the blooms.

[41] The results from culture experiments with *E. huxleyi* presented in this paper confirm the link between coccolithophore production and coccolith weight: there is a clear relationship between cell density and coccolith weight (Figure 12). This needs to be confirmed in other strains of *E. huxleyi*

and/or other species, but this could have interesting applications. If the correlation proves to be consistent it would be possible, first, to estimate (at least indirectly) growth rates in the past using morphometric measurements of biominerals in deep-sea sediments. Another important consequence would be that the density of aggregates would increase not only due to an increase in coccolith production but also due to increase in their weight. This implies that when coccolithophore production increases, the transfer of organic carbon would be increased significantly by this process in the manner proposed by [Klaas and Archer, 2002].

4. Conclusions

[42] A major aim of this study was to monitor the effect of coccolithophore blooms on coccolith production, sedimentary processes such as dissolution, and transport through the water column. We found that:

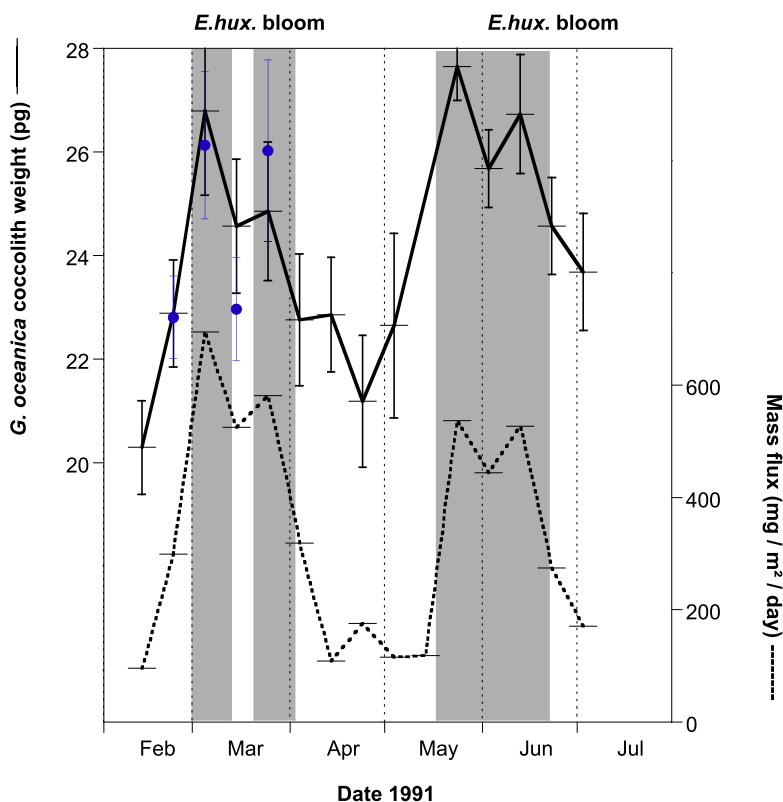


Figure 11. Five month record of the evolution of the weight of *G. oceanica* and of the mass flux at 2500 m in the Mesotroph EUMELI traps. The blue dots correspond to the weight of *G. oceanica* at 250 m at the same location. The grey areas correspond to bloom periods.

[43] 1. The weight of coccoliths did not change significantly during experimental acid attacks. Therefore the weight of coccoliths in sediments can be considered to represent their original weight on the coccosphere (in the absence of recrystallization and diagenesis).

[44] 2. *Florisphaera profunda* and placoliths are equally resistant to dissolution. The relative abundance of coccoliths of these two groups did not significantly change during dissolution. Therefore study of the relative abundance between these groups in deep-sea cores is meaningful.

[45] 3. Coccoliths were not subject to dissolution during settling between 250 and 2500 m. This does not contradict the proposal of Milliman *et al.* [1999], who suspected carbonate dissolution above 1000 m. However, this result suggests that dissolution occurs in the photic zone, above 200 m. This finding supports one of the two reasons forwarded by Milliman *et al.* [1999] for supra-lysoclinal dissolution: dissolution takes place during aggregate formation (i.e., into the guts of grazers).

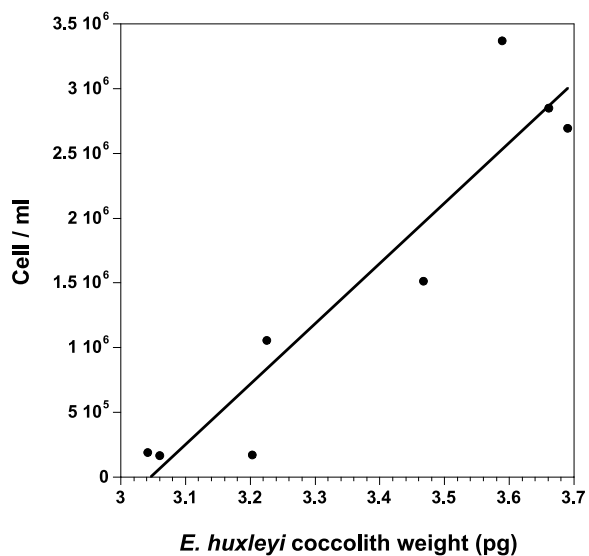


Figure 12. Comparison of the weight (pg) of coccoliths of *E. huxleyi* to the number of cells per milliliter obtained during several cultures of the strain TW1 in different nutrient settings (Table 1). Values are average of two replicates. The regression line is plotted ($R = 0.94$).

[46] 4. When *E. huxleyi* blooms the relative weight of coccoliths in the settling particle assemblage increases. The relative weight of *E. huxleyi* in the coccolith assemblage becomes dominant. The contribution of CaCO_3 to the mass flux also increases. Consequently the density of the aggregates increases and the speed of settling particles reaches values as high as 750 m/day. This shows that *E. huxleyi* plays a critical role the carbon burial by ballasting aggregates and therefore increasing the rate at which organic matter is exported from the upper ocean. This diminishes the time during which the organic matter can be recycled in the water column.

[47] 5. During bloom events the relative weight of coccoliths per weight of sediment doubled. This implies an increase in the density of aggregates. Consequently, coccoliths are important contributors to the ballasting of aggregates during blooms. The fact that coccolith weight increased in relation with coccolithophore production implies further amplification of the ballasting effect during blooms.

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