

# Impact of a *Coccolithus braarudii* bloom on the carbonate system of Portuguese coastal waters

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**Abstract** In the adjacent Tagus and Sado coastal waters (off SW Portugal), a phytoplankton bloom dominated by diatoms (up to 1000 cells ml<sup>-1</sup>) was observed, in spring 2002, under upwelling conditions. Overlapping with the phytoplankton bloom, but spatially differentiated from the diatoms and other phytoplankton groups, a pronounced development of the coccolithophore *Coccolithus braarudii* (up to 60 cells ml<sup>-1</sup>) occurred, associated with a thermally stratified water-mass localised in the Tagus Bay. Some cells of the *C. braarudii* hyalinus-phase were also observed. A production of about five tons of calcite was calculated for the upper 30m of the water-column, based on the integration over depth of a total of 6.7g CaCO<sub>3</sub> m<sup>-2</sup>, covering 0.7km<sup>2</sup> of the Tagus Bay. An average CaCO<sub>3</sub> precipitation rate of 11.2mmol CaCO<sub>3</sub> m<sup>-2</sup> d<sup>-1</sup> was estimated, resulting in the release of 7.4mmol CO<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> during the *C. braarudii* bloom. These results indicate that the studied coastal system contributed an additional source of CO<sub>2</sub> to the water, and eventually to the atmosphere.

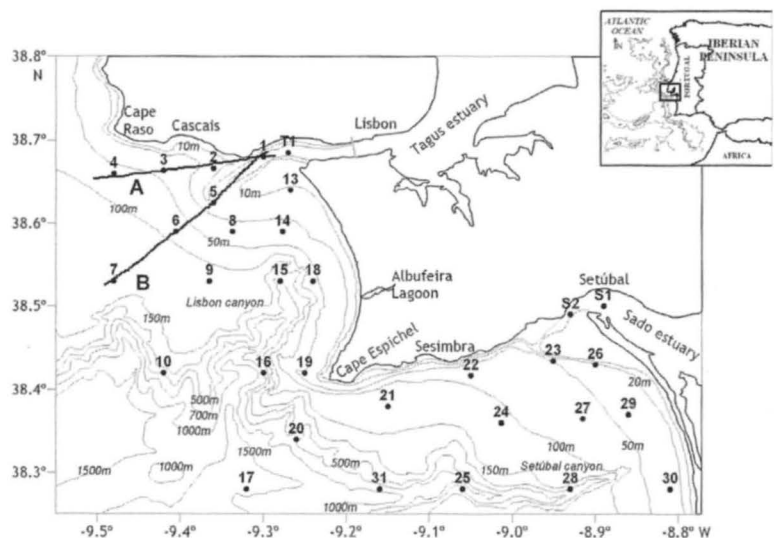
**Keywords** *Coccolithus braarudii*, calcification, alkalinity, temperature, coastal waters, upwelling

## 1. Introduction

Coccolithophores are one of the major phytoplankton groups in the oceans. They potentially play a unique role in the global climate system, mainly through three mechanisms: (1) by affecting the carbonate system of sea-water, involving the production of both particulate inorganic carbon, such as calcium carbonate (CaCO<sub>3</sub>) through calcification, and particulate organic carbon (POC) through photosynthesis (Armstrong *et al.*, 2002; Zondervan *et al.*, 2002; Rost & Riebesell, 2004); (2) by altering the heat exchange between sea-water and the atmosphere, due to increased light scattering by detached coccoliths (Buitenhuis *et al.*, 1996); and (3) by contributing to cloud albedo, with emissions of dimethylsulphide (DMS) (Buitenhuis *et al.*, 1996). The precipitation-dissolution of CaCO<sub>3</sub> by calcifying organisms has an impact on oceanic cycles, in terms of both dissolved inorganic carbon (DIC) and alkalinity (TA), and plays, thus, a significant role in the buffering capacity of sea-water and its functioning as a sink or a source of carbon dioxide (CO<sub>2</sub>) to the atmosphere (Zondervan *et al.*, 2001; Rost & Riebesell, 2004). Besides the fixation of dissolved CO<sub>2</sub> leading to a decrease of total CO<sub>2</sub> and inorganic nutrients in the water-column, coccolithophore production of CaCO<sub>3</sub> leads to an increase of surface CO<sub>2</sub> pressure (pCO<sub>2</sub>) and induces a parallel drop in TA which, in turn, shifts the DIC equilibrium in the

direction of CO<sub>2</sub> (Robertson *et al.*, 1994).

The purpose of this paper is to discuss a *Coccolithus braarudii* bloom episode associated with upwelling conditions, and its potential effect on the carbonate system, namely on CaCO<sub>3</sub> production and alkalinity patterns in the water-column. In addition, quantification of biocalcification is done in the CO<sub>2</sub> source/sink context.



**Figure 1:** Map of the study site showing sampling stations. A = Transect A (Stations 1, 2, 3, 4); B = Transect B (Stations 1, 5, 6, 7)

### 1.1 Study area

The present investigation was carried out along the Portuguese coast between 38.25° and 38.75°N and 8.85° and 9.60°W, mainly covering the continental shelf off-

shore Tagus and Sado Estuaries (Figure 1). The coastline of this region is interrupted by large capes (Raso and Espichel) and by pronounced embayments (Tagus and Sado). The hydrogeomorphological features are deeply marked by the intense discharge of the Tagus River, with an annual mean flow of  $350\text{ m}^3\text{ s}^{-1}$ , while the Sado's annual mean flow is only  $5\text{ m}^3\text{ s}^{-1}$ . The bottom topography of the area is dominated by the deep submarine canyons of Lisbon and Setúbal, which almost reach the coastline, thus creating strong bathymetric discontinuities. Seasonally, wind-driven upwelling events occur in the area (Fiúza, 1984). South of Lisbon, the upwelled water exhibits thermohaline (Fiúza & Halpern, 1982) and nutrient characteristics (Brogueira *et al.*, 1994), corresponding to the North Atlantic Central Water, whereas the thermohaline properties within the Setúbal canyon region indicate the presence of Mediterranean water (Ambar, 1983).

## 1.2 Previous work on phytoplankton

In past years, a number of studies have been undertaken on phytoplankton temporal and spatial variability along the Portuguese coast, and specifically on the coastal waters adjacent to the Tagus and Sado Estuaries (Abrantes & Moita, 1999; Cabeçadas *et al.*, 1999, 2000; Moita, 2001). In this paper, we focus on the phytoplankton group of coccolithophores, considering its potential importance on the biogeochemical cycling of carbon and the lack of information concerning their ecology in these waters.

For the Portuguese shelf, there are frequent records of the coccolithophore species *Coccolithus pelagicus* (up to  $4\text{ cells ml}^{-1}$ ) associated with the Iberian upwelling season and with temperature ranges between  $12$  and  $17^\circ\text{C}$  (Cachão & Moita, 2000). Recently, the larger cell-sized species from temperate waters have been identified as a different species named *Coccolithus braarudii* (Geisen *et al.*, 2004 and references therein), therefore this denomination will be adopted throughout this work.

## 2. Material and methods

The present investigation was carried out on board the *RV Mestre Costeiro* from 29th May to 7th June, 2002 (spring season) in the study area, where a total of 32 stations were sampled.

### 2.1 Physical, chemical and biological parameters

Temperature profiles were taken with a SeaBird-CTD probe calibrated with a high-accuracy reversed thermometer and an AutoSal salinometer. The Mixed Layer Depth (MLD) corresponded to the depth of water where the temperature between surface and depth was  $\geq 1^\circ\text{C}$ .

pH was measured immediately after collecting the samples using a Metrohm 704 pH-meter and a combination electrode (Metrohm), standardised against NBS buffers pH6.865 and pH9.180. Precision of the pH-measurements was  $\pm 0.01$ .

Discrete water-samples were taken using a CTD-

rosette system (SeaBird), equipped with 12 Niskin bottles (8l each) for determination of nutrients ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ),  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  (referred to herein as  $\text{NO}_3$ ,  $\text{NH}_4$  and  $\text{PO}_4$ , respectively) and  $\text{Si}(\text{OH})_4$ , dissolved oxygen (DO), chlorophyll *a* (Chla) and TA. Nutrient samples were filtered with MSI Acetate Plus filters ( $0.45\mu\text{m}$ ) and analyses were carried out on a Traacs Autoanalyser, following Tréguer & Le Corre (1975). DO was analysed onboard, following the Winkler method (Carrit & Carpenter, 1966), using a whole-bottle manual titration. The coefficient of variation associated with the method was 0.8-0.25%.

Samples for Chla and phytoplankton were collected from surface to bottom at shallow stations and from surface to 50m depth at deeper stations. Chla was measured by filtering triplicate aliquots of 50-100ml water-samples through Whatman GF/F filters. The filters were frozen immediately and later extracted in 90% acetone for analysis in a Perkin Elmer Fluorometer, using the modified protocol by Lorenzen (1967). Commercial solutions (Sigma Chemical Company) of Chla were used to calibrate the fluorometer.

TA was determined by potentiometric titration, according to Dickson & Goyet (1994). The 50ml GF/F (24 hours at  $450^\circ\text{C}$ ) filtered samples were titrated with HCl ( $\sim 0.25\text{M}$  HCl solution prepared with 1M Mallinckhodt standard solution in  $0.45\text{M}$  NaCl) past the end-point of 4.5. TA measurements with an accuracy of  $\pm 4\mu\text{mol kg}^{-1}$  were standardised against reference materials (CRMs) provided by Dr. Dickson of the Scripps Institution of Oceanography.  $p\text{CO}_2$  was estimated from pH and TA data, and calculations were performed with a program developed by APO (unpublished data). Constants were taken from Roy *et al.* (1993) and Weiss (1974). The error on the  $p\text{CO}_2$  calculation was estimated to be less than  $6\mu\text{atm}$ .

### 2.2 Phytoplankton samples

Phytoplankton samples were preserved with alkaline Lugol's solution and stored in the dark at  $4^\circ\text{C}$ . Phytoplankton cells were identified and counted (including large coccolithophore coccospheres) under an inverted Zeiss IM35 microscope, equipped with phase-contrast, by the Utermöhl technique (Hasle, 1988).

A 160x magnification was used to enumerate larger phytoplankton cells in the entire bottom-chamber, while smaller cells were counted in two transects with a 400x magnification. Single coccoliths were disregarded.

### 2.3 Coccolithophores

A 50ml sample was filtered on a 25mm-diameter Millipore filter (nominal pore-size of  $0.45\mu\text{m}$ ) immediately after arrival, using a 25mm Millipore filtration apparatus and under low vacuum. Filters were placed on a microscope slide and covered with a drop of immersion oil followed by a coverslip. The clarified filter was examined with a Zeiss Standard Axioskop Mod-2 microscope,

using an oil-immersion 100x objective, 2.5x optovar lens and 10x oculars. The material observed consisted mostly of *Coccolithus braarudii* coccospheres, some cells of its alternate motile *Crystallolithus* stage, and small coccospheres not identified ( $<5\mu\text{m}$  diameter). In the present study, 'cell density' numbers, as well as the  $\text{CaCO}_3$  estimations, concern *C. braarudii* calcified cells.

Smaller coccolithophore species ( $<5\mu\text{m}$  diameter) were disregarded, taking into account their relatively low contribution in terms of mass (2.3pg at mean length) when compared to the much greater per specimen mass (248pg at mean length) of *C. braarudii* species.

## 2.4 *Coccolithus braarudii* volume, calcite mass and organic carbon biomass

*Coccolithus braarudii* coccosphere size was measured by semiautomatic image analysis (Zeiss-KS100 3.0 software). The measured average coccosphere diameter ( $d\mu\text{m}$ ) was  $22.5\mu\text{m}$  for the sampled population, and mean coccosphere volume ( $V = d^3\pi/6$ ) was estimated as  $6157\mu\text{m}^3$ . The calcified volume of  $3079\mu\text{m}^3$ , corresponding to ~50% of total volume, was converted to mass by multiplying it by calcite density. An estimation of 8.3ng  $\text{CaCO}_3$  mass per coccosphere was obtained using the Young & Ziveri (2000) formula.

*C. braarudii* (coccosphere) was converted to biomass by using a conversion factor of 233pg C per cell, based on literature values for Atlantic samples, considering the measured average cell-size (Holligan *et al.*, 1993).

## 2.5 Particulate organic and inorganic carbon integrations along Transect B

The carbon biomass of total phytoplankton (including coccolithophores) was estimated from Chl *a* concentrations based on a carbon:chlorophyll ratio of 76, calculated from the linear regression of POC vs Chl *a* (measured values). Amounts of total phytoplankton carbon biomass were estimated from the carbon:chlorophyll ratio. *Coccolithus braarudii* organic and inorganic carbon were calculated, respectively, from abundance counts and the published carbon-per-cell conversion factor, and from counts to mass conversion. Total phytoplankton carbon, *C. braarudii* organic and inorganic carbon estimations were submitted to trapezoidal integration from surface to bottom, or 50m depth, at each station of Transect B and expressed as  $\text{mg C m}^{-2}$ . These estimations were extrapolated to the whole transect, considering its length (22.8km) and mean upper-column depth (31m). Total phytoplankton carbon and *C. braarudii* carbon, concerning the studied transect, are expressed in tons.

## 3. Results

### 3.1 Oceanographic and chemical conditions

The present investigation took place in a period of mod-

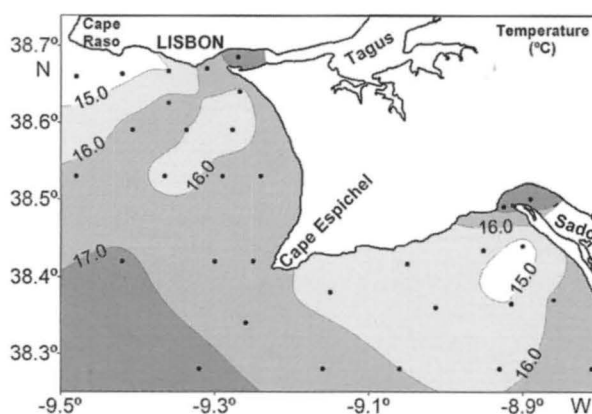


Figure 2: Surface temperature ( $^{\circ}\text{C}$ ) distribution covering continental shelf, offshore Tagus and Sado Estuaries, late spring, 2002

erate but persistent upwelling conditions, revealed by means of the Bakun Index ( $350\text{m}^3\text{ s}^{-1}\text{ km}^{-1}$ ) and by satellite images (Coastwatch, 2003). At surface, temperature distribution shows clearly the colder upwelled water along Transect A (Lisbon–Cape Raso), and a marked resurgence offshore Sado Estuary reveals temperatures below  $15^{\circ}\text{C}$  (Figure 2). The upwelled water induced sharp surface temperature gradients ( $14\text{--}18^{\circ}\text{C}$ ).

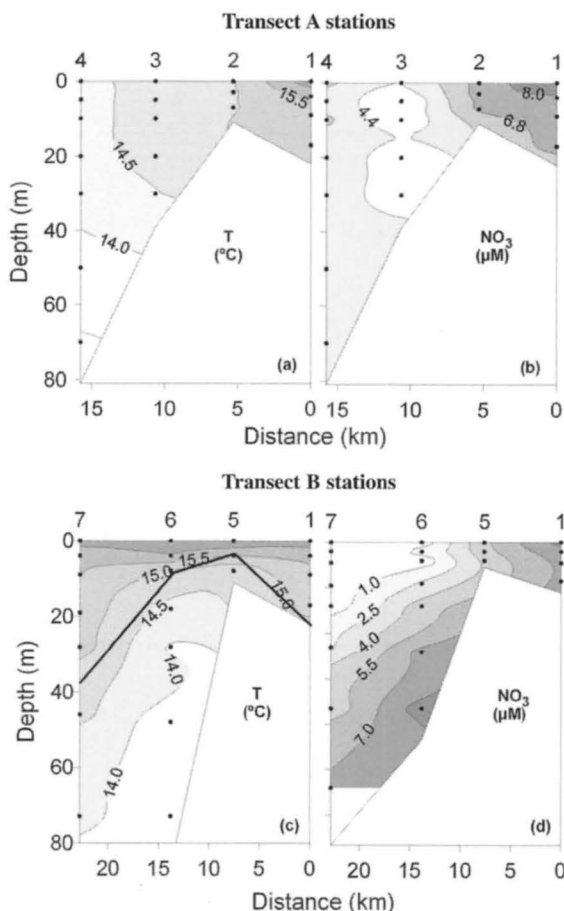


Figure 3: (a) Vertical temperature ( $^{\circ}\text{C}$ ) distribution and (b) vertical nitrate ( $\mu\text{M}$ ) distribution along Transect A; (c) vertical temperature ( $^{\circ}\text{C}$ ) distribution (emphasised line represents Mixed Layer Depth, based on temperature) and (d) vertical nitrate ( $\mu\text{M}$ ) distribution along Transect B

Along the studied Transects A (Stations 1, 2, 3, 4) and B (Stations 1, 5, 6, 7), vertical profiles of water temperature and nitrate (shown in Figure 3) evidenced also the upwelled cold water-mass enriched in nutrients from depth. Vertical patterns of phosphate and silicate were similar to the one of nitrate (not shown).

Along Transect A, the water-column was in general mixed, while along Transect B, owing to thermal stratification, MLD increased from inshore to offshore, reaching a depth of 28m at the most offshore station (Station 7). Nearly depleted nutrient conditions were noticed offshore in surface-waters and oxygen supersaturation was always present with values up to 115%. By that time, river plumes were very weak, indicating low discharge from both rivers:  $46\text{ m}^3\text{ s}^{-1}$  from the Tagus and  $1.0\text{ m}^3\text{ s}^{-1}$  from the Sado.

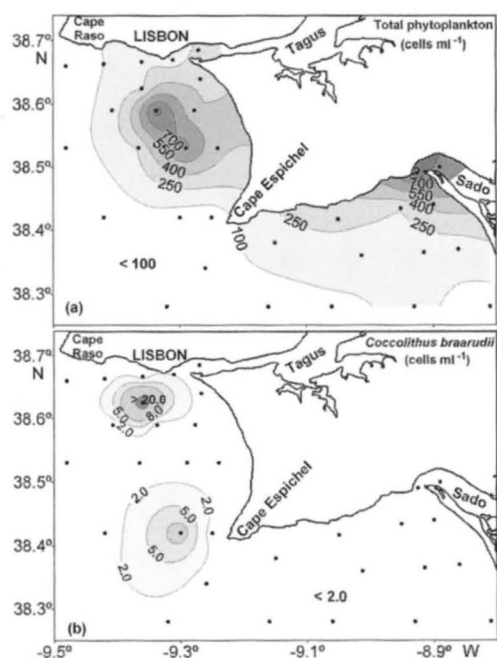
### 3.2 Phytoplankton abundance

The phytoplankton bloom was dominated by chain-forming diatoms, such as *Guinardia striata*, *Detonula pumila*, *Dactyliosolen fragilissimus* and *Pseudonitzschia* spp. The highest abundance was observed at the surface in the vicinity of the Lisbon submarine canyon head, reaching concentrations of up to 1000 cells  $\text{ml}^{-1}$ , which represents 74% of the total phytoplankton (Figure 4a). In this region, nutrients were almost exhausted ( $0.1 < \text{NO}_3 < 0.2\text{ }\mu\text{M}$ ;  $0.5 < \text{NH}_4 < 1.1\text{ }\mu\text{M}$ ;  $0.06 < \text{PO}_4 < 0.11\text{ }\mu\text{M}$ ;  $0.4 < \text{Si}(\text{OH})_4 < 1.4\text{ }\mu\text{M}$ ) and the water-column was stratified. MLD varied between 10 and 20m.

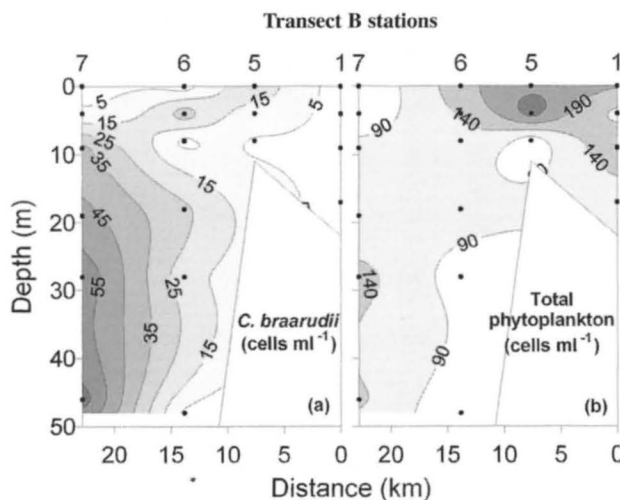
Spatially differentiated from the diatoms occurred a proliferation of the coccolithophore *Coccolithus*

*braarudii*. At the surface, offshore Tagus Estuary, *C. braarudii* densities of 22.5 cells  $\text{ml}^{-1}$  were attained, while south of that area, close to Cape Espichel, densities of 1 cell  $\text{ml}^{-1}$  (Figure 4b) were registered. The nutrient regimen for the first area was influenced by the Tagus Estuary plume ( $4.0 < \text{NO}_3 < 5.2\text{ }\mu\text{M}$ ;  $0.4 < \text{NH}_4 < 1.0\text{ }\mu\text{M}$ ;  $0.6 < \text{PO}_4 < 0.8\text{ }\mu\text{M}$ ;  $1.6 < \text{Si}(\text{OH})_4 < 2.3\text{ }\mu\text{M}$ ), while the second area showed concentrations typical of an offshore region ( $0.1 < \text{NO}_3 < 0.5\text{ }\mu\text{M}$ ;  $0.2 < \text{NH}_4 < 0.3\text{ }\mu\text{M}$ ;  $0.04 < \text{PO}_4 < 0.09\text{ }\mu\text{M}$ ;  $0.3 < \text{Si}(\text{OH})_4 < 0.4\text{ }\mu\text{M}$ ). Across both areas, MLD varied between 5 and 10m.

*C. braarudii* highest densities occurred along Transect B, showing densities of up to 60 cells  $\text{ml}^{-1}$  at the most offshore station (St.7) between 30 and 50m depth (Figure 5a) where nutrient concentrations were quite low ( $0.1 < \text{NO}_3 < 2.3\text{ }\mu\text{M}$ ;  $0.1 < \text{NH}_4 < 0.2\text{ }\mu\text{M}$ ;  $0.09 < \text{PO}_4 < 0.20\text{ }\mu\text{M}$ ;  $1.0 < \text{Si}(\text{OH})_4 < 1.3\text{ }\mu\text{M}$ ) and the MDL deeper (27m). In contrast, total phytoplankton reached higher abundances inshore at subsurface (up to 240 cells  $\text{ml}^{-1}$ ), showing an opposite spatial pattern to that observed for the coccolithophores (Figure 5b).



**Figure 4:** Surface distributions in Tagus and Sado adjacent coastal waters, late spring, 2002. (a) Total phytoplankton (cells  $\text{ml}^{-1}$ ); (b) *Coccolithus braarudii* (cells  $\text{ml}^{-1}$ )



**Figure 5:** Transect B distributions. (a) Vertical *Coccolithus braarudii* (cells  $\text{ml}^{-1}$ ); (b) vertical total phytoplankton (cells  $\text{ml}^{-1}$ )

### 3.3 Phytoplankton organic and inorganic carbon estimations for Transect B

For the entire Transect B, an amount of ~5 tons of POC was estimated for the total phytoplankton biomass (including coccolithophores). Considering that *Coccolithus braarudii* corresponded to 0.1 ton of organic carbon, it can be roughly inferred that this species accounted for 2% of the total phytoplankton biomass.

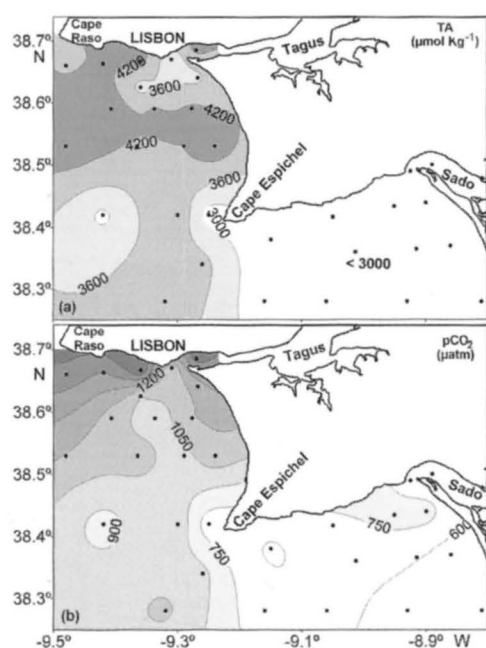
Integration of *C. braarudii*  $\text{CaCO}_3$  over depth yielded a total of  $6.7\text{ g CaCO}_3\text{ m}^{-2}$  for the area defined by Transect B. Considering the referred area, we estimate ~5 tons of calcite were produced in the upper 30m of water.

### 3.4 The carbonate system

A striking feature in the alkalinity distribution of the stud-



ied waters was the obvious difference in pattern between the areas north and south of Cape Espichel. North Cape Espichel and adjacent to the Tagus Estuary, TA displayed values as high as  $4600 \mu\text{mol kg}^{-1}$ , while south of that cape, and adjacent to the Sado Estuary, TA showed values ranging from 2300 to  $2600 \mu\text{mol kg}^{-1}$  (Figure 6a). The two estuarine sources have exhibited quite different alkalinities (Tagus:  $3560 \mu\text{mol kg}^{-1}$ ; Sado:  $2620 \mu\text{mol kg}^{-1}$ ), revealing differences in the transported material to the coastal waters. From TA and pH values, DIC concentrations were determined by using Roy *et al.*'s (1993) set of constants. A similar pattern of distribution to that obtained for TA is observed, with values ranging from 2050 to  $4200 \mu\text{mol kg}^{-1}$ . Both TA and DIC behaved non-conservatively. The entire study area exhibited elevated values of TA and DIC, which were probably related to these upwelled waters enriched in TA and DIC. South of Cape Espichel and adjacent to the Sado Estuary, values were lower, since the upwelled water indicates the presence of Mediterranean Water while north of that cape and adjacent to the Tagus Estuary, the upwelled water corresponded to the North Atlantic Central Water.



**Figure 6:** Distributions in Tagus and Sado adjacent coastal waters, late spring, 2002. (a) Surface TA ( $\mu\text{mol kg}^{-1}$ ); (b)  $p\text{CO}_2$  ( $\mu\text{atm}$ )

It is clear from Figure 6b that the entire study area was supersaturated with respect to  $p\text{CO}_2$  ( $470\text{--}1770 \mu\text{atm}$ ), with oversaturation with respect to atmospheric equilibrium (presently  $\sim 360 \mu\text{atm}$ ) close to 490%. Oversaturation of  $p\text{CO}_2$  was probably due to the transport of higher quantities of inorganic carbon from deep, colder upwelled water. This feature was more striking along Transect A, although induced remineralisation due to the presence of a peak in organic matter (not shown) may also have

accounted for the elevated  $p\text{CO}_2$  values (up to  $1770 \mu\text{atm}$ ).

#### 4. Discussion

The described scenario seems representative of a declining bloom episode, taking place during late spring in coastal waters under upwelling conditions, showing nutrients, to a certain extent, exhausted throughout the water-column. However, satellite images clearly point out that upwelling conditions persisted during the phytoplankton bloom until the end of June. Diatoms proliferated well throughout turbulent waters, while coccolithophore development required more stable conditions and coincided with lower nutrient levels. Apparently, coccolithophores found optimal growth conditions close to a frontal zone established between a deep, colder, upwelled mixed water-mass, located alongshore Lisbon–Cape Raso, and a thermally stratified water-mass which occupies the Tagus and Sado Bays (Figures 4a, b). Some cells of *Crystallolithus braarudii* (*Coccolithus braarudii* alternate motile stage), clearly exhibiting flagella and crystalloliths, were observed at Transect A, associated with a colder and mixed, upwelled water-mass (Figure 2), probably indicating that the motile phase could have been an important component of the Tagus Bay population. The simultaneous occurrence of *C. braarudii* and its alternate motile *Crystallolithus* stage have never been reported for the Portuguese shelf. The growth of these motile cells could have been triggered and amplified by the local environmental conditions over the non-motile *C. braarudii* population and are reflected, to a certain extent, in the alkalinity distribution in the area (Figure 6a). As a matter of fact, TA distribution at Transect A (especially Stations 2, 3 and 4) and B (particularly Stations 6 and 7) cannot be explained only by a variable alkalinity estuarine source, alkalinity changes due to biological activity, and upwelled waters with high TA. Considering that a mean rain ratio (the ratio of particulate inorganic to organic carbon in exported biogenic matter) of  $\sim 1$  was achieved in Transect B waters, revealing that photosynthesis and calcification processes were in equilibrium, the sharp alkalinity rise in surface-waters adjacent to the Tagus Estuary could possibly be related to the dissolution of *C. braarudii* cells. At this transect, the  $\sim 5$  tons of calcite produced in the upper 30m of water confirm that significant  $\text{CaCO}_3$  deposition can occur in coastal temperate ecosystems. Holligan *et al.* (1993) estimated  $7.2 \times 10^4$  tons of calcite in the upper 60m of water for a bloom of coccolithophores ( $40 \text{ g CaCO}_3 \text{ m}^{-2}$ ) covering  $7200 \text{ km}^2$ , which he mentions to be an order of magnitude smaller than the annual  $\text{CaCO}_3$  production for the European shelf by coccolithophores. Considering a sinking rate for *C. braarudii* coccospheres of about  $7 \text{ m d}^{-1}$  (Young, 1994), an average  $\text{CaCO}_3$  precipitation rate of  $1.1 \text{ g CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$  can be estimated along the referred transect, this being a value within the ranges of carbonate production found in the literature for coccolithophores (Fernández *et al.*, 1993; Holligan *et al.*, 1993). Actually, a

calcification rate of  $1.7 \text{ g CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$  was observed during a coccolithophore bloom survey in the NE Atlantic (Holligan *et al.*, 1993).

Concerning Transect B, and using the molar ratio (Y) of  $\text{CO}_2$  released versus  $\text{CaCO}_3$  precipitated (Frankignoulle *et al.*, 1994), which attained the mean value of 0.66, it was possible to calculate the fraction of  $\text{CO}_2$  released to the water during calcification. Through the  $11.2 \text{ mmol CaCO}_3 \text{ m}^{-2} \text{ d}^{-1}$  estimated for the total population of *C. braarudii*, a value of  $7.4 \text{ mmol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$  was calculated, indicating that the calcification process constitutes an additional source of  $\text{CO}_2$  to the water and, eventually, to the atmosphere.

*C. braarudii* calcification/dissolution in the surface layer modifies upper-ocean alkalinity and directly affects air/sea  $\text{CO}_2$  exchanges. The results obtained are in agreement with recent works (e.g. Rost & Riebesell, 2004), which indicate that productivity and distribution of coccolithophores are sensitive to  $\text{CO}_2$ -related changes in environmental conditions, both directly through acidification of surface sea-water, and indirectly through increasing upper-ocean thermal stratification.

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