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COCCOLITHOPHORES

IN COASTAL WATERS: LISBON BAY, PORTUGAL

COCOLITÓFOROS EM ÁGUAS COSTEIRAS: BAIA DE LISBOA, PORTUGAL

Alexandra Maria Azinheira Duarte Silva

Tese orientada por

Professora Doutora Vanda Brotas

Doutora Teresa Moita

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NOTA

Na presente dissertação incluem-se trabalhos que foram objecto de publicação em colaboração com M.T. Moita, V. Brotas, S. Palma, P.B. Oliveira e C.R. Mendes.

De acordo com o paragrafo 1 do Artigo 40, Capitulo V, do Regulamento de Estudos Pós Graduados da Universidade de Lisboa, publicado no Diário da Répubblica – II Série No. 153, de 5 de Julho de 2003, clarifico que os artigos científicos publicados (3) e submetido (1) em revistas científicas indexadas, compõem na totalidade a presente dissertação. Tendo estes trabalhos sido feitos em colaboração, a candidata delara que interveio na concepção e elaboração do trabalho experimental, na interpretação dos resultados e na responsabilidade da redacção dos manuscritos enviados para publicação.

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Alexandra Maria Azinheira Duarte Silva

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RESUMO

A presente dissertação foca o estudo de cocolitóforos em águas costeiras da baia de Lisboa. Com o objectivo de identificar espécies indicadoras de diferentes massas de água e processos oceanográficos, foram estudados os padrões de distribuição e abundância sazonal e inter-anual dos cocolitóforos (Capitulos 2 and 3). Analisaram-se possíveis relações com diferentes parâmetros ambientais e qual o nicho ecológico ocupado pelos cocolitóforos (Capitulo 4), em relação a outros membros da comunidade de fitoplâncton (diatomáceas e dinoflagelados). Procedeu-se à determinação de pigmentos por HPLC (High Performance Liquid Chromatography) para avaliar a aplicação desta técnica no estudo de variações temporais da comunidade fitoplanctónica (Capitulo 5).

De Julho de 2001 a Maio de 2005, numa estação fixa da baía de Lisboa (38º41'N, 09º24'W), foram efectuadas colheitas semanais de água do mar, uma hora antes do pico da maré-cheia, e foram medidos e determinados diferentes parâmetros físico-químicos e biológicos (afloramento, temperatura, salinidade, nutrientes, clorofila *a* e fitoplâncton). As amostras de água para análise de cocolitóforos foram filtradas e as espécies foram identificadas, contadas e medidas com um microscópio óptico com luz polarizada e com um microscópio electrónico de varrimento. As amostras de água para análise de outros grupos de fitoplâncton foram preservadas com formol neutro e as células foram identificadas e enumeradas pela método de Utermöhl, utilizando um microscópio invertido com contraste de fase. No último ano de amostragem foram também efectuadas colheitas para análise de pigmentos por HPLC. A representatividade do local de amostragem enquanto indicador das variações do fitoplâncton a nível regional foi comprovada. A presença de espécies indicadoras de afloramento ou convergência assegura a influência de distintos processos costeiros. A reduzida profundidade do local amostrado e efeitos antropogénicos foram considerados durante a interpretação dos resultados em particular na análise das temperaturas de superfície no Verão e aumento de nutrientes no Inverno.

Durante os quatro anos de estudo a comunidade de fitoplâncton apresentou alterações sazonais e inter-anuais. A concentração máxima dos principais grupos reflectiu o ciclo hidrológico sazonal das regiões temperadas. Concentrações máximas desde a Primavera até ao Outono associadas a períodos de turbulência variável e

mínimas no Inverno associadas a períodos de mistura e maior disponibilidade de nutrientes que os restantes períodos. A persistência das condições de afloramento e condições associadas poderá ter estado na base do aumento da biomassa (Chl *a*) ao longo dos quatro anos (0,76 $\mu g.l^{-1}$). A Chl *a* reflectiu as principais variações na comunidade fitoplanctónica e os pigmentos detectados por HPLC apresentaram uma boa correlação com os diferentes grupos identificados. Nos anos de 2002 e 2004 registaram-se as abundâncias mais elevadas de fitoplâncton. Em 2002, os períodos de afloramento e convergência foram bem distintos e a precipitação foi reduzida. A comunidade foi dominada por diatomáceas nos períodos de turbulência e por cocolitóforos nos períodos de advecção de águas oceânicas. Em 2004 observaram-se longos períodos de afloramento fraco mas persistente. A comunidade foi dominada por diatomáceas e em vez de cocolitóforos, por dinoflagelados que apresentaram dois picos curtos mas com a máxima concentração registada. Em 2003 os longos períodos de intensa precipitação e consequente aumento da escorrência costeira e as baixas salinidades (<30) e temperaturas resultaram numa diminuição das concentrações máximas de fitoplâncton, observadas mais tarde no ano.

Ao longo dos 4 anos os nutrientes estiveram regularmente disponíveis, em parte devido às características de retenção da baia de Lisboa e do local de amostragem em particular. As oscilações mais marcadas coincidiram com períodos de intensa precipitação e associada escorrência costeira (e.g. silicatos) e com máximos fitoplanctónicos. A influência do afloramento no input de nutrientes ao ponto amostrado não foi directamente observada.

A comunidade de fitoplâncton foi maioritariamente composta por diatomáceas, dinoflagelados e cocolitóforos que representaram mais de 90% dos indivíduos contabilizados. Os pigmentos fucoxantina, peridinina e 19'-hexanoiloxifucoxantina surgiram como bons indicadores de diatomáceas, dinoflagelados e cocolitóforos, respectivamente. Os pigmentos apresentaram uma variação sazonal e uma correlação positiva significativa com cada um dos grupos de que são indicadores. Além disso, vários outros pigmentos como a clorofila *b*, zeaxantina, violaxantina, neoxantina, prasinoxantina e aloxantina foram também identificados e representam uma comunidade de indivíduos das divisões Euglenophyta, Clorophyta, Cianophyta, Prasinophyta e Criptophyta, respectivamente, não tendo sido os indivíduos das quatro últimas divisões identificados por microscopia óptica.

As diatomáceas foram o grupo mais abundante durante a Primavera e a sua variabilidade pareceu condicionada pela persistência das condições de afloramento, luz e disponibilidade de silicatos. A predominância de ciclos/pulsos curtos de afloramento ao longo dos 4 anos pareceram desfavoráveis à manutenção e desenvolvimento de diatomáceas típicas de estádios mais avançados da sucessão fitoplanctónica.

Os cocolitóforos foram o segundo grupo mais abundante e enquanto grupo tipicamente "oceânico", revelaram uma ampla tolerância aos diferentes processos costeiros como a turbulência. A distribuição e desenvolvimento desta comunidade sugerem um nicho ecológico de características intermédias, entre as diatomáceas e os dinoflagelados. A concentração do grupo decresceu ao longo dos 4 anos, devido à diminuição da intensidade e duração dos períodos de convergência e do aumento do número de dias com afloramento, em especial entre o Outono e o Inverno. A abundância de nitratos, em traços gerais, pareceu condicionar o desenvolvimento do grupo contudo cada espécie surgiu associada a diferentes proporções dos vários nutrientes determinados. Em oposição às diatomáceas, a composição dos cocolitóforos apresentou uma variação sazonal e o grupo desenvolveu-se num intervalo mais amplo de condições oceanográficas. As espécies mais abundantes durante o Verão foram *Helicosphaera carteri*, *Coronosphera mediterranea*, *Rhabdosphaera clavigera*, *Syracosphaera pulchra*, *E. huxleyi* e *Gephyrocapsa* spp.. As primeiras quatro espécies representaram a comunidade de Verão - Outono indicadora da presença costeira de águas subtropicais. *E*. *huxleyi*, *G*. *muellerae* e *G*. *ericssonii* surgiram associadas a águas frias do inicio do estação de afloramento, durante a Primavera, enquanto que *G*. *oceanica* dominou os períodos de produtividade durante o Verão. A presença de *E*. *huxleyi* e *Gephyrocapsa* spp. pode ser indicadora de ambientes produtivos associados a áreas de afloramento. Sendo uma espécie indicadora de frentes de afloramento, a abundância de *Coccolithus pelagicus* na baía de Lisboa pode expressar a posição da pluma de afloramento localizada no cabo da Roca, em relação ao local de amostragem. As espécies *Calcidiscus quadriperforatus* e *Calcidiscus leptoporus*, dominaram a comunidade de fitoplâncton no Inverno e revelaram ser indicadoras da advecção de águas superficiais oceânicas para a zona costeira, durante o Inverno e início da Primavera. Uma intensificação das condições de afloramento é desfavorável ao desenvolvimento costeiro destas espécies.

Os dinoflagelados, tal como alguns cocolitóforos, desenvolveram-se preferencialmente em condições de fraca turbulência ou estratificação termica,

apresentando máximos durante o Verão. As baixas concentrações registadas ao longo dos quatro anos parecem reflectir uma baixa tolerância à intensificação das condições de afloramento ou uma desvantagem competitiva relativamente ao cocolitóforos.

As colheitas semanais permitiram uma observação pormenorizada do fitoplâncton e de como as espécies variam em resposta aos pulsos de afloramento e dinâmica costeira. A amostragem intensiva foi crucial para determinar associações entre as espécies e os regimes oceanográficos locais. A abordagem quimio-taxonómica demonstrou ser uma forma útil e expedita de analisar alterações gerais na comunidade de fitoplâncton, e de reconhecer a presença de taxa difíceis de identificar e enumerar por microscopia. Contudo, a análise microscópica mostrou-se decisiva na confirmação exacta da assinatura dos pigmentos marcadores dos grupos de fitoplâncton e na interpretação dos valores máximos, permitindo um estudo efectivo da estrutura e dinâmica da comunidade de fitoplâncton.

Palavras-chave: cocolitóforos, fitoplâncton, sucessão temporal, afloramento, pigmentos marcadores, Portugal.

ABSTRACT

The present thesis is focus on coccolithophores in coastal waters of Lisbon bay. Their biodiversity, abundance and distribution patterns were analyzed in order to identify potential proxies for different local water bodies and environmental conditions (Chapters 2 and 3). The seasonal and interannual distribution patterns of the different species and their relationships with environmental parameters are addressed. It was also investigated the ecological niche of coccolithophores (Chapter 4), as a group, in relation to other members of the phytoplankton community (diatoms and dinoflagellates) as well as the use of pigments, determined by HPLC (High Performance Liquid Chromatography), as chemo-taxonomic tools to monitor time scale changes of phytoplankton groups (Chapter 5).

From July 2001 to May 2005 seawater samples were collected once a week at a fixed station in Lisbon bay (38º41' N, 09º24' W), one hour before high tide, and physical-chemical and biological parameters measured and determined (upwelling, temperature, salinity, nutrients, chlorophyll *a* and phytoplankton). Samples for coccolithophores were filtered and species were identified, counted and measured with an optical microscope under cross-polarized light and with a scanning electron microscope. Samples for analyzes of other phytoplankton groups were preserved with buffered formalin and cells were identified and enumerate by the Utermöhl technique, using an inverted microscope with phase contrast and bright field illumination. During the last year, samples were also collected for pigment analysis by HPLC. It was proved that the sampling site was an indicator of the phytoplankton variations at a regional scale. The presence of species typical from upwelling or convercence situations insures the influence of distintic coastal processes. The shallow depth of the sampling station and antropogenic effects were considered during the interpertation of the results in particular the analisis of SST during summer and the increase in nutrients during winter.

During these four years of the study, the phytoplankton community varied from seasonal to interannual scales. Maxima of major groups reflected the seasonal hydrographic cycle of a temperate region. Maxima from spring to autumn associated with different levels of turbulence and minima related to water mixture and a greater availability of nutrients than in the other seasons.

The persistence of upwelling and higher SST (sea surface temperature), observed earlier in the year, were probably responsible by an increase in biomass (Chl *a*), through the years (0,76 $\mu g.l^{-1}$). Chlorophyll *a* reflected the major trends in phytoplankton development and the pigments detected under the HPLC showed a good correlation with phytoplankton identifications. During 2002 and 2004 the highest phytoplankton concentrations were recorded. In 2002 the upwelling and downwelling seasons were clearly distinguished and precipitation was low. The community was dominated by diatoms under prevailing turbulence and by coccolithophores during onshore advection. Contrasting, 2004 was characterized by longer periods of mild upwelling and the assemblage was dominated by diatoms and, instead of coccolithophores, by dinoflagellates with two short and expressive peaks. In 2003, the longer periods of intense precipitation and consequent increase of river runoff, and and lower salinities (<30) and temperatures resulted in minor phytoplankton concentrations that were observed later in the year.

During the four years nutrients were normally available in part due to the retention caracteistics of Lisbon Bay and from the sampling site in particular. The strongest fluctuations coincided with periods of intense precipitation and runoff (e.g. silicates) and with phytoplankton maxima. The influence of upwelling in the input of nutrients in the sampling station was not directly observed.

The phytoplankton community was mainly composed by diatoms, dinoflagellates and coccolithophores which represented more than 90% of the assemblage. Fucoxantin, peridinin and 19'-hexanoyloxyfucoxanthin appeared as good indicators, for diatoms, dinoflagellates and coccolithophores, respectively, with synchronized seasonal variations and significant positive correlations. In addition several minor pigments were also detected by chromatography, such as chlorophyll *b*, zeaxanthin, violaxanthin, neoxanthin, prasinoxanthin and alloxanthin which we considered as representing an assembly of euglenophytes, chlorophytes, cyanobacteria, prasinophytes and cryptophytes. Cells from the last four divisions were not identified by microscopy.

Diatoms were the most abundant group during spring and species variability seemed influenced by the persistence of upwelling conditions, light and availability of silicates. The dominance of short upwelling pulses through the 4 years seemed unfavourable for diatoms maintenance and for the development of diatoms from later stages of phytoplankton sucession.

Coccolithophores were the second most abundant group, usually considered as an oceanic group, appeared capable of resisting to coastal processes such as turbulence. The distribution and development of this assemblage suggest an ecological niche with intermediate features, between diatoms and dinoflagellates. The group decreased throughout the sampling probably as a result of shorter and less intense convergence periods and of an increase in the number of days with upwelling, especially from autumn until winter. The availability of nitrites, in general terms seemed to influence the development of this group however each specie appeared associated with differente nutrient ratios

In opposition to diatoms, coccolithophore composition changed seasonally and the group thrived in a remarkable variety of oceanographic conditions. The most abundant species during summer – autumn were *Helicosphaera carteri*, *Coronosphera mediterranea*, *Rhabdosphaera clavigera*, *Syracosphaera pulchra*, *E. huxleyi* and *Gephyrocapsa* spp.. The first four species represent a summer-autumn assemblage reliable to trace the presense over the shelf of subtropical waters. *E. huxleyi*, *G. muellerae* and *G. ericssonii* indicated the presence of colder waters associated with the beginning of the upwelling season usual during spring while *G. oceanica* was particularly indicative of productive periods during summer. The presence of *E. huxleyi* and *Gephyrocapsa* spp. can be used as a proxy of highly productive environments generated by upwelling and surrounding areas of an upwelling center. As a proxy for the presence of an upwelling front, the abundance of *Coccolithus pelagicus* in Lisbon bay can indicate the position of the upwelling plume rooted at Cape Roca, in relation to the Cascais site. The species *Calcidiscus quadriperforatus* and *Calcidiscus leptoporus* dominated the phytoplankton community during winter and can be pointed out as tracers for the advection of surface offshore subtropical waters over the shelf, during winter and early spring. An intensification of the upwelling conditions seemed unfavourable to the coastal development of these species

Dinoflagellates, like coccolithophores, preferred stratified conditions in warmer waters, with maxima during summer. This group reached lower concentrations through the four years what was related to the decrease of lasting convergence periods, indicating a narrow tolerance to changes in turbulence and temperature.

Sampling on a weekly basis allowed exhaustive observations of phytoplankton composition and how species varied significantly as a response to upwelling pulses and coastal dynamics. Such an effort was crucial to determine accurate associations

between species and different regional oceanographic regimes. The chemotaxonomic approach was a helpful and faster way of analyze larger changes of the phytoplankton community, and to recognize the presence of phytoplankton taxa difficult to identify and enumerate by microscopy. However, microscopic studies were critical to an exact assignment of marker pigments to phytoplankton taxa and in the interpretation of peaks and thus permiting a reliable study of phytoplankton community structure and dynamics.

Keywords: coccolithophores, phytoplankton, time-series, upwelling, biomarker pigments, Portugal.

CHAPTER 1: INTRODUCTION

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1.1. COCCOLITHOPHORES

Coccolithophores are one of the main groups of marine phytoplankton, widespread throughout the oceans. They are important primary producers in the photic zone, directly dependent on changing gradients in surface waters (Henriksson, 2000) and with a significant role in biogeochemical cycles and climatic processes. Sournia et al. (1991) refered the marine phytoplankton is composed by 5000 species. Among the major taxonomic groups, and those considered in the present study, are the calcareous nannoplankton or coccolithophores (Prymnesiophyceae), the subject of this thesis, the diatoms (Bacillariophyceae) and the dinoflagellates (Dinophyceae). In a review of modern coccolithophore taxonomy Young et al. (2003) described 280 coccospheres types. These include previously described and many non-described species, along with informally described morphotypes and approximately 80 holococcolith-bearing taxa. Based on this data set, Young et al. (2005) estimated extant coccolithophore diversity at between 200 (the number of known forms excluding holococcolithophores) and 500 (possible total diversity assuming a moderate degree of cryptic speciation).

Classification and terminology

Coccolithophores are formally classified in the Kingdom Chromista, phylum Haptophyta and class Prymnesiphyceae (Guiry and Guiry, 2008). The group is distinctive from other phytoplankton in that at some point in their life-cycle they precipitate $CaCO_3$ in the form of calcite platelets or coccoliths, which surround the cell to form the exoskeleton or coccosphere. The coccosphere shape can vary considerably and may be spherical to ovoid to ellipsoidal in form or display elaborate modified coccolith appendages. The taxonomy of coccolithophores is primarily based on the morphology of the coccoliths and morphometric studies of species revealed a high morphological variability that can be associated with environmental parameters, genetic variability or ecophenotype (Knappersbusch et al., 1997; Bollmann, 1997; Renaud and Klaas, 2001; Sáez, et al., 2003). The calcareous exosqueleton or coccosphere of motile coccolithophores possesses a flagellar opening at the apical pole thought which the flagella and haptonema pass, and an opposite antiapical pole

(Figure 1). Besides to the body coccoliths which cover the cell, polar coccoliths, morphologically differentiated, can occur at both poles. Those surrounding the flagellar pore are termed circum-flagellar or apical coccoliths and those at the opposite pole, antapical coccoliths. Species with coccospheres possessing just one morphologically distinct type are refered to as monomorphic, those comprise of two discrete forms on a single coccosphere as dimorphic, and those presenting more then two discrete types as polymorphic.

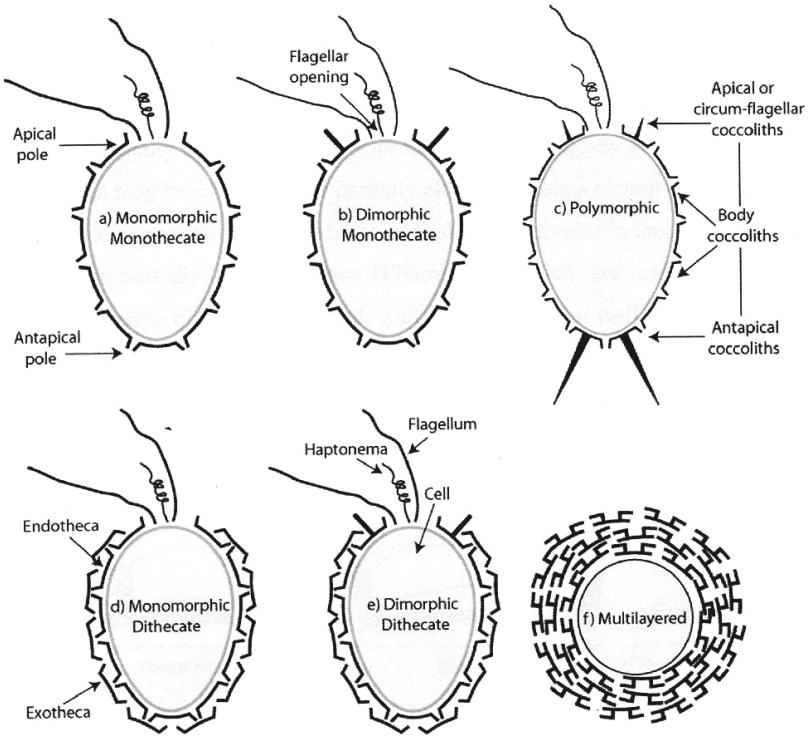

Figure 1 - Coccosphere characteristics and classification according to coccolith types (redrawn from Young et al. 1997).

Coccolithophores may have as well one or more distinct layers of coccoliths, or theca. A single layer of coccoliths compose a monothecate coccosphere while a dithecate coccosphere have two discrete layers formed from different coccolith types; an inner endotheca with endothecal coccoliths and an outer exotheca composed of exothecal coccoliths. *Emiliania huxleyi*, for instance, has two or more layers with no coccolith differentiation and is termed multilayered (Figure 1).

Two main types of coccoliths can be observed heterococcoliths and holococcoliths, according to the biomineralisation mode thought which they are formed (Figure 2a, b).

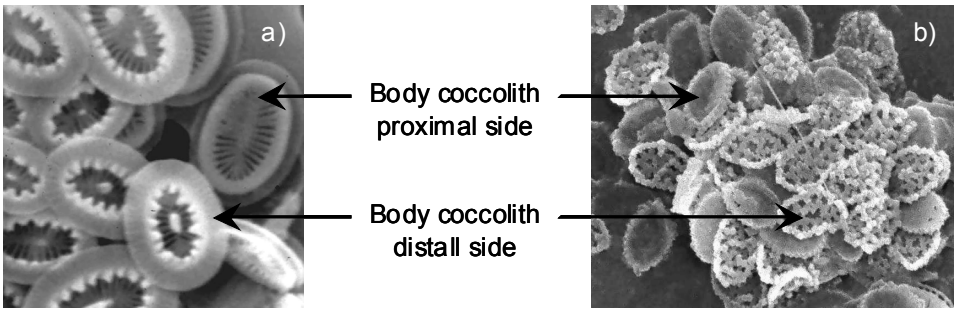

Figure 2 – Representatives of three main two main coccolithophore types: a) heterococcoliths and b) holococcoliths.

Lacking the typical features of hetero- or holococcoliths, but displaying features generic to coccolith structure, are a third minor group termed nannoliths. These calcareous structures are of uncertain origin and are thought to be formed by a different mode of biomineralisation (Young et al., 1999). Although many representatives exist in the fossil record only two extant nannolith-bearing families exist, Braarudosphaeraceae and Ceratolithaceae.

Heterococcoliths are the more commonly found type of coccoliths, composed of a radial array of complex crystal units, of variable shapes and sizes (Young et al., 1997). These in general exhibit an inner central area surrounded by an outer margin or rim. The central area may be completely open or in part closed, include a central opening or show a protruding spine or process. Three shapes occur frequently across genera which are useful in the description of the group (Figure 3): placoliths exhibit a rim with two or more developed shields, muroliths display a prominent, wall-like rim and are essentially bowl shaped and planoliths display a low rim so the coccolith is essentially planar.

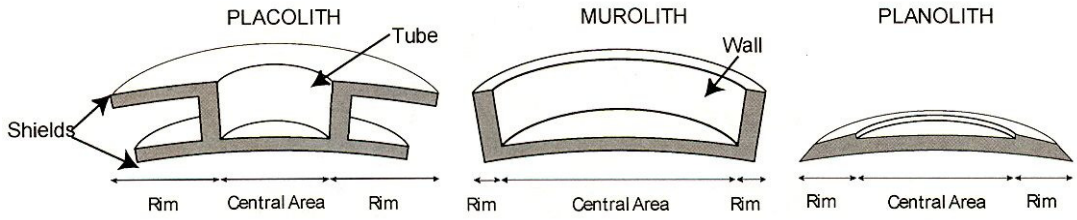

Figure 3 – Structural morphology of heterococcoliths (redrawn from Young et al., 2003).

Moreover, Young et al. (1997) also refer other specific cases with informal descriptive terms for coccoliths based on the morphology of the taxa as helicoliths which exhibit a helical flange characteristic of Helicosphaeraceae; rhabdoliths are planoliths from Rhabdosphaeracea and caneoliths, endothecal muroliths of Syracosphaeraceae.

In contrast, holococcoliths are formed by numerous minute identical euhedral crystallites arranged in continuous arrays (Figure 2b). The arrangement pattern of crystallites includes a simple hexagonal array, hexagonal meshwork with regular crystallite perforations or rhomboid crystallites in a rhombohedral array. The description of the group include three main forms, cavate coccoliths, with a near-continuous rim which covers a large cavity; septate coccoliths, where the area inside the rim is subdivided by walls or septae; and solid coccoliths, formed from a mass of crystallites, with or without depressions or pores. Unlike hetecoccoliths, informal descriptive terms for holococcolith morphotypes are not taxa restricted but occur independently across the group (Young et al., 1997). These terms are useful in a purely descriptive manner (Figure 4). Laminoliths are formed of several layers and can be solid or exhibit pores; calyptroliths are formed from an almost continuous domal cavate distal-cover; crystalloliths are disc-like solid holococcoliths formed of one to two layers of crystallites; zygoliths have a wall extended by a bridge shaped distal cover; which in helladoliths expands distally into a double layered leaf-like process. Fragarioliths possess a proximal layer of crystallites surmounted by a large blade like process and.

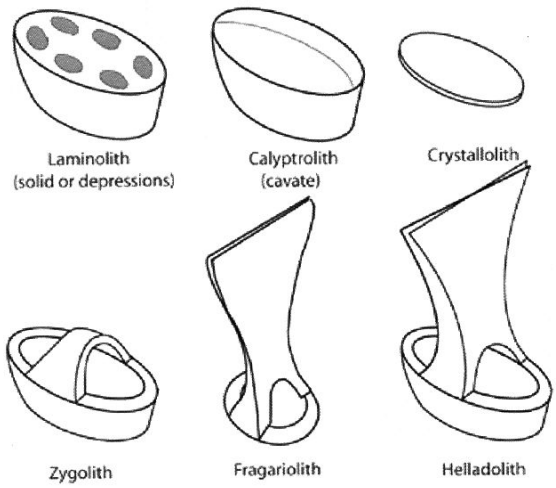

Figure 4 – Three main forms of holococcoliths (redrawn from Young et al., 1997).

Biology

Investigations into the biology of coccolithophores began in the late 19th and early 20th centuries (Murray and Blackman, 1898; Lohmann, 1902; Kamptner, 1927) and the introduction of scanning electron microscope in coccolithophore studies (Braarud et al., 1952; Braarud and Deflangre, 1955) renewed the interest in the group. Members of the calcareous nannoflora coccolithophore cells are minute, and generally range in size from 3-30 μm. As explained before, two major groups of coccoliths exist, produced by different modes of biomineralisation, holococcoliths and heterococcoliths. The two forms were previously regarded as belonging to independent species however it is now clear that holococcolithophores represent the haploid phase in the complex heterococcolithophore life-cycle. The heterococcoliths are produced intracellularly (Pienaar, 1994) while holococcoliths calcify outside of the cell (Rowson et al., 1986; Young et al., 1999). Calcification is believed to have evolved as a biotic response to cell toxicity caused by rapidly increasing oceanic calcium levels ca. 600 million years ago (Holligan and Balch, 1991). As the dominant calcifying plankton in the world's oceans, the group plays a uniquely significant role in the biogeochemical cycling of various elements on a global scale, as in the marine carbon cycle. Through the process of photosynthesis, the inorganic fixation of carbon in the upper sunlit layers of the water column and its sedimentation to depth, results in a net draw down of atmospheric CO_2. The sedimentation of detached coccoliths makes an important contribution in the transport of inorganic carbon to the sea floor. This process termed the organic carbon

pump is common to all marine primary producers, however in the case of calcareous nannoplankton it is partially counteracted by that of the carbonate counter pump, whereby the production of calcium carbonate and its transport to depth alters seawater alkalinity, which results in the release of CO_2 in the surface layers (see Rost and Riebesell, 2004 for full review). The relative strength of these two pumps, termed the rain ratio, largely determines the flux of CO_2 between the oceans and the overlying atmosphere (Rost and Riebesell, 2004). On geological time scales, the calcite contributes to the formation of massive sedimentary rocks, which is the major sink for mobile carbon on Earth (Falkowski and Raven 1997).

Coccolithophores are also important components of the sulphur cycle. They are one of the main producers of dimethylsulphoniopropionate (DMSP), a compound used for cellular osmoregulation, and the precursor of dimethyl sulphide (DMS) (Lovelock et al., 1972). DMS is the primary natural source of atmospheric sulphate (contributing up to 60%), and acts as nuclei for cloud condensation, which is essential for the formation of clouds thus increasing the earth's albedo and influencing climate (Charlson et al., 1987).

Some coccolithophores, in particular species such as *Gephyrocapsa oceanica* and *Emiliania huxleyi*, can form blooms so extensive that are detectable by remote sensing techniques due to the reflective nature of coccoliths (Holligan et al., 1983). Coccolithophores comprise one of the most abundant and stratigraphically complete records for any fossil group (Bown et al., 2005) and provide key geological records for the reconstruction of past oceanographic, biological and environmental events. In addition, long-chain alkenones produced by a small group of coccolithophores have been utilised as organic biomarkers and geochemical paleoproxies for past environmental change.

The cytological aspects of coccolithophores have been extensively studied and detailed comprehensive reviews are available (Pienaar, 1994; Inouye, 1997; Billard and Inouye, 2004a). Briefly, the coccolithophore cell is composed of nucleus and one to two golden brown chloroplasts which capture available light and contain chlorophylls *a*, c_1, c_2, c_3, fucoxanthin, diadinoxanthin, diatoxanthin, 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin (Figure 5).

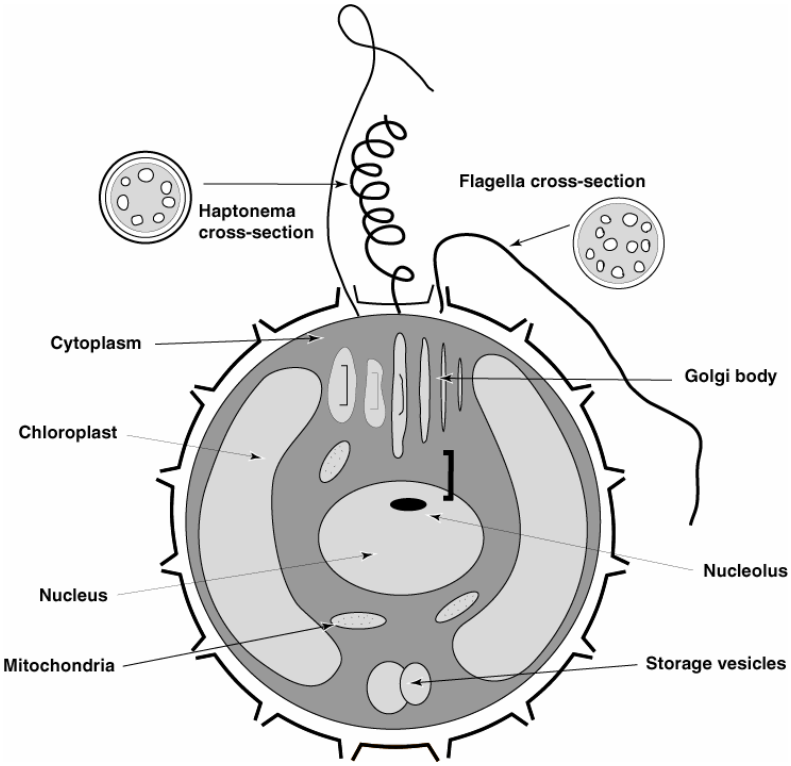

Figure 5 - Schematic representation of the cell structure of coccolithophores (redrawn from Bown and Young, 1998).

The cell also contains mitochondria that produce enzymes which provide the energy necessary for cell function, vacuoles which deal with waste products and the Golgi body which is the site of heterococcolith secretion in many species. Chloroplasts are the centers of carbon dioxide fixation. Motile cells produce two tapering flagella subequal to unequal in size. The flagella are smooth, lacking any hair-like appendages or surface scale ornamentation. Unique to members of the haptophyta is the flagella-like haptonema, which is generally coiled and differs from the flagella in its microtubular sub-structure and basal attachment. The exact function of the haptonema is not known but is thought to act as a sensory tactile organelle and has been shown to play a role in food capture in non-calcifying haptophytes. Both the flagella and haptonema emerge from a shallow depression in the apical region of the cell. The cell membrane or periplast is usually composed of one or more layers of overlapping, oval organic scales proximal to the plasma membrane, though in some species such as *E. huxleyi* these have been shown to be absent. Organic scales can be characterized by surface patterns which appear as concentric ridges on the distal surface and radiating rings proximally (Billard and Inouye, 2004a). Base plate scales are covered with

polysaccharide which is thought to play a role in attachment (Pienaar, 1994). Organic scales appear to form a base for the precipitation of calcite holococcoliths which are produced by the Golgi apparatus. The role of the cell-covering structures, the coccoliths, in the interaction between individual phytoplankton cells and their environment is still little understood. The two most widely suggested types of function are protection against predation and flotation-regulation (Young, 1994). Spines may increase cell diameter preventing predation by smaller zooplankton and reducing nutritive value. Continuous cover may protect the cell against osmotic, chemical or physical shocks and bacterial infestation. Flotation is important since all phytoplankton need to stay within the photic zone. Heavy coccospheres may cause rapid sinking and allow faster nutrient uptake and in contrast, aspherical coccospheres and spines may reduce sinking rates and possibly allow variation of sinking rates. More specialized possible functions include light concentration trough larger areas over which light is collected, coccoliths may refract light into the cell, allowing life lower in the water column or they may reflect ultraviolet light away from the cell, permitting life in lower depths. Lastly, it is further possible that calcification acts as a source of carbon dioxine for photosynthesis so the two reactions could be linked (Paasche, 1962).

Life cycles

The principal mode of reproduction in coccolithophores is asexual mitotic division, following which coccoliths are redistributed between daughter cells (Billard and Inouye, 2004b). It is now well established that most, if not all, coccolithophore species also exhibit a complex heteromorphic life-cycle (Figure 6) in which two or more morphologically distinct stages are represented by the alternation of haploid and diploid phases (Billard, 1994). Traditionally regarded as separate species, these phases are now known to be alternate stages in the life-cycle of a single species.

Typically, the diploid phase is characterised by heterococcolith bearing non-motile cells, while the motile haploid phase bears holococcoliths. In each of these phases cells are capable of asexual (mitotic) reproduction, which allows a rapid population growth in periods of optimum environmental conditions. In some species, the alternate holococcolith phase may be replaced by a non-calcifying motile stage (e.g. *E. huxleyi*) or a naked benthic stage (e.g. *Pleurochrysis* spp.). Other heterococcolithophores, such as *Alisphaera* and *Ceratolithus*, have been shown to

produce haploid phases displaying nannoliths of aragonite (Cros and Fortuño, 2002) or calcite (Sprengel and Young, 2000), respectively.

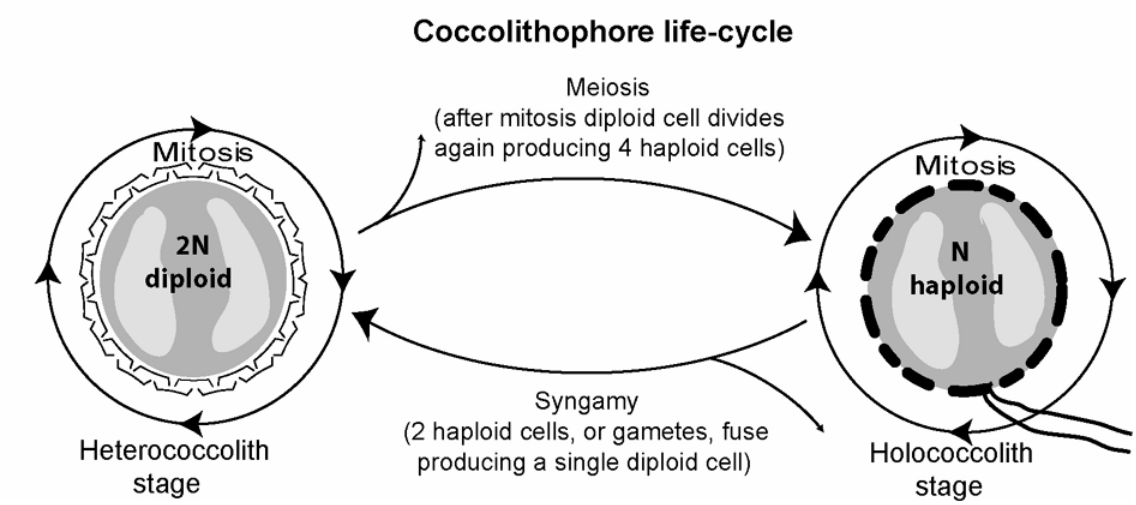

Figure 6 - Schematic representation of coccolithophore life-cycles with the diploid phase covered in heterococcoliths and the motile haploid stage in holococcoliths (adapted from Young et al., 2003).

Evidence for the existence of a haplo-diplontic life-cycle has been demonstrated directly through phase transition in live cultures, in which meiosis (Parke and Adams, 1960) and syngamy (Gayral and Fresnel, 1983) have been observed. Further confirmation has been derived from flow cytometric analyses of the relative DNA content of ploidy levels (Green et al., 1996; Houdan et al., 2004), nuclear staining and relative chromosome counts (Fresnel, 1994). Combination coccospheres (Figure 7) essentially capture the instant of phase change, and bear the two different coccolith types. In recent years, the observation in water samples collected in field studies corroborate the evidence of combination coccospheres and more combinations have been reported for a growing number of species (Cortés, 2000; Cros et al., 2000; Cortés and Bollmann, 2002; Cros and Fortuño, 2002, Geisen et al., 2002; Triantaphyllou and Dimiza, 2003; Triantaphyllou et al., 2003, 2004). In addition to the approximately twenty observed combinations involving heterococcolith and holococcolith bearing phases, six cases have been recorded in which a single heterococcolithophore species has formed separate associations with two or more holococcolith phases (Cros et al., 2000; Geisen et al., 2002). Geisen et al. (2002) proposed non-genotypic intra-specific variation in the

degree of holococcolith calcification, and cryptic speciation or fine-scale variation in the heterococcolith phase as mechanisms to explain this phenomenon.

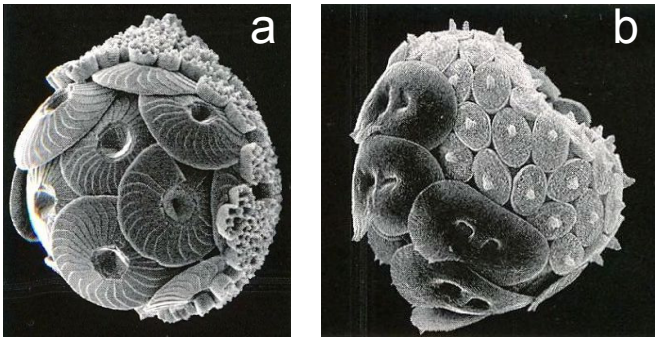

Figure 7 – Examples of combined coccospheres: a) *Calcidiscus quadriperforatus* (heterococcolith) and *Syracolithus quadriperforatus* (holococcolith) and b) *Helicosphaera carteri* (heterococcolith) and *Syracolithus catilliferus* (holococcolith) (Adapted from Young et al., 2003)

Ecology

In general and for the Portuguese coast in particular, the sequence of succession progresses from an initial phase, where strong vertical mixing favours chain-forming diatoms, to mature phases, where stability of the water column is exploited by dinoflagellates and other flagellates that can utilise their limited mobility to take advantage of zones of increased light or nutrients (Margalef, 1978). Diatoms dominate spring blooming phytoplankton, owing to their rapid growth rates, high nutrient demands, and the tolerance of these organisms to the turbulent conditions which prevail throughout this period. This rapid increase in biomass strips surface waters of nutrients resulting in progressive nutrient depletion in the euphotic zone. The continuous increase of the surface layers warming, during late spring and early summer causes the onset of stratification. The presence of the thermocline inhibits mixing and forms a “barrier” between the nutrient-depleted surface mixed-layer and nutrient-rich bottom waters. Increased stability of the water column causes a shift from a diatom dominated assemblage to that of small flagellates or other ‘r’ strategists which have high growth rates but require high energy inputs (Harris, 1986). Within the thermocline nutrient diffusion from the bottom mixed layer is sufficient to support phytoplankton growth and a chlorophyll maximum may develop there dominated by monospecific blooms of dinoflagellates. During winter, the combined effects of heat

loss to the atmosphere, and strong wind induced turbulence causes vertical mixing of the water column from surface to bottom along the continental shelf (Holligan, 1987). Phytoplankton is mixed vertically and spends considerable periods at depth where there is insufficient light for photosynthesis. Therefore, although nutrient concentrations are at their maxima, the depth of mixing exceeds the critical depth (Sverdrup, 1953) and no net production can take place.

Within this annual succession there is a temporal window in which a class of phytoplankton known as Haptophytes flourishes. It occurs just after the spring bloom, especially during late summer-autumn. During these seasons, they are significant contributors to the phytoplankton community in mature upwelled waters, characterized by reduced turbulence but nutrients provided by upwelling still available (Mitchell-Innes and Winter, 1987; Winter, 1985; Giraudeau, 1992; Ziveri et al. 1995). However, several authors also pointed out this group preference for oligotrophic conditions in warm and stratified waters from low and middle latitude regions (McIntyre and Bé, 1967; Honjo and Okada, 1974; Cortés et al., 2001). The ecological distribution of coccolithophores has traditionally been linked to water temperature although this is more likely due to the fact that, in many early field investigations, temperature and salinity measurements were the only environmental parameters recorded (Baumann et al., 2005). Additional environmental conditions such as upwelling, nutrients and light are important controlling factors of the seasonal dynamics of the main coccolithophore taxa. They are sensitive indicators of surface water conditions being important markers of oceanographic changes and proxies of environmental conditions as sea surface water-masses and temperatures, productivity and past climate changes, as pointed out by several authors (detailed in advance). Like other marine phytoplankton, coccolithophores have limited mobility and are therefore dependant on the water shifts and an environment which provides adequate irradiance and nutrients for growth. The primary nutritional mode of coccolithophores is photosynthesis and the group is therefore restricted to the upper layers of the water column or the euphotic zone. The extent of this zone is dependant on the penetration depth of surface irradiance which is related to the amount of suspended particles in the water column. This tends to deepen towards lower latitudes; for example, in highly productive temperate regions the depth at which 1% of the surface irradiance is found is about 30 m, whereas in the less productive subtropical areas it may be as deep as 100-200 m (Winter et al., 1994). With the exception of *E. huxleyi*, most coccolithophore species experience photoinhibition in surface waters. It is probable however that species such as

Florisphaera profunda which dwell in the lower photic zone have a lower light requirement, but given that they are difficult to culture, experimental evidence is lacking (Brand, 1994). Coccolithophores also utilise certain nutrients for biochemical reactions and growth, of which nitrate and phosphate are particularly important. Nitrate for instance is necessary for calcification and other nutrients are also believed to influence bloom development. Low silicates or their depletion has been investigated as a possible explanation for the timing and biogeographical distribution of blooms (Brown and Yoder, 1994).

Margalef (1978) defined the interactive effects of nutrient conditions and habitat turbulence for three major groups of phytoplankton using a simple two-dimensional model, or mandala (Figure 8).

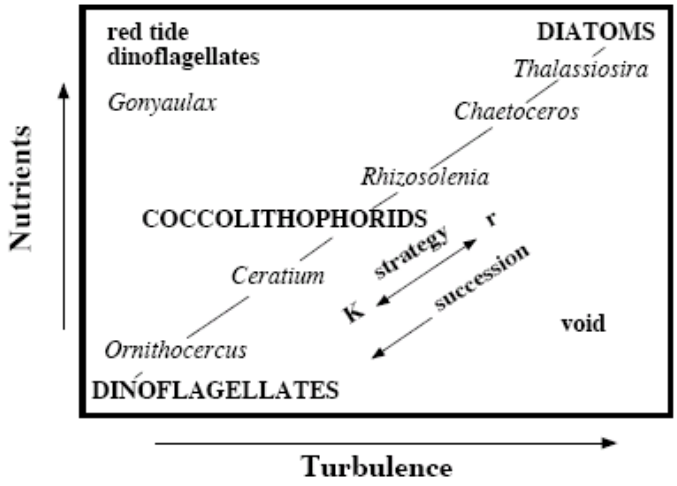

Figure 8 - Margalef's mandala. Graphic representation of the main phytoplankton life forms in an ecological space defined by nutrient concentration and turbulence. From Margalef (1978).

Along a template of r versus K growth strategies, diatoms (r-selected) exploited well-mixed, turbulent, nutrient-rich conditions, while dinoflagellates (K-selected) dominated in stable, stratified waters with low nutrient regimes (Margalef, 1978). In between these two boundaries were placed coccolithophores, which appear to be associated with intermediate turbulence, and nutrient regimes. In accordance with Margalef's model, Young (1994) additionally related the ecological distribution of coccolithophores to their morphology, and found the lowest diversity and abundances both in strongly eutrophic environments and in extreme oligotrophic conditions, while

the highest were in intermediate conditions. In a synthesis of data from studies in the Pacific (Okada and Honjo, 1973; Honjo and Okada, 1974; Honjo, 1977), four distinct groupings were recognised. Placolith-bearing species were shown to dominate assemblages in nutrient rich, turbulent environments such as upwelling areas, coastal and shelf seas, and constituted the main bloom-forming coccolithophores. These were recognised as early succession r-selected species like *E. huxleyi*. Conversely, umbelliform species (e.g. *Umbilicosphaera irregularis*, *U. tenuis*) were considered to be a K-selected group, dominating assemblages in the upper waters (0-100 m) of oligotrophic, low turbulence mid-ocean environments. Floriform coccolithophores (e.g. *Florisphaera profunda*) predominate in the deep photic-zone (ca. 150-200 m) in stable stratified waters where nutrients are high but light availability is low. A final group, termed miscellaneous, included species from intermediate environments which rarely dominate assemblages and showing a tendency towards weak K selection. In general, the model of r versus K-selection is supported by the biogeographical distribution of coccolithophore species with broad latitudinal limits, related to regional temperature and trophic regimes.

The biogeographical distribution of coccolithophores has been the subject of many investigations involving surveys of both the plankton and surface sediments (for reviews see Brand, 1994; Winter et al., 1994; Young, 1994). These have included a number of large-scale quantitative studies, carried out in the Pacific Ocean (McIntyre et al., 1970; Okada and Honjo, 1973; Honjo and Okada, 1974; Roth and Berger, 1975; Okada and McIntyre, 1977), the Indian Ocean (Friedinger and Winter, 1987; Kleijne et al., 1989; Kleijne, 1991; 1992; 1993), Atlantic ocean (McIntyre and Bé, 1967; Winter et al., 1994; Okada and McIntyre, 1979; Okada and McIntyre, 1977; Jordan, 1988; Henriksson, 2000; Knappertsbusch and Brummer,1995; Broerse et al.,2000; Toledo et al., 2007) and the Mediterranean Sea (Knappertsbusch, 1990; Kleijne, 1991; 1992; Cros, 2001; Flores et al, 1997). Other, more small scale, regional studies have focused on the marginal seas of the western Pacific (Okada and Honjo, 1975; Zhang and Siesser, 1986), eastern Pacific (Jordan and Winter, 2000; Hernández-Becerril et al., 2001), the North and South Equatorial currents (Reid, 1980; Hagino and Okada, 2004).

Others have investigated upwelling areas of the Arabian Sea (Andruleit and Rogalla, 2002; Rogalla and Andruleit, 2005; Andruleit et al., 2003, 2005; Schiebel et al., 2004), Nordic Sea (Andruleit, 1997; Baumann et al., 2000; Samtleben and Schroder, 1992; Samtleben et al., 1995). the Gulf of Elat (Winter et al., 1979), Australian waters (Conley, 1979; Hallegraeff, 1984; Takahashi and Okada, 2000;

Findlay and Flores, 2000, Northeast Taiwan (Yang et al., 2001), African waters (Mitchell-Innes and Winter, 1987; Giraudeau,1992; Giraudeau et al., 1993; Giraudeau and Rogers, 1994; Giraudeau and Bayley, 1995), the California system (Winter, 1985; Ziveri et al., 1995; Ziveri and Thunnel, 2000; De Bernardi et al., 2005) and Bay of Biscay (Beaufort and Heussner, 1999; Beaufort and Heussner, 2001).

Along the Portuguese coast, coccolithophores are a widely under-reported component of the phytoplankton in water samples. Sometimes the use of inadequate preservation and observation techniques, due to other aims of investigation, has resulted in poor records of coccolithophore species in the area. Coccolithophore studies have been carried out using sediment assemblages and sinking material (Alday et al., 2006; Narciso et al., 2006; Parente et al., 2004). Although these studies provide similar information concerning the taxonomic composition of living communities (Haydar et al., 2000), plankton skeletons are influenced by processes as destruction and/or dissolution (Eynaud et al., 1999; Sprengel et al., 2000) and the results integrate time and spatial variability of coccolithophores. These studies were sometimes combined with water column samples (Abrantes and Moita, 1999; Cachão and Moita, 2000; Cachão et al., 2000; Ferreira et al., 2008; Silva et al., 2008) which provide further and valuable information on short time and spatial scales of variability. Hence, due to high spatial and temporal changes in plankton communities, a comparison between living and fossil floras can only provide an instantaneous picture of the actual consistency between the two (Andruleit, 1997). Regional studies on living coccolithophores are needed to calibrate species-specific ecological tolerances and to assess the potential for paleoceanographic reconstructions in each studied area (Andruleit, 2007). A better understanding of modern coccolithophore ecology and diversity is needed in order to use them successfully as a biotic proxy of past global change and to assess the quality and accuracy of the information preserved in sedimentary records.

McIntyre and Bé (1967) were the first to extensively document the biogeographical provinces of coccolithophore populations in the Atlantic Ocean. They identified five coccolithophore floral zones, associated with the movements of distinct water masses, and termed them Subarctic, Temperate, Subtropical, Tropical, and Subantarctic zones (Figure 9).

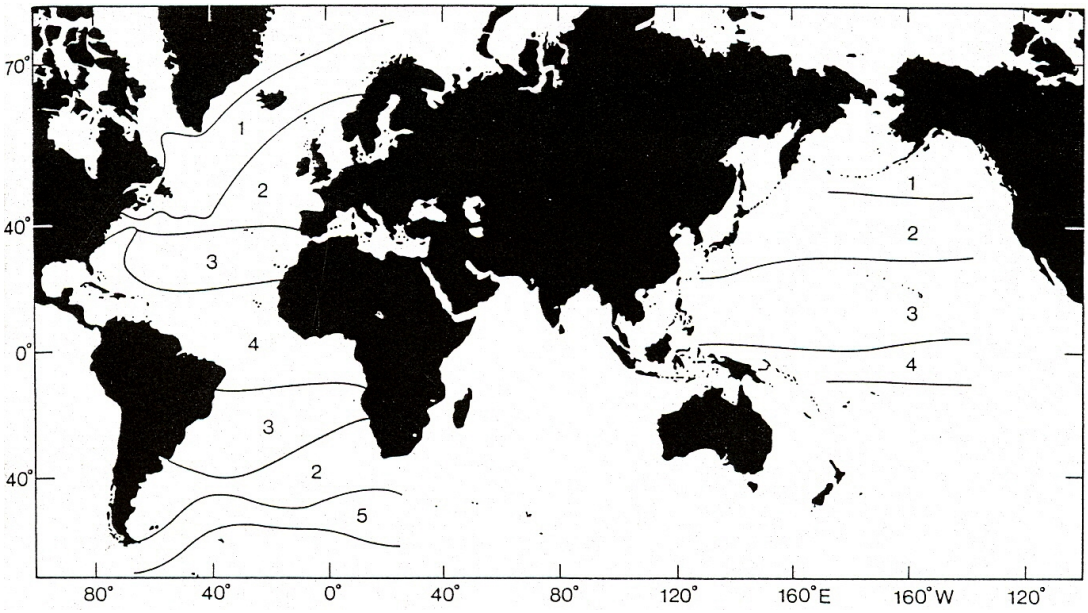

Figure 9 – Biogeographic coccolithophore zones from the Atlantic and Pacific oceans redrawn from McIntyre and Bé (1967) and Okada and Honjo (1973) respectively. 1 = Subarctic, 2 = Temperate (Transitional), 3 = Subtropical (Central), 4 = Tropical (equatorial) and 5 = Subantarctic regions (from Winter et al., 1994).

A later study by Okada and Honjo (1973) examined the horizontal and vertical distribution of coccolithophore flora in the north and central Pacific, and defined six coccolithophore zones with similar patterns to those of the Atlantic: Subarctic, Transitional (equates to the temperate zone of McIntyre and Bé, 1967), Central (or Subtropical), Equatorial (or Tropical) and Subantarctic. Each zone is characterized by a different coccolithophore assemblage according to differences in temperature, nutrients and light availability and distinct assemblages in a particular zone of one hemisphere have classically being viewed as being similar to their counterparts in the opposing hemisphere (Winter et al., 1994). According to Winter et al. (1994), in coastal regions of the Subarctic and Subantarctic zones, where low temperatures and salinities prevailed, the only living coccolithophores are those belonging to the Pappospheraceae and the partially calcified genera. In the open-ocean of the Subarctic zone, mainly during summer months, *Coccolithus pelagicus* (and its motile form *Crystallolithus hyalinus*) and *Calciopapus caudatus* are the only coccolithophores present. In waters of Atlantic origin *Emiliania huxleyi* and *Algirosphaera robusta* may be common. The Temperate zone is dominated most or all year by *E. huxleyi* and by *Gephyrocapsa muellerae,* common to abundant, especially during summer months, only in this zone (or upwelling waters). Flora is also characterized by other placoliths-bearing species. The Subtropical zone has a high diversity with vertical zonation and is characterized by

holococcolithophores, *Discosphaera tubifera*, *Rhabdosphaera clavigera*, *Umbellosphaera* spp., *Florisphaera profunda*, *Thorosphaera flabellata* and *Syracosphaera* spp.. Finally, the Tropical zone is dominated by placoliths-bearing species, especially *E. huxleyi*, *Calcidiscus leptoporus* and *Gephyrocapsa oceanica*. *Umbellosphaera* spp., *Florisphaera profunda* and *Thorosphaera flabellate* are also present. *Reticulofenestra sessilis* is only found in this zone.

The floral macroscale zones are however, a simplistic overview of coccolithophore biogeography. They do not take into account mesoscale oceanographic phenomena such as areas of episodic upwelling, the edges of subtropical central gyres, eddy or coastal currents, responsible for shorter scale changes within the coccolithophore assemblage. For instance at west coast of Portugal, upwelling generally occurs seasonally, and the area lies on the boundary between the temperate and subtropical coccolithophore biogeographical zones (McIntyre and Bé, 1967).

1.2. MAIN FEATURES OF PORTUGUESE HIDROLOGY

The Atlantic coast of the Iberian Peninsula is the northern limit of the upwelling area located along the eastern side of the north Atlantic anticyclone gyre (Wooster et al., 1976) and is a hydrographically complex region characterized by the confluence of different water masses (Fiúza, 1984; Rios et al., 1992; Fiúza et al., 1998) and currents (Frouin et al., 1990; Haynes and Barton, 1990; Fiúza et al., 1998). Wind-driven coastal upwelling occurs when equatorward winds induce net offshore surface Ekman transport, resulting in transport divergence near the coast. In turn, downwelling occurs when poleward winds induce net onshore surface Ekman transport, resulting in transport convergence near the coast. A particular interest of upwelling and downwelling circulations concerns the role of their secondary, cross-shelf circulation, which redistributes not only heat and salt, but also nutrients and biological fields.

Along the Portuguese coast, the wind regime induces seasonal upwelling with different patterns along the coast determined by coastal morphology, the continental shelf/upper slope bathymetry and local winds (Fiúza, 1983). At west coast of Portugal upwelling generally occurs seasonally, from April to September, under northerlies, while advection of warmer oligotrophic oceanic waters is observed during autumn and winter, when southerly winds begin to dominate and there is an intensification of waters

flowing poleward (Fiúza et al., 1982; Haynes and Barton, 1990; Peliz et al., 2005). Episodes of reverse winds can occur during both seasons. Upwelling filaments extending more than 100 km offshore are often rooted at capes (Haynes et al., 1993) and Moita et al. (2003) evidenced the presence of an upwelling shadow area in the lee side of Cape Roca (Figure 10).

The upper ocean mixed layer and seasonal thermocline varies widely according to the season and there is considerable zonal variability in the upwelling-related flow field off western Iberia. North of Lisbon the waters upwelled have characteristics similar to those of the Western North Atlantic Central Water sub polar branch (ENACWsp) while, according to Rios et al. (1992), south of the Nazaré canyon, the main upwelling source is the Eastern North Atlantic Central Water sub tropical branch (ENACWst) and thus influencing Lisbon bay. This branch can be present along the Portuguese continental margin as far north as 40ºN (Fiúza et al., 1982). The influence of ENACWst, which overlays the ENACWsp, decreases gradually towards the north.

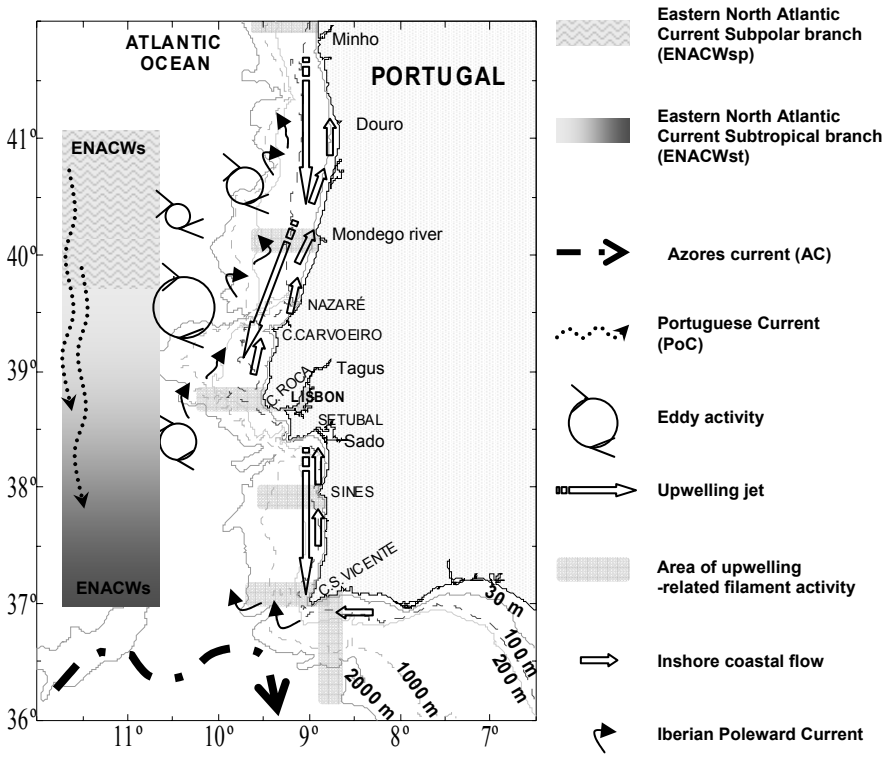

Figure 10 – Schematic representation of the main hydrological features influencing the Portuguese continental coast (redrawn from Mason et al., 2005)

The main large-scale surface currents associated to this Central Water distribution are the Portugal Current (PoC) and the Azores Current (AC). Below the surface, a poleward undercurrent, relatively narrow and weak (Haynes and Barton, 1990; Frouin et al., 1990), is consistently present over the slope (Huthnance et al., 2002). This flow has been referred to both as the Iberian Poleward Current (IPC) (Peliz et al., 2003, 2005) and the Portugal Coastal Counter Current (PCCC) (Ambar and Fiúza, 1994) and often extends to the surface during winter. Peliz et al. (2002) reported a second coastal counter-flow attached to the coast, partly attributable to buoyancy input from the many regional rivers (the Douro, Minho and Mondego Rivers, other smaller rivers, and the Rías Baixas). They named this low-salinity water lens, a year-round feature which extends along the coast the Western Iberia Buoyant Plume (WIBP). The WIBP influences the structures related to the upwelling by increasing stratification over the shelf and by the creation of an inshore frontal region that promotes northward baroclinic transport. The Western Iberia Buoyant Plume is a particularly important feature during winter, owing to the maximum regional rainfalls, when it may be associated with strong poleward transport over the shelf.

The sampling site, Cascais, is located at the northern side of Lisbon bay, south of cape Roca. North of the bay, an upwelling filament rooted at cape Roca recurrently occurs during the northerly wind periods (upwelling favourable), typically extending in the south and westward direction. The studied area is also under the influence of Tagus River discharges, being an import source of nutrient supply, especially during winter. This bay represents an important coastline discontinuity being considered an upwelling shadow area where phytoplankton species can be accumulated through different retention mechanisms (Graham and Largier, 1997, Moita et al., 2003; Oliveira et al., 2008). Coastal upwelling was identified as the major source of seasonal and spatial phytoplankton variability along the Iberian Atlantic coast (Estrada, 1984; Varela, 1992; Moita, 2001). The phytoplankton from the sampling site is thus influenced either by upwelled waters of the Roca filament or by warmer and mature surrounding waters, depending on the intensity and persistence of the upwelling favourable winds and the offshelf mesoscale structures which control the offshore extension and position of the upwelling filament. Weak upwelling conditions allow a larger influence of warmer and more stratified waters into the bay.

1.3. SCOPE OF THE THESIS

This study is focus on coccolithophores in coastal waters of Lisbon bay. Their biodiversity, abundance, and distribution patterns were analyzed in order to identify potential proxies for different local water bodies and environmental conditions.

Water samples were collected on a weekly basis through four years (July 2001 – May 2005), one hour before high tide and the relations between the group and local physical and chemical oceanographic regimes were investigated to define their role as tracers of water masses (Chapter 2). Chapter 3, in particular, is a detailed description of two coccolithophore species from the genus *Calcidiscus*, with a recurrent winter-spring pattern through the times series and largely present in samples from a summer cruise, in 2005. The species, *C. quadriperforatus* and *C. leptoporus* appeared to be associated with different physical-chemical conditions allowing an interesting comprehensive study. In chapter 4, the objective of the study was to describe the ecological niche of coccolithophores, as a group, in relation to other members of the phytoplankton community (diatoms and dinoflagellates). During the last year of the study (April 2004 – May 2005) it was also investigated the use of pigments as chemo-taxonomic tools to monitor time scale changes of phytoplankton groups (Chapter 5). The present thesis resulted in four manuscripts and the major conclusions of all this work are gathered and highlighted in Chapter 6. Finally, in Chapter 7 are presented SEM micrographs of some coccolithophores from Lisbon bay.

References

Abrantes, F. and Moita, M.T. 1999. Water column and recent sediment data on diatoms and coccolithophorids, off Portugal, confirm sediment record of upwelling events. Oceanologica Acta, 22 (3): 319-336.

Alday, M. Cearreta, A., Cachão, M., Freitas, M.C., Andrade, C. and Gama, C. 2006. Micropalaeontological record of Holocene estuarine and marine stages in the Corgo do Porto rivulet (Mira River, SW Portugal). Estuarine, Coastal and Shelf Science, 66 (3-4): 532-543.

Ambar, I.; Fiúza, A.F.G. 1994. Some features of the Portugal Current System: A poleward slope undercurrent, an upwelling related southward flow and an autumn-winter poleward coastal surface current. In: 2nd International Conference on Air-Sea Interaction and Meteorology and Oceanography of the Coastal Zone, American Meteorological Society: 286-287.

Andruleit, H., 2007. Status of the Java upwelling area (Indian Ocean) during the oligothrophic northern hemisphere winter monsoon season as revealed by coccolithophores. Marine Micropaleontology, 64 (1-2): 36-51.

Andruleit, H. 1997. Coccolithophore fluxes in the Norwegian-Greenland Sea: Seasonality and assemblage alterations. Marine Micropaleontology, 31 (1-2): 45-64.

Andruleit, H. and Rogalla, U. 2002. Coccolithophores in surface sediments of the Arabian Sea in relation to environmental gradients in surface waters. Marine Geology, 186 (3-4): 505-526.

Andruleit, H., Rogalla, U. and Stager, S. 2005. Living coccolithophores recorded during the onset of upwelling conditions off Oman in the western Arabian Sea. Journal of Nannoplankton Research, 27 (1): 1-14.

Andruleit, H., Stager, S., Rogalla, U. and Cepek, P. 2003. Living coccolithophores in the northern Arabian Sea: ecological tolerances and environmental control. Marine Micropaleontology, 49 (1-2): 157-181.

Baumann, K.-H., Andruleit, H. and Samtleben, C. 2000. Coccolithophores in the Nordic Seas: comparison of living communities with surface sediment assemblages. Deep Sea Research Part II: Topical Studies in Oceanography, 47 (9-11): 1743-1772.

Baumann, K.H., Andruleit, H., Bockel, B., Geisen, M. and Kinkel, H. 2005. The significance of extant coccolithophores as indicators of ocean water masses, surface water temperature, and palaeoproductivity: a review. Palaeontologische Zeitschrift, 79 (1): 93-112.

Beaufort, L. and Heussner, S. 2001. Seasonal dynamics of calcareous nannoplankton on a West European continental margin: the Bay of Biscay. Marine Micropaleontology, 43 (1-2): 27-55.

Beaufort, L. and Heussner, S. 1999. Coccolithophorids on the continental slope of the Bay of Biscay - production, transport and contribution to mass fluxes. Deep Sea Research Part II: Topical Studies in Oceanography, 46 (10): 2147-2174.

Billard, C. 1994. Life Cycles. In: Green, J.C. and Leadbeater, B.S.C. (eds.), The Haptophyte Algae. Clarendon Press, Oxford, p. 167-186.

Billard, C. and Inouye, I. 2004a. What is new in coccolithophore biology? In: Thierstein, H.R. and Young, J.R. (eds.), Coccolithophores: From Molecular Processes to Global Impact. Springer-Verlag, Berlin, p. 1-29.

Billard, C. and Inouye, I. 2004b. What is new in coccolithophore biology? In: Thierstein, H.R. and Young, J.R. (eds.), Coccolithophores: From Molecular Processes to Global Impact. Springer-Verlag, Berlin, p. 565.

Bollmann, J. 1997. Morphology and biogeography of Gephyrocapsa coccoliths in Holocene sediments. Marine Micropaleontology, 29: 27-55.

Braarud, T. and Deflangre, G. 1955 Terminology, nomenclature, and systematics of the Coccolithophoridae. Micropaleontology, 1 (2): 157-159.

Braarud, T., Gaarder, K.R., Markali, J. and Nordli, E. 1952 Coccolithophorids studied in the electron microscope. Observations on *Coccolithus huxleyi* and *Syracosphaera carterae*. Nytt Mag Bot, 1: 129-134.

Brand, L.E. 1994. Physiological ecology of marine coccolithophores. In: Winter, A. and Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press.

Broerse, A.T.C., Ziveri, P., van Hinte, J.E. and Honjo, S. 2000. Coccolithophore export production, species composition, and coccolith-CaCO3 fluxes in the NE Atlantic (34° N 21° W and 48° N 21° W). Deep-Sea Research II, 47: 1877-1905.

Brown, C.W. and Yoder, J.A. 1994. Coccolithophorid blooms in the global ocean. Journal of Geophysical Research, 99: 7467-7482.

Cachão, M. and Moita, M.T. 2000. *Coccolithus pelagicus*, a productivity proxy related to moderate fronts off Western Iberia. Marine Micropaleontology, 39: 131-155.

Cachão, M., Oliveira, A. and Vitorino, J. 2000. Subtropical winter guests, offshore Portugal. Journal of Nannoplankton Research, 22 (1), 19-26.

Charlson, R.J., Lovelock, J.E., Andreae, M.O. and Warren, S.G. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature, 326: 655-661.

Conley, S.M. 1979. Recent coccolithophores from the Great Barrier Reef- Coral sea region. Micropaleontology, 25 (1): 20-43.

Cortés, M.Y. 2000. Further evidence for the heterococcolith-holococcolith combination Calcidiscus leptoporus-Crystallolithus rigidus. Marine Micropaleontology, 39: 35-37.

Cortés, M. and Bollmann, J. 2002. A new combination coccosphere of the heterococcolith species *Coronosphaera mediterranea* and the holococcolith species *Calyptrolithophora hasleana*. European Journal of Phycology, 37 (1): 145-146.

Cortés, M.Y., Bollmann, J. and Thierstein, H.R. 2001. Coccolithophore ecology at the HOT station ALOHA, Hawaii. Deep-Sea Research II, 48: 1957-1981.

Cros, L. 2001 Planktonic coccolithophores of the NW Mediterranean. Ph. D. Thesis. Universitat de Barcelona, Barcelona, p. 389.

Cros, L. and Fortuño, J.M. 2002. Atlas of Northwestern Mediterranean Coccolithophores. Scientia Marina, 66 (Supplement 1): 7-182.

Cros, L., Kleijne, A., Zeltner, A., Billard, C. and Young, J.R. 2000. New examples of holococcolith-heterococcolith combination coccospheres and their implications for coccolithophorid biology. Marine Micropaleontology, 39 (1-4): 1-34.

De Bernardi, B., Ziveri, P., Erba, E. and Thunell, R.C. 2005. Coccolithophore export production during the 1997–1998 El Niño event in Santa Barbara Basin (California). Marine Micropaleontology, 55 (1-2): 107-125.

Estrada, M. 1984. Phytoplankton composition off Galicia (North west Spain). Journal of Plankton Research, 6: 417-434.

Eynaud, F., Giraudeau, J., Pichon, J.J. and Pudsey, C.J. 1999. Sea-surface distribution of coccolithophores, diatoms, silicoflagellates and dinoflagellates In south Atlantic Ocean during the late austral summer 1995. Deep-Sea Research I, 46: 451-482.

Falkowski, P.G. and Raven, J.A. 1997. Aquatic Photosynthesis. Malden, MA, Blackwell Science.

Ferreira, J., Cachão, M., and González R. 2008 Reworked calcareous nannofossils as ocean dynamic tracers: The Guadiana shelf case study (SW Iberia). Estuarine, Coastal and Shelf Science, 79 (1): 59-70.

Findlay, C.S. and Flores, J.A. 2000. Subtropical Front fluctuations south of Australia (45º09'S, 146º17'E) for the last 130 ka years based on calcareous nannoplankton. Marine Micropaleontology, 40: 403-416.

Fiúza, A.F.G. 1984. Hydrology and Dynamics of the Portuguese coastal waters. Ph.D. Thesis. University of Lisbon, Lisbon, unpublished.

Fiúza, A.F.G. 1983. Upwelling patterns off Portugal. In: Suess, E. and Thied, J. (eds.), Coastal upwelling, its sediment Record. Part A. Responses of the sedimentary regime to present coastal upwelling. Plenum, New York, p. 85-98.

Fiúza, A.F.G., Macedo, M.E. and Guerreiro, M.R. 1982. Climatological space and time variation of the Portuguese coastal upwelling. Oceanologica Acta, 5 (1): 31-40.

Fiúza, A.F.G., Hamman, M., Ambar, I., Diaz del Rio, G., Gonzalez, N. and Cabanas, J.M. 1998. Water masses and their circulation off western ibéria during May 1993. Deep-sea Research I, (45): 1127-1160.

Flores, J.A., Sierro, F.J., Francês, G., Vasquez, A. and Zamarreño, I. 1997. The last 100,000 years in the western Mediterranean: sea surface water and frontal dynamics as revealed by coccolithophores. Marine Micropaleontology, 29: 351-366.

Fresnel, J. 1994. A heteromorphic life cycle in two coastal coccolithophorids, *Hymenomonas lacuna* and *Hymenomonas coronata* (Prymnesiophyceae). Canadian Journal of Botany, 72: 1455-1462.

Friedinger, P.J.J. and Winter, A. 1987. Distribution of modern coccolithophore assemblages in the southwest Indian Ocean off southern Africa. Journal of Micropaleontology, 6 (1): 49-56.

Frouin, R., Fiúza, A.F.G., Ambar, I. and Boyd, T.J. 1990. Observations of a poleward surface current off the coast f Portugal and Spain during winter. Journal of Geophysical Research, 95: 679-691.

Gayral, P. and Fresnel, J. 1983. Description, sexualite et cycle de development d'une nouvelle Coccolithophoracee (Prymnesiophyceae): *Pleurochrysis pseudoroscoffensis* sp. nov. Protistologica, 19: 245-261.

Geisen, M., Billard, C., Broerse, A.T.C., Cros, L., Probert, I. and Young, J. 2002. Lifecycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? European Journal of Phycology, 37: 531-550.

Giraudeau, J., 1992. Distribution of recent nannofossils beneath the Benguela system: Southwest African continental margin. Marine Geology, 108 (2): 219-237.

Giraudeau, J., Bayley, G.W. 1995. Spatial dynamics of coccolithophore communities during an upwelling event in the Southern Benguela system. Continental Shelf Research, 15: 1825-1852.

Giraudeau, J. and Rogers, J. 1994. Phytoplankton biomass and sea-surface temperature estimates from sea-bed distribution of Nanofossils and Planktonic Foraminifera in the Benguela upwelling system. Micropaleontology, 40 (3): 275-285.

Giraudeau, J., Monteiro, P.M.S., Nikodemus, K. 1993. Distribution and malformation of living coccolithophores in the northern Benguela system off Namíbia. Marine Micropaleontology, 22: 93-110.

Graham, W.M. and Largier, J.L. 1997. Upwelling shadows as nearshore retention sites: the example of northern Monterey Bay. Continental Shelf Research, 17: 509-532.

Green, J.C., Course, P.A. and Tarran, G.A. 1996. The life-cycle of Emiliania huxleyi: A brief review and a study of relative ploidy levels analysed by flow cytometry. Journal of Marine Systems, 9 (1-2): 33-44.

Guiry, M.D., Guiry, G.M. 2008. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 26 November 2008

Hagino, K. and Okada, H. 2004. Floral responses of coccolithophores to progressive oligotrophication in the South Equatorial Current, Pacific Ocean. In: Shiyomi, M., Kawahata, H., Koizumi, H., Tsuda, A. and Awaya, Y. (eds.), Global Environmental Change in the Ocean and on Land. Terrapub, Tokyo.

Haidar, A.T., Thierstein, H.R. and Deuser, D.G. 2000. Calcareous phytoplankton standing fluxes and accumulation in Holocene sediments off Bermuda (N. Atlantic). Deep-Sea Research II, 47: 1907-1938.

Hallegraeff, G.M. 1984. Coccolithophorids (Calcareous Nanoplankton) from Australian waters. Botanica Marina, 27: 229-247.

Harris, G.P. 1986 Phytoplankton Ecology: Structure, Function and Fluctuation. Chapman and Hall, New York: Henry, R. and Simão, C. A. (eds.), pp.384.

Haynes, R. and Barton, E.D. 1990. A poleward flow along the Atlantic coast of the Iberian Peninsula. Journal of Geophysics Research, 95 (C7): 11425-11441.

Haynes, R., Barton, E.D.; Pilling, I., 1993. Development, persistence and variability of upwelling filaments off the atlantic coast of the Iberian peninsula. Journal of Geophysical Research, 98: 22681-22692.

Henriksson, A. S. 2000. Coccolithophore response to oceanographic changes in the equatorial Atlantic during the last 200,000 years. Palaeogeography, Palaeoclimatology, Palaeoecology, 156: 161-173.

Hernández-Becerril, D. U., Bravo-Sierra, E. and Ramírez-Valdéz, Y. 2001. Coccolithophorids from the west coast of Baja California, Mexico. Hydrobiologia, 452: 31-45.

Holligan, P. M. 1987. The physical environment of exceptional phytoplankton blooms in the NE Atlantic. . Rapport P-V Réunion du Conseil Internationale de Exploration du Mer, 187: 9-18.

Holligan, P. M. and Balch, W. M. 1991. From the oceans to cells: coccolithophore optics and biogeochemistry. In: Demers, S. (ed), Particle Analysis in Oceanography. Springer-Verlag Berlin Heidelberg, G 27: 301-324.

Holligan, P., Viollier, M., Harbour, D. S., Camus, P. and Champagne-Philippe, M. 1983. Satellite and ship studies of coccolithophore production along a continental shelf edge. Nature, 304: 339-342.

Honjo, S. 1977. Biogeography and provincialism of living Coccolithophorids in the Pacific Ocean. In: Ramsey, A.T.S. (ed), Oceanic Micropaleontology. Academic Press, London, p. 951-972.

Honjo, S. and Okada, H. 1974. Community structure of coccolithophores in the photic layer of the Mid-pacific. Micropaleontology, 29 (2): 209-230.

Houdan, A., Billard, C., Fabrice Not, D. M., Sáez, A. G., Young, J. R. and Probert, I. 2004. Holococcolithophore-heterococcolithophore (Haptophyta) life cycles: flow cytometric analysis of relative ploidy levels. Systematics and Biodiversity, 1 (4): 453-465.

Huthnance, J.M.; Van Aken, H.M.; White, M.; Barton, E.D.; Le Cann, B.; Coelho, E.F.; Fanjul, E.A.; Miller, P.; Vitorino, J., 2002. Ocean margin exchange-water flux estimates. Journal of Marine Systems, 32: 107-137.

Inouye, I. 1997. Systematics of haptophyte algae in Asia-Pacific waters. Algae, The Korean Journal of Phycology, 12: 247-261.

Jordan, R. W. 1988 Coccolithophorid Communities in the North East Atlantic. PhD thesis, University of Surrey, Guildford.

Jordan, R. W. and Winter, A. 2000. Assemblages of coccolithophorids and other living microplankton off the coast of Puerto Rico during January-May 1995. Marine Micropaleontology, 39 (1-4): 113-130.

Kamptner, E. 1927. Beitrag zur Kenntnis adriatischer Coccolithophoriden. Archiv fűr Protistenkunde, 58: 173-184.

Kleijne, A. 1993. Coccolithophorid Biogeography. In: Morphology, taxonomy and distribution of extant coccolithophorids (Calcareous nannoplankton). Free University, Amsterdam, 297-317.

Kleijne, A. 1992. Extant Rhabdosphaeraceae (coccolithophorids, class Prymnesiophyceae) from the Indian Ocean, Red sea, Mediterranean Sea and North Atlantic Ocean. Scripta Geologica , 100: 1-63.

Kleijne, A. 1991. Holococcolithophorids from the Indian Ocean, Red Sea, Mediterranean Sea and North Atlantic Ocean. Marine Micropaleontology ,17: 1-76.

Kleijne, A., Kroon, D. and Zevenboom, W. 1989. Phytoplankton and foraminiferal frequencies in northern Indian Ocean and Red Sea surface waters. Netherlands Journal of Sea Research, 24 (4): 531-539.

Knappertsbusch, M. W. 1990 Geographic distribution of modern coccolithophores in the Mediterranean Sea and morphological evolution of *Calcidiscus leptoporus*. Swiss Federal Institute of Technology, Zurich ETH.

Knappertsbusch, M. and Brummer, G-J. A. 1995. A sediment trap investigation of sinking coccolithophorids in the North Atlantic. Deep Sea Research Part I: Oceanographic Research Papers, 42 (7): 1083-1109.

Knappertsbusch, M., Cortes, M. Y. and Thierstein, H. R. 1997. Morphologic variability of the coccolithophorid *Calcidiscus leptoporus* in the plankton, surface sediments and from the Early Pleistocene. Marine Micropaleontology, 30: 293-317.

Lohmann, H. 1902. Die Coccolithophoridae, eine Monographie der Coccolithen bildenden Flagellaten, Zugleich ein Beitrag zur Kenntnis des Mittelmeerauftriebs. Arch. Protistenk., 1: 89-165.

Lovelock, J. E., Maggs, R. L. and Rasmussen, R. A. 1972. Atmospheric dimethylsulphide and the natural sulphur cycle. Nature, 237: 452–453.

Margalef, R. 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanologica Acta., 1 (4): 493-509.

Mason, E., Coombs, S. and Oliveira, P. B. 2005. An overview of the literature concerning the oceanography of the eastern North Atlantic region. Relatório Cientifico e Técnico, Instituto Investigação das Pescas e do Mar, nº 33, 58 pp.

McIntyre, A. and Bé, A. W. H. 1967. Modern coccolithophoridae of the Atlantic Ocean – I. Placoliths and Cyrtoliths. Deep-Sea Research, 14: 561-597.

McIntyre, A., Bé, A. W. H. and Roche, M. B. 1970. Modern Pacific Coccolithophorida: a palaeontological thermometer. Transactions of the New York Academy of Sciences, Series II, 32 (6): 720-731.

Mitchell-Innes, B.A. and Winter, A. 1987. Coccolithophores: a major phytoplankton component in mature upwelled waters off the Cape Peninsula, South Africa in March, 1983. Marine Biology, 95: 25-30.

Moita, M.T. 2001. Estrutura, variabilidade e dinâmica do Fitoplâncton na costa de Portugal Continental. Ph.D. Thesis. University of Lisbon, Lisbon, unpublished.

Moita, M.T., Oliveira, P. B., Mendes, J.C. and Palma, A.S. 2003. Distribution of chlorophyll a and *Gymnodinium catenatum* associated with coastal upwelling plumes off central Portugal. Acta Oecologica, 24: S125-S132.

Murray, G. and Blackman, V.H. 1898. On the nature of the Coccospheres and Rhabdospheres. Philosophical Transactions of the Royal Society 190B: 427-441.

Narciso, A., Cachão, M. and Abreu, L. 2006. Coccolithus pelagicus subsp. pelagicus versus *Coccolithus pelagicus* subsp. *braarudii* (Coccolithophore, Haptophyta): A proxy for

surface subarctic Atlantic waters off Iberia during the last 200 kyr. Marine Micropaleontology, 59 (1): 15-34.

Okada, H. and McIntyre, A. 1979. Seasonal distribution of modern coccolithophores in the western North Atlantic Ocean. Marine Biology, 54: 319-328.

Okada, H. and McIntyre, A. 1977. Modern coccolithophores of the Pacific and North Atlantic oceans. Micropaleontology, 23: 1-55.

Okada, H. and Honjo, S. 1975. Distribution of coccolithophores in marginal seas along the western Pacific Ocean and in the Red Sea. Marine Biology, 31: 271-285.

Okada, H. and Honjo, S. 1973. The distribution of oceanic coccolithophorids in the Pacific. Deep Sea Research, 20: 355-374.

Oliveira, P.B., R. Nolasco, J. Dubert, T. Moita and A.J. Peliz. 2008. Surface temperature, chlorophyll and advection patterns during a summer upwelling event off central Portugal. Continental Shelf Research, doi:10.1016/j.csr.2008.08.004

Paasche, E. 1962. Coccolith formation. Nature, 193: 1094-1095

Parente, A., Cachão, M., Baumann, K-H., Abreu, L. and Ferreira, J. 2004. Morphometry of *Coccolithus pelagicus* s.l. (Coccolithophore, Haptophyta) from offshore Portugal, during the last 200 kyr. Micropaleontology, 50 (1): 107-120.

Parke, M. and Adams, I. 1960. The motile (Crystallolithus hyalinus Gaarder and Markali) and non-motile phases in the life history of *Coccolithus pelagicus* (Wallich) Schiller. Journal of the Marine Biological Association of the U.K., 39: 263-274.

Peliz, Á.J.; Dubert, J.S.; Haidvogel, D.B. 2003. Subinertial response of a density-driven eastern boundary poleward current to wind forcing. Journal of Physical Oceanography, 33: 1633-1650.

Peliz, Á.J., Rosa, T.L., Santos, A.M.P. and Pissarra, J.L. 2002. Fronts, jets and counter-flows in the western Iberian upwelling system. Journal of Marine Systems, 35: 61-77.

Peliz, A., Dubert, J., Santos, A.M.P., Oliveira, P.B., Le Cann, B. 2005. Winter upper ocean circulation in the Western Iberian Basin - Fronts, Eddies and Poleward Flows: an overview. Deep Sea Research I, 52: 621-646.

Pienaar, R.N. 1994. Ultrastructure and calcification of coccolithophores. In: Winter, A. and Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press, Cambridge, p. 13-37.

Reid, F.M.H. 1980. Coccolithophorids of the North Pacific Central Gyre with notes on their vertical and seasonal distribution. Micropaleontology, 26 (2): 151-176.

Renaud, S. and Klaas, C. 2001. Seasonal variations in the morphology of the coccolithophore *Calcidiscus leptoporus* off Bermuda (N. Atlantic). Journal of Plankton Research, 23: 779-795.

Rios, A. F., Pérez, F. F. and Fraga, F. 1992. Water masses in the upper and middle North Atlantic Ocean east of the Azores. Deep-Sea Research, 39 (3/4): 645-658.

Rogalla, U. and Andruleit, H. 2005. Processional forcing of coccolithophore assemblages in the northern Arabian Sea: Implications for monsoonal dynamics during the last 200,000 years. Marine Geology, 217: 31-48.

Rost, B. and Riebesell, U. 2004. Coccolithophores and the biological pump: responses to environmental changes. In: Thierstein, H.R. and Young, J.R. (eds.), Coccolithophores: From molecular processes to global impact. Springer-Verlag Berlin, 99-125.

Roth, P.H. and Berger, H. 1975. Distribution and dissolution of coccoliths in the South and central Pacific. In: Sliter, W.V., Bé, A.W.H., Berger, W.H. (eds.), Dissolution of Deep-Sea Carbonates. Cushman Found. Foraminiferal Research, Special Publication, 13: 87-113.

Rowson, J.D., Leadbeater, B.S.C. and Green, J.C. 1986. Calcium carbonate deposition in the motile (Crystallolithus) phase of Coccolithus pelagicus (Prymnesiophyceae). British Phycological Journal, 21: 359-370.

Sáez, A.G., Probert, I., Geisen, M., Quinn, P., Young, J. and Medlin, L.K. 2003. Pseudocryptic speciation in coccolithophores. Proceedings of the National Academy of Sciences of the U.S.A., 100: 7163-7168.

Samtleben, C. and Schroder, A. 1992. Living coccolithophore communities in the Norwegian-Greenland Sea and their record in sediments. Marine Micropaleontology, 19: 333-354.

Samtleben, C., Baumann, K.-H. and Schroder-Ritzrau, A. 1995. Distribution, composition and seasonal variation of coccolithophore communities in the northern North Atlantic. In: Flores, J.A. and Sierro, F.J. (eds.), Proceedings of the 5th INA Conference. Universidad de Salamanca, Salamanca, p. 219-235.

Schiebel, R., Zeltner, A., Treppke, U.F., Waniek, J..J., Bollmann, J., Rixen, T. and Hemleben, C. 2004. Distribution of diatoms, coccolithophores and planktic foraminifers along a trophic gradient during SW monsoon in the Arabian Sea. Marine Micropaleontology, 51 (3-4): 345-371.

Silva, A., Palma, S. and Moita, M.T. 2008. Coccolithophores in the upwelling waters of Portugal: Four years of weekly distribution in Lisbon bay. Continental Shelf Research, 28: 2601-2603.

Sournia, A., Chretiennot-dinet, M.J. and Ricard, M. 1991. Marine phytoplankton how many species in the world ocean? Journal of Plankton Research, 13: 1093-1099.

Sprengel, C. and Young, J.R. 2000. First direct documentation of associations of Ceratolithus cristatus ceratoliths, hoop-coccoliths and Neosphaera coccolithomorpha planoliths. Marine Micropaleontology, 39 (1-4): 39-41.

Sprengel, C., Baumann, K.-H and Neuer, S. 2000. Seasonal and interannual variation of coccolithophore fluxes and species composition in sediment traps north of Gran Canaria (29ºN 15ªW). Marine Micropaleontology, 39: 157-178.

Sverdrup, H.U. 1953. On conditions for the vernal blooming of phytoplankton. Journal Conseil International d' Exploration du Mer, 18: 287-295.

Takahashi, K. and Okada, H. 2000. Environmental control on the biogeography of modern coccolithophores in the southeastern Indian Ocean offshore of Western Australia. Marine Micropaleontology, 39 (1-4): 73-86.

Toledo, F.A.L, Cachão, M., Costa, K.B. and Pivel, M.A.G. 2007. Planktonic foraminifera, calcareous nannoplankton and ascidian variations during the last 25 kyr in the Southwestern Atlantic: A paleoproductivity signature? Marine Micropaleontology, 64 (1-2): 67-79.

Triantaphyllou, M.V. and Dimiza, M.D. 2003. Verification of the Algirosphaera robusta-Sphaerocalyptra quadridentata (coccolithophores) life-cycle association. Journal of the Micropalaeontology, 22: 107-111.

Triantaphyllou, M.V., Dimiza, M.D. and Dermitzakis, M. 2004. *Syracosphaera halldalii* and *Calyptrolithina divergens* var. *tuberosa* life-cycle association and relevant taxonomic remarks. Micropaleontology, 50 (1): 121-126.

Triantaphyllou, M.V., Dimiza, M.D. and Dermitzakis, M. 2003. A new coccolithophore life-cycle association: *Syracosphaera halldalii* (Heterococcolithophore) and *Calyptrolithina divergens* var. *tuberosa* (Holococcolithophore). Gaia, 11: 77-80.

Varela, M. 1992. Upwelling and phytoplankton ecology in Galician (NW Spain) rías and shelf waters. Boletim do Instuto Espanhol de Oceanografia., 8 (1): 57-74.

Winter, A. 1985. Distribution of living coccolithophores in the California current system, Southern California borderland. Marine Micropaleontology, 9: 385-393.

Winter, A., Jordan, R.W. and Roth, P. 1994. Biogeography of living coccolithophores in ocean waters. In: Winter, A. and Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press, Cambridge, p. 161-177.

Winter, A., Reiss, Z. and Luz, B. 1979. Distribution of living coccolithophore assemblages in the Gulf of Elat ('Aqaba). Marine Micropaleontology, 4: 197-223.

Wooster, W.S., Bakun, A. and McLain, D.R. 1976. The seasonal upwelling cycle along the eastern boundary of the North Atlantic. Journal of Marine Research, 34: 131-141.

Yang, T., Wei, K. and Gong, G. 2001. Distribution of coccolithophorids and coccoliths in surface ocean off northeastern Taiwan. Botanical Bulletin of Academic Sciences, 42: 287-302.

Young J.R., Geisen M. and Probert I. 2005. A review of selected aspects of coccolithophore biology with implications for palaeodiversity estimation. Micropaleontology, 51: 267-288.

Young, J.R. 1994. The functions of coccoliths. In: Winter, A. and Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press, Cambridge, 63-82.

Young, J.R., Bergen, J.A., Bown, P.R., Burnett, J.A., Fiorentino, A., Jordan, R.W., Kleijne, A., van Niel, B., Romein, A.J.T. and von Salis, K. 1997. Guidelines for coccolith and calcareous nannofossil terminology. Palaeontology, 40 (4): 875-912.

Young, J.R., Davis, S.A., Bown, P.R. and Mann, S. 1999. Coccolith Ultrastructure and Biomineralisation. Journal of Structural Biology, 126 (3): 195-215.

Young, J.R., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I. and Østergaard, J.B. 2003. A guide to extant coccolithophore taxonomy. Journal of Nannoplankton Research (Special Issue 1), p. 125.

Zhang, J. and Siesser, W.G. 1986. Calcareous nannoplankton in continental-shelf sediments, East China Sea. Micropaleontology, 32 (3): 271-281.

Ziveri, P. and Thunell, R.C. 2000. Coccolithophore export production in Guaymas Basin, Gulf of California: response to climate forcing. Deep-Sea Research II, 47: 2073-2100.

Ziveri, P., Thunell, R. and Rio, D. 1995. Export production of coccolithophores in an upwelling region: Results from San Pedro Basin, Southern California Borderlands. Marine Micropaleontology, 24: 335-358.

CHAPTER 2: COCCOLITHOPHORES IN THE UPWELLING WATERS OF PORTUGAL: FOUR YEARS OF WEEKLY DISTRIBUTION IN LISBON BAY

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Silva, A*, Palma S., Moita M.T.

Instituto Nacional de Recursos Biológicos (INRB-IPIMAR), Av. Brasília s/n, 1449-006 Lisboa, Portugal. Tel: +351213027121, Fax: +351213015948

* Corresponding author: amsilva@ipimar.pt

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ABSTRACT

From July 2001 to May 2005, seawater samples were collected once a week at a fixed station in Lisbon bay (38º41'N, 09º24'W) in order to describe the ecological dynamics of the coccolithophore community. The seasonal and interannual distribution patterns of the different species and their relationships with environmental parameters are addressed. The present work aimed to identify potential proxies for different local water bodies and environmental conditions. Throughout the four years, the upwelling events were weak and progressively more persistent. High sea surface temperatures (SST) were observed earlier in the year; summers and winters were gradually warmer and colder, respectively. Salinity variations reflected the different weather conditions as they are strongly influenced by rainfall and thus by the Tagus river flow. The extended periods of weak upwelling and the overall increase in SST resulted in the development of phytoplankton populations as measured by chlorophyll *a*. However, the persistence of the upwelling, and thus shorter convergence periods, favoured other phytoplankton groups than coccolithophore populations as these decreased towards the end of the sampling period.

The annual structure of the coccolithophore assemblage showed a pronounced and recurrent seasonal variability, mainly related with the intensity and persistence of upwelling. The highest cell densities were recorded from spring to autumn. An overall preference by most species for mature upwelled waters and low turbulent conditions was observed associated with high temperatures and salinities, although the species develop in different windows with mismatching maxima. The coccolithophores observed were capable of withstanding coastal processes such as turbulence and were well adapted to an environment rich in nutrients provided by both continental runoff and upwelling.

The consistency of the results enabled local oceanographic tracers to be defined. *Emiliania huxleyi* and *Gephyrocapsa* species can be used as proxies of surface productivity waters during spring and summer while *Coccolithus pelagicus* indicates the presence of upwelling fronts. *Calcidiscus leptoporus* is a tracer of the convergence of subtropical oceanic waters onto the shelf, during winter while *Coronosphaera mediterranea*, *Syracosphaera pulchra*, *Helicosphaera carteri* and *Rhabdosphaera clavigera* revealed the presence of those waters during the short period that characterized the transition from upwelling to downwelling seasons.

Keywords: Coccolithophores ecology; seasonal and interannual changes; time series; Iberia upwelling system.

1. INTRODUCTION

Coccolithophores are one of the major groups of marine phytoplankton. They are primary producers in the photic zone and directly dependent on changing gradients in surface waters (Henriksson, 2000). In general, coccolithophores prefer oligotrophic conditions in warm and stratified waters from low and middle latitude regions (McIntyre and Bé, 1967; Honjo and Okada, 1974; Cortés et al., 2001). However, they are also important contributors to the phytoplankton community in mature upwelled waters (Mitchell-Innes and Winter, 1987; Winter, 1985; Giraudeau, 1992; Ziveri et al., 1995). Widespread throughout the oceans, coccolithophores are important markers of oceanographic changes, as they appear to be sensitive indicators of surface water conditions. Each coccolithophore species has specific spatial and temporal distributions in surface waters. Environmental conditions such as upwelling, temperature, salinity, nutrients and light are important controlling factors of the seasonal dynamics of the main coccolithophore taxa. Thus, this group can serve as proxies of environmental conditions such as sea-surface water masses and temperatures, productivity and global to past climate and environmental changes (Mitchell-Innes and Winter, 1987; Kleijne, 1990; Winter et al., 1994; Ziveri et al., 1995; Ziveri and Thunell, 2000; Andruleit, 1997; Broerse et al., 2000; Sprengel et al., 2000; Beaufort and Heussner, 2001; Andruleit et al., 2003, 2005; Rogalla and Andruleit, 2005; De Bernardi et al., 2005).

Most coccolithophore studies have been carried out using sediment assemblages and sinking material, which consist mainly of isolated coccoliths. Although these studies provide similar information concerning the taxonomic composition of living communities (Haidar et al., 2000), plankton skeletons are influenced by processes as destruction and/or dissolution (Eynaud et al., 1999; Sprengel et al., 2000) and the results integrate time and spatial variability of coccolithophores. Thus, due to high spatial and temporal changes in plankton communities, a comparison between living and fossil floras can only provide an instantaneous picture of the actual consistency between the two (Andruleit, 1997). Water column studies provide further and valuable information on short time and spatial scales of variability. A better understanding of modern coccolithophore ecology and diversity is needed in order to use them successfully as a biotic proxy of past global change and to assess the quality and accuracy of the information preserved in sedimentary records. Regional studies on

living coccolithophores are needed to calibrate species-specific ecological tolerances and to assess the potential for paleoceanographic reconstructions in each studied area (Andruleit, 2007). Along the Portuguese coast, very few studies have been conducted on water column samples (Cachão and Moita, 2000; Cachão et al., 2000). Studies on the dynamics of this group in this area are relevant since it lays on the boundary between the temperate and subtropical coccolithophore biogeographical zones (McIntyre and Bé, 1967). The present work aims to describe the ecological dynamics of the coccolithophore community in Lisbon bay and is based on weekly seawater samples over 4 years. The seasonal and interannual distribution patterns of the different species and their relationships with environmental parameters will be addressed as well as their role as regional oceanographic tracers.

2. MATERIALS AND METHODS

From July 2001 to May 2005 seawater samples were collected once a week, at a fixed station located in Lisbon bay (Cascais: 38º41'N, 09º24'W) (Figure 1), from surface and bottom (5m depth) waters 1 h before high tide. Because the surface and bottom values of temperature, salinity, chlorophyll a and coccolithophore abundances did not differ significantly an average was calculated. Samples for chlorophyll *a* and coccolithophores evaluation were collected. Sampling was conducted 1 h before high tide in order to minimize the direct influence of estuarine waters on the area. Temperature, salinity and depth were determined in situ with a Quanta CTD. Wind data were obtained from the meteorological weather station of Cape Carvoeiro, located 50km north of Cascais (Figure 1) and upwelling indices (negative values indicate upwelling) were based on the northward wind stress component and calculated according to Bakun (1973). Monthly river discharge of the Tagus River and monthly precipitation were obtained from the National Water Institute from a public database (www.inag.pt).

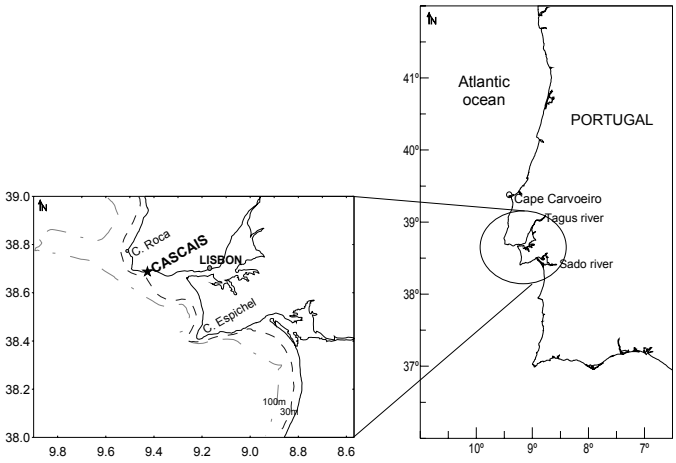

Figure 1 - Location of the Cascais sampling site 38º41′N and 09º24′W. (*) - Lisbon bay.

To evaluate the chlorophyll a concentration, 250 ml seawater samples were filtered and pigments extracted with 90% acetone and determined on the Perkin–Elmer spectrofluorometer (Holm-Hansen et al., 1965).

For coccolithophore studies, water samples (750 or 1000 ml) were filtered through a 47mm nitrate cellulose membrane (Whatman) with a 0.45 μm nominal pore size. A strip of the membrane was cut from the center to the rim and slides were rendered transparent with a drop of Entellan mounting medium. Coccospheres were identified and counted up to a maximum of 300 cells of all taxa per sample (Fatela and Taborda, 2002) on an area of 2.2mm^2 of the filter, with a Zeiss optical microscope under cross-polarized light at a magnification of 1250x. Classification of species followed Young et al. (2003). Holococcolithophores and other coccolithophores with morphological features difficult to recognize were counted and grouped into the category of ‘‘others’’ to be subsequently identified using a scanning electron microscope JEOL JSM-5200.

Although sampling was conducted 1 h before high tide, the study site is at a coastal location; it is shallow and is influenced by estuarine waters and anthropogenic effects. Thus, in order to validate the site, 10 supplementary surface samples were simultaneously collected once a month, 4 km offshore from the Cascais station. A Mann–Whitney *U*-test was performed with the 10 samples. The null hypothesis was that each pair of samples is drawn from a single community and therefore the medians are equal. The main phytoplankton groups were observed and quantified for the test and the p-level found for each pair was always above 0.1, meaning there was an

overlap between the two distributions. The study site was then considered representative of the inner shelf community.

In order to support and describe the associations between the coccolithophore species, a Principal Component Analysis (PCA) was performed using the software NTSYSpc version 2.02i from 1997 by Applied Biostatistics, Inc. The analysis was carried out with the species that occurred at least in 10% of the samples during the 4 years. From the 11 species identified only *Braarudosphaera bigelowii* was excluded due to a relative low frequency (5%). The category of ‘‘others’’ was also excluded from the PCA analysis because of the ecological heterogeneity of the group. Despite the number of zeros (species absence), the analysis did not distort the structure of the original data since the correlation (Mantel test) between Euclidian distances of the original data and the projections of species in the PCs axes showed a good fit ($r = 0.8$, $n = 10$).

3. HYDROGRAFY

The west coast of Portugal is the northern limit of the North Atlantic upwelling system (Wooster et al., 1976; Fiúza et al., 1982). In this region upwelling generally occurs seasonally, from April to September, under strong and steady north winds. In turn, convergence is observed during autumn and winter, although reverse winds can occur during both periods. At the end of the upwelling season, southern winds begin to dominate and there is an increase of waters flowing poleward as well as convergence towards the coast of warmer, oligotrophic oceanic waters (Fiúza, 1984; Haynes and Barton, 1990).

According to Fiúza (1984) and Rios et al. (1992), the main upwelling source water influencing Lisbon bay is the Eastern North Atlantic Central Water subtropical branch (ENACWst). The rivers Tagus and Sado also drain into this area, although with limited influence on local hydrology (Fiúza, 1984). Lisbon bay represents an important discontinuity in the north–south coastline orientation and is an upwelling shadow area where phytoplankton species can accumulate through different retention mechanisms (Graham and Largier, 1997). For the area, Moita et al. (2003) identified upwelling as the major source of seasonal and spatial variability of phytoplankton. An intensification of upwelling induces localized centers of colder water rooted at cape Roca and

Espichel. The upwelled waters of cape Roca can displace from the coast to form a filament, the cape Roca plume that can extend southwards and westwards. On the northern side of the upwelling plume high velocities advect any plankton out of the area; along its southern side, weaker current velocities result in a low net advection towards the coast that can induce plankton accumulation on the northern shore of Lisbon bay. The phytoplankton from the sampling site, Cascais (Figure 1), located on the northern shore of Lisbon bay and south of cape Roca is thus influenced either by upwelled waters of the cape Roca plume or by warmer and mature surrounding waters depending on the upwelling intensity and position of the plume. Weak upwelling conditions mean warmer and more stratified waters have a greater influence on the bay.

4. RESULTS AND DISCUSSION

4.1. Seasonal and interannual variability of the environmental conditions, phytoplankton biomass and coccolithophores

According to Bakun (1990) and Santos et al. (2005) for the Iberian margin and McGregor et al. (2007) for NW Africa, there has been a regional increase in coastal upwelling during the last decades of the 20th century. During the sampling period, the upwelling was in general weak and progressively more persistent. From 2002 to 2004 an increase of 55 days of upwelling was observed (Table 1).

Upwelling values were normally higher than -1000m^3 s^{-1} km^{-1}, ranging between -3142 and 2743m^3 s^{-1} km^{-1} in November 2001 and March 2002, respectively (Figure 2a). However, from autumn to spring the number of single strong events (<-2000m^3 s^{-1} km^{-1}) increased, as in November 2001, April 2002, February, May and November 2003 and Mars, May and November 2004. During spring, upwelling was always intermittent. The convergence periods were shorter and less intense over the years and there was an increase in the number of days with upwelling, especially from early autumn until winter (Table 1). The longest convergence period was during early winter 2002 (Figure 2a).

Upwelling index ($m^3 s^{-1} Km^{-1}$)	2001	2002	2003	2004	2005
Summer					
Season average	-272	-368	-258	-290	-
Nº of days with upwelling (-UI)	56	69	76	72	-
Total coccolithophores ($x10^3$ cells.l^{-1})	432	236	634	564	-
Autumn					
Season average	9	63	-90	-56	-
Nº of days with upwelling (-UI)	43	40	46	60	-
Total coccolithophores ($x10^3$ cells.l^{-1})	238	695	274	404	
Winter					
Season average	-	26	9	-136	-412
Nº of days with upwelling (-UI)	-	41	44	49	69
Total coccolithophores ($x10^3$ cells.l^{-1})	-	293	230	139	67
Spring					
Season average	-	-434	-239	-412	-98
Nº of days with upwelling (-UI)	-	71	62	86	31
Total coccolithophores ($x10^3$ cells.l^{-1})	-	476	255	202	296
Nº of days with upwelling per year	106	216	234	271	89
Total coccolithophores ($x10^3$ cells.l^{-1}) per year	635 (**)	1735	1401	1310	363 (**)

values from 2,5 months

**values from 5 months

Table 1 - Interannual variability of upwelling index ($m^3 s^{-1} km^{-1}$) and total coccolithophores ($x10^3 cells.l^{-1}$) from July 2001 until May 2005.

Sea surface temperatures (SST) that normally are sensitive indicators of changes in upwelling intensity and prevailing winds (Wooster et al., 1976; Nykjaer and Van Camp, 1994) did not vary directly with the increase in upwelling persistence. This was probably related to the location and depth of the sampling site, a shallow upwelling shadow area, which is more suited influenced by variations in air temperature (8.5–26.7ºC in www.inag.pt). Temperature ranged between 11.5 and 20.41ºC, respectively, in January 2003 and October 2002 (Figure 2b). SST varied at both seasonal and interannual time scales. The lowest temperatures were observed from late-autumn until spring, while the highest were during summer and early autumn (Figure 2b, Table 2). Summers were progressively warmer with longer periods above 17ºC. Summer 2002 was 1ºC lower than the other years and summer 2003 had a warmer minima and higher temperatures earlier in the season (Figure 2b, Table 2). According to Díaz et al. (2006), for the period 1991–2003, the summer 2003 (July and August) was exceptionally hot, especially in most of Western Europe. In Lisbon, the air temperature was ~4ºC higher than that in August 2002. These high temperatures influenced SST, with temperature anomalies of ~+1.5ºC in relation to the other summers. Furthermore,

high SST were observed earlier in the year, during the spring, where the maxima increased by 1ºC. Temperature amplitude increased during late spring 2003 and 2004.

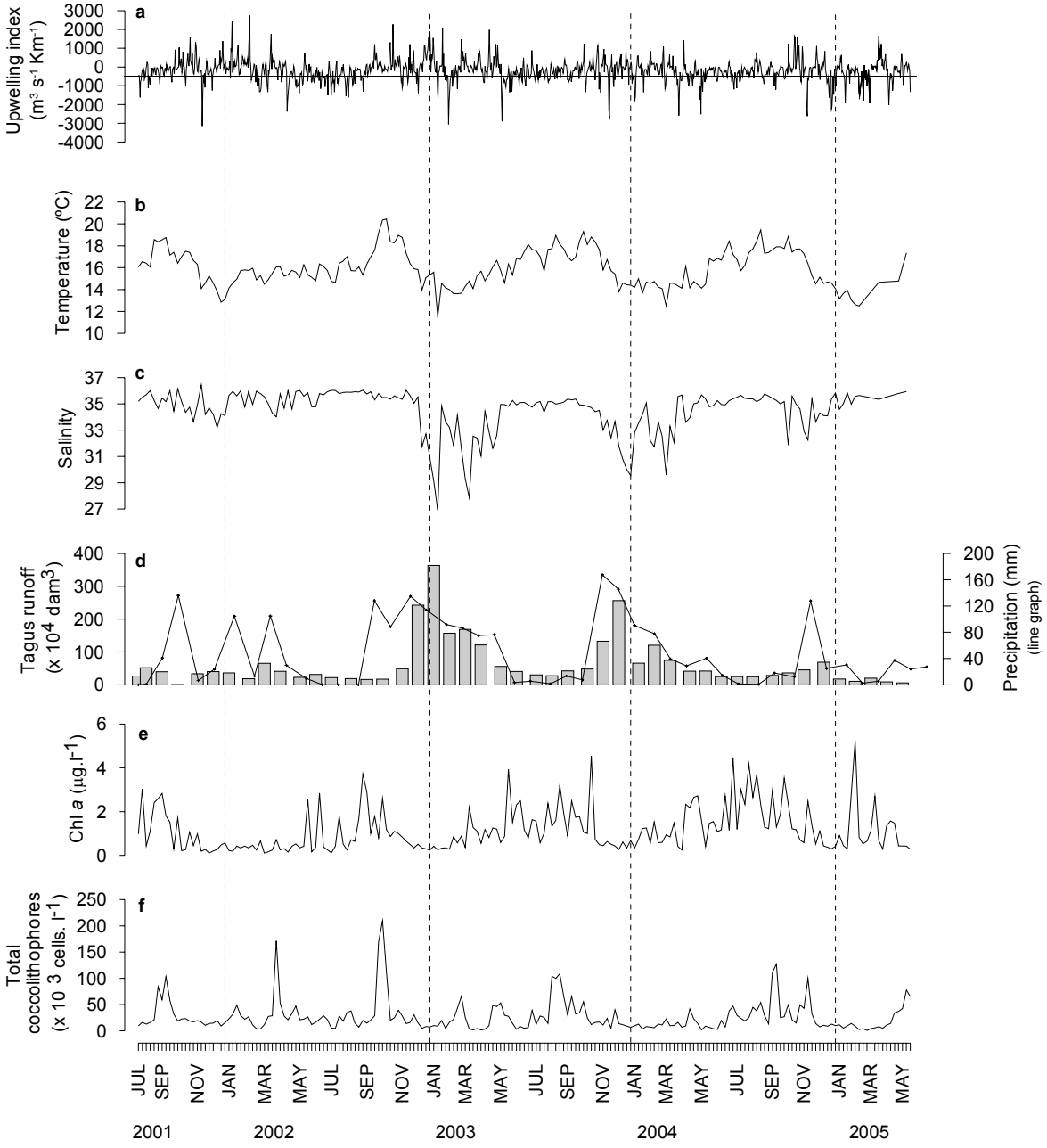

Figure 2 - Daily distribution of (a) upwelling index (negative values indicate upwelling), weekly distribution of (b) sea surface temperature and (c) salinity, monthly distribution of (d) Tagus runoff (bar chart) and precipitation (line graph) and weekly distribution of (e) chlorophyll a and (f) total coccolithophores, during the sampling period (July 2001–May 2005).

Autumns had values of 15–20ºC and 2002 and 2003 were the warmest with greater temperature amplitudes. Autumn 2002 was 1.5ºC higher then the other years. The lowest temperatures were recorded at the end of the season decreasing towards winter, when temperatures ranged from 12 to 15ºC (Figure 2b, Table 2). Winters became progressively colder, by about 1.5ºC and the maxima decreased over the years. Winter temperatures were never higher than 16ºC and the strongest differences apparently related to the increment of precipitation due to an intensification of the SW winds were in 2003 (www.inag.pt). This is usual for the autumn–winter period.

Salinity also presented a seasonal pattern and interannual variability ranging from 26.9 in January 2003 to 36.5 in October 2001, and was usually higher then 34.5 (Figure 2c). Higher and relatively constant values were observed from spring to early autumn while lower values were observed from late-autumn to winter. The interannual variation in salinity highlights the different weather conditions since salinity is strongly influenced by rainfall and thus by the Tagus river flow (Figure 2c, d). Rainfall is normally considered a proxy of seasonal and interannual climate change and the Tagus river flow is an indication of precipitation over the whole river basin (Trigo and DaCamara, 2000; Trigo et al., 2004). During the period of study, higher salinities were recorded in 2002 than the following years, which can be explained by the observed low precipitations and river runoff (Figure 2c, d, Table 2).

TEMPERATURE (ºC)	2001	2002	2003	2004	2005
Summer					
Minima	16,1	14,6	16,6	15,7	-
Maxima	18,8	17,0	19,0	19,5	-
Season average	17,4	16,0	17,5	17,5	-
Amplitude	2,7	2,4	2,3	3,7	-
Autumn					
Minima	13,9	14,0	13,8	14,5	-
Maxima	17,5	20,4	19,3	18,9	-
Season average	16,0	17,8	16,9	16,6	-
Amplitude	3,7	6,5	5,5	4,3	-
Winter					
Minima	-	12,9	11,5	12,5	12,2
Maxima	-	16,0	15,6	15,0	14,6
Monthly average	-	14,8	14,1	14,3	13,3
Amplitude	-	3,1	4,1	2,5	2,4
Spring					
Minima	-	14,8	14,1	14,1	14,7
Maxima	-	16,4	17,5	17,5	17,4
Season average	-	15,6	15,8	15,5	15,5
Amplitude	-	1,6	3,4	3,5	2,7
values from 2,5 months					

SALINITY	2001	2002	2003	2004	2005
Summer					
Minima	34,7	35,7	34,4	35,2	-
Maxima	36,0	36,0	35,4	35,7	-
Season average	35,4	35,9	35,1	35,4	-
Amplitude	1,4	0,4	1,0	0,6	-
Autumn					
Minima	33,2	31,7	30,8	31,9	-
Maxima	36,5	36,0	34,9	35,6	-
Season average	34,8	35,2	33,6	34,1	-
Amplitude	3,3	6,5	4,1	3,7	-
Winter					
Minima	-	34,1	26,9	29,5	35,0
Maxima	-	36,0	34,1	35,1	35,9
Season average	-	35,3	31,4	32,3	35,4
Amplitude	-	3,1	7,2	5,5	0,8
Spring					
Minima	-	34,0	31,0	33,6	35,4
Maxima	-	36,0	35,1	35,7	36,3
Season average	-	35,2	33,8	35,0	35,9
Amplitude	-	1,5	4,1	2,1	0,9
values from 2,5 months					

Table 2 - Interannual variability of temperature (ºC) and salinity from July 2001 until May 2005.

On the other hand, the lowest salinities were during winter 2003 when precipitation and river flow were the highest. In 2004 and 2005 salinity maxima gradually decreased in accordance with the rainfall. Precipitation varied from 0.1mm in July 2002 to 167.6mm in October 2003 (Figure 2d). The Tagus flow varied between $1x10^4$ m^3s^{-1} in October 2001 and $364x10^4$ m^3s^{-1} in January 2003, directly reflecting the increases in precipitation immediately prior (Figure 2d).

Throughout the 4 years studied the increase of both persistent weak upwelling conditions and SST resulted in the development of phytoplankton populations as measured by chlorophyll a (Figure 2e). Chlorophyll a varied between 0.1 $\mu g.l^{-1}$ in November 2003 and 5.24 $\mu g.l^{-1}$ in February 2005. These values are consistent with those observed by Moita (2001) along the coast of Portugal. Spring and summer were the two most productive seasons with a rise in biomass of ~1 $\mu g.l^{-1}$ throughout the years (Table 3), most probably related to the persistence of upwelling. A similar increase was also recorded for the winter. Biomass (Chl *a*) through the autumn remained constant and around 0.8 $\mu g.l^{-1}$. The maxima observed, are however 30 times lower than during bloom events. At Cascais bay extreme values of 160 $\mu g.l^{-1}$ have been recorded in a *Mesodinium rubrum* patch (Cabeçadas et al., 1983).

Chl *a* ($\mu g.l^{-1}$)	Anual average	Summer	Autumn	Winter	Spring
2001	1,0	1,9	0,6	-	-
2002	0,8	1,3	0,8	0,3	0,7
2003	1,2	1,7	0,8	0,7	1,5
2004	1,5	2,4	0,9	1,0	2,0
2005	1,3	-	-	1,3	0,8
values from 2.5 months					

Table 3 - Interannual variability of total biomass represented by chlorophyll a (Chl a) from July 2001 until May 2005.

The increase of phytoplankton biomass presented above was not followed by the coccolithophore populations that decreased towards the end of the sampling period (Figure 2f). The persistence of the upwelling conditions seems to have favoured other phytoplankton groups (unpublished results), more adapted to turbulence, such as

diatoms. The total abundance of coccolithophores decreased in accordance with the increase in the persistence and number of days with upwelling (Table 1). The variability in the total coccolithophores distribution revealed several peaks in all seasons. These peaks were always related to weak upwelling, upwelling relaxation or even convergence conditions, preferably associated with high temperatures and salinities. Minima were normally observed during winter related to lower temperatures and salinities and persistence of upwelling. In 2004 and 2005 the lowest concentrations ($0.9x10^3$ cells l^{-1} in February 2005) were observed in contrast with 2002 and 2003, years characterized by well established downwelling conditions (Table 1).

Maxima occurred during the summer–autumn short transition period from upwelling to downwelling seasons. During this period of about 1 month (Figueiras et al., 2002), coastal turbulence is reduced but nutrients provided by upwelling are still available. These conditions are characteristic of mature upwelled waters, where coccolithophores are most favoured (Margalef, 1978; Giraudeau et al., 1993; Kleijne, 1993; Ziveri et al., 1995). The highest abundances were recorded during summer 2003 characterized by high SST and very weak and persistent upwelling conditions (Tables 1 and 2). At the end of each summer, convergence was established and SST increased, resulting in higher coccolithophore concentrations. The highest peak ($210x10^3$ cells l^{-1}) was registered in autumn 2002, the warmest of the study period (Figure 2b, f) with less upwelling days (Tables 1 and 2). During winter–spring, other lower and shorter coccolithophore peaks were observed. These maxima seemed related to convergence periods or relaxation of upwelling events and lower temperatures, but with salinities higher than 34. From winter 2002 to winter 2005 coccolithophores maxima decreased four times due to an increase in the persistence and intensity of upwelling (Table 1).

4.2. The coccolithophore assemblage

Eleven coccolithophore species were identified and the SEM revealed ten additional species grouped in the ‘‘others’’ category, made up of holococcolithophores and disintegrated cells or free coccoliths from the genus *Syracosphaera*. Due to the coastal location of the sampling site, all the species observed are representative of the upper photic zone. Coccolithophores from the deeper photic zone such as *Florisphaera profunda*, *Oolithotus antillarium*, *Algirosphaera robusta* and *Gladiolithus flabellatus*

(Andruleit, 2007), which have been observed further offshore in deeper layers (unpublished results) were not found during this work. The structure of the coccolithophore assemblage (CA) in general changed throughout the year showing seasonal fluctuations mainly related to the intensity and persistence of upwelling (Figure 2a and 3, Table 4). Most coccolithophore maxima were within a range of temperatures of 13.6–20.4ºC and with salinity values between 31.6 and 36.0. The winter community was characterized by maxima of *Calcidiscus leptoporus* and *Coccolithus pelagicus* (Figure 3). During spring, the maximum abundances were of *Emiliania huxleyi*, *Gephyrocapsa* spp. and there were lower concentrations of *C. pelagicus*, *C. leptoporus* and *Coronosphaera mediterranea* (Figure 3). The most abundant species during summer–autumn were *Helicosphaera carteri*, *C. mediterranea*, *Rhabdosphaera clavigera* and *Syracosphaera pulchra*, *E. huxleyi* and *Gephyrocapsa* spp. (Figure 3).

E. huxleyi occurred in larger numbers and usually dominated the CA. Maxima of this highly eutrophic species (Roth, 1994) were during spring and late summer close to very weak upwelling events (Figure 2a and 3, Table 4). This opportunistic behaviour, characterized by rapid growth during the two most productive periods (spring and summer), reinforce the use of *E. huxleyi* as a proxy for productivity (Knappertsbusch, 1993; Giraudeau and Bayley, 1995; Broerse et al., 2000; Andruleit and Rogalla, 2002; Bárcena et al., 2004). *E. huxleyi* was distributed between 11.5 and 20.4ºC and salinities of 26.9–36.3 (Figure 6). This species reached a maximum of $156x10^3$ cells l^{-1}, with relative abundances higher than 80%, during March 2002 (Table 4). The warmest year of 2003, associated with very weak and intermittent upwelling, favoured a regular development of the species that dominated the assemblage for almost all the year (Figure 2a and 3).

The three species of *Gephyrocapsa* (*G. oceanica*, *G. muellerae* and *G. ericsonii*), similar to *E. huxleyi*, were always present but higher abundances were observed after very weak and intermittent upwelling episodes, especially from late spring until autumn (Figure 2 and 3, Table 2). A close relationship was observed between the genus and highly productive environments.

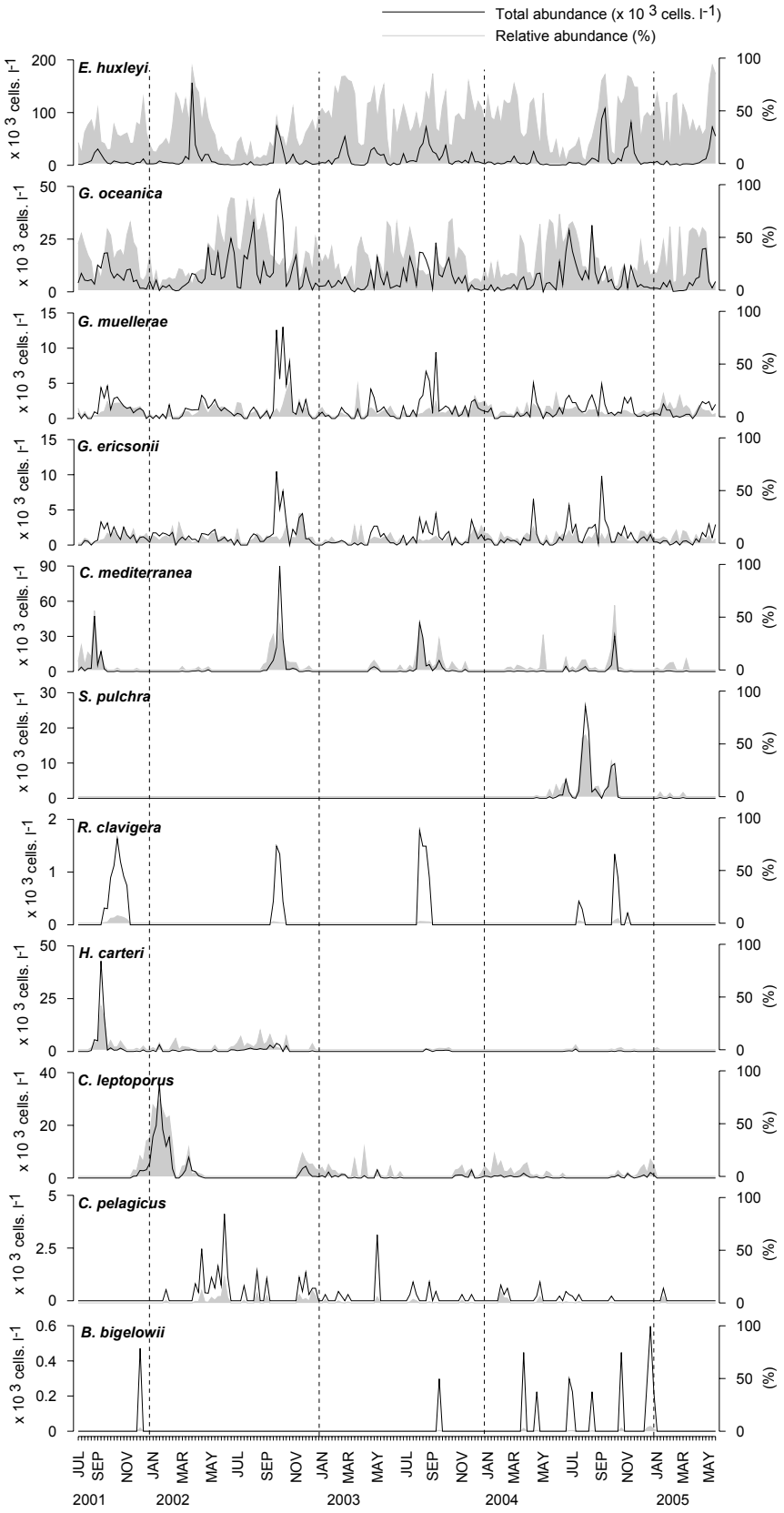

Figure 3 - Weekly distribution (x10^3 cells l^{-1}) of the ten coccolithophores observed from July 2001 to May 2005.

Therefore, *Gephyrocapsa* spp., like *E. huxleyi*, are good proxies for coastal productivity generated by upwelling and nutrient availability (Giraudeau, 1992; Young, 1994; Ziveri et al., 1995; Bollmann, 1997; Broerse et al., 2000; Hagino et al., 2000; Bárcena et al., 2004; Rogalla and Andruleit, 2005). Despite the same opportunistic behaviour and similar ecological preferences, maxima of these species do not coincide. Most maxima of the genus *Gephyrocapsa* were during summer–autumn and after *E. huxleyi*, which preferentially develops during spring. The interannual distribution revealed that all the *Gephyrocapsa* species had maxima in autumn 2002, the warmest autumn (~+1.5ºC) of the present time series (Tables 2 and 4). This work also regarded *E. huxleyi* and *Gephyrocapsa* spp. as coastal species since these coccolithophores were always observed in samples from Lisbon bay. *Gephyrocapsa* spp. occurred between 11.5–20.4ºC and 26.9–36.3 of salinity (Figure 6).

G. oceanica reached 48 x10^3 cells l^{-1} in September 2002 (23–86% CA) and was the most abundant species during summer (Figure 2 and 3, Table 2). This subtropical–tropical coccolithophore Ziveri and Thunell, 2000; Álvarez et al., 2005) prefers warmer and less turbulent waters compared to *G. muellerae* and *G. ericsonii*. These two species appeared earlier in the year, in April-May, though were less abundant (4–16% CA) and tolerate lower temperatures (Figure 2a,b and 3, Table 4). This agrees with Findlay and Flores (2000), Colmenero-Hidalgo et al. (2004) and Bollmann (1997), who considered *G. mullerae* an indicator of cold water and/or waters of moderate to high productivity. The species is also regarded as a marker of the temperate biogeographic coccolithophore zone (Zone 2 of McIntyre and Bé, 1967; Winter et al., 1994). *G. mullerae*, reached 13x10^3 cells l^{-1} in October 2002. In this work *G. ericsonii* can be considered ecologically similar to *G. muellerae* since it showed similar preferences. The species reached a maximum of 10x10^3 cells l^{-1} in September 2002 and 2004 (Figure 2a,b and 3, Table 4).

Another group of less opportunistic coccolithophores occurred during the summer–autumn and was composed of *C. mediterranea*, *S. pulchra*, *R. clavigera* and *H. carteri*, subtropical species that coexisted from August to October. These species preferred high temperatures and salinities combined with relaxation periods at the end of the upwelling season or the beginning of the downwelling period (Figure 2a, b and 3, Table 4). However, the present data also suggest that during these periods the assemblage seems to tolerate colder waters under weak upwelling conditions. All the taxa except *R. clavigera* tolerated coastal turbulence. These species seem to thrive in environments resulting from the confluence of warmer and oligotrophic oceanic waters

with coastal and low turbulent but still nutrient enriched waters. This group almost dominates the CA (40–60%) during summer and was also probably favoured by low net advection or even retention conditions, responsible for plankton maintenance and accumulation at this time of the year.

C. mediterranea occurred in high abundances during late summer under convergence of warmer waters or during very weak upwelling conditions. The highest concentration observed was of $90x10^3$ cells l^{-1} (43% CA) in October 2002, the warmest autumn associated with well established convergence (Figure 2a and 3, Table 4). The species was also observed during spring in small concentrations under low temperatures. *C. mediterranea* developed in salinities between 31.6 and 36.0 and in a wide range of temperatures 12.5–20.4ºC (Figure 6). Due to shorter convergence periods the species decreased in abundance towards the end of the sampling period. *C. mediterranea* was the species with the widest range of temperature and salinity tolerance from the summer–autumn assemblage.

S. pulchra appeared for the first time in June 2004 under very weak upwelling conditions and reached $26x10^3$ cells l^{-1} (58% CA) in 2004 during August, dominating the coccolithophore community (Figure 2a and 3, Table 3). *S. pulchra* occurred between 12.3–19.5ºC and 31.9–35.8 of salinity (Figure 6). Our data agree with Beaufort and Heussner (2001) who suggested that *S. pulchra* is an autumn species capable of increasing in abundance from August to November and which with *R. clavigera* prefers stable stratified waters (Hagino et al., 2000). Findlay and Giraudeau (2000) also observed that *Syracosphaera* species preferred warm waters and does not seem to tolerate temperatures below 10ºC (Samtleben et al., 1995).

R. clavigera was only observed during the summer, always in very low abundances, reaching $2x10^3$ cells l^{-1} in October 2001 and August 2003 (7% and 2% CA, respectively) (Figure 2a and 3, Table 4). *R. clavigera* revealed a low tolerance to turbulence since the presence of this species inshore was always associated with the absence of northerlies, i.e. upwelling relaxation. Whenever very weak and intermittent upwelling episodes took place, the species disappeared. *R. clavigera* was found between 15.3 and 20.4ºC and salinities between 31.9 and 36.1 (Figure 6), and can be considered a warm water species as stated by other authors (McIntyre and Bé, 1967; Okada and Honjo, 1973; Kleijne, 1993; Winter et al., 1994; Haidar and Thierstein, 2001). Cachão et al. (2000) reported this subtropical species during winter off Portugal

along with *C. mediterranea* and *S. pulchra* and considered the group indicative of the influence of the ENACWst water mass off Portugal.

H. carteri maxima were during summer and early autumn under very weak upwelling and high temperatures ($43x10^3$ cells l^{-1} in September 2001; 41% CA) (Figure 2 and 3, Table 2). This species developed between 13.9–20.4ºC and 31.7–36.2 of salinity (Figure 6) and several authors refer to this species as a coastal thermophilic taxon (McIntyre and Bé, 1967; Okada and McIntyre, 1979; Winter et al., 1979). *H. carteri* could tolerate moderate turbulence generated by persistent events of weak upwelling that appear to be in agreement with other works that consider the species as a marker of moderate nutrient levels (Roth and Berger, 1975; Giraudeau, 1992; Ziveri et al., 1995). We also observed that *H. carteri* occurred in a narrower range of temperatures and do not seem to respond so quickly to nutrient enrichment as *C. mediterranea* and *S. pulchra*, although the three species can be associated with productivity. Nevertheless, *H. carteri* has the advantage of being a robust coccolithophore, whose coccoliths remain better preserved in the sediment, making this species a valuable target for future research. This group of species can also be considered characteristic of the shelf, as they are observed inshore whenever enriched and stable conditions occur. For example, *H. carteri* approached the coast once in January 2002, a winter characterized by higher temperatures and salinities with downwelling events, conditions not recorded in the following rainy winters (Figure 2a, b and 3).

In contrast to the above summer group, *C. leptoporus* presented maxima mainly during winter ($36x10^3$ cells l^{-1} in January 2002, 73% CA) under convergence conditions and lower temperatures (Figure 2a and 3, Table 4). A second peak occurred near the coast during spring ($3x10^3$ cells l^{-1} in May 2003, 6% CA) whenever the upwelling pulses relaxed. *C. leptoporus* developed between 11.5 and 18.9ºC and salinities of 26.9–36.0 and concentrations decreased over the years directly related to a decrease in convergence periods during winter and also to strong fluctuations in salinity due to rainfall (Figure 6). *C. leptoporus* is considered by several authors as being characteristic of tropical to subtropical oligotrophic warm-water masses (McIntyre and Bé, 1967; Okada and Honjo, 1973; Kleijne, 1993; Winter et al., 1994). However, other authors pointed out that the species prefer low turbulent and cold waters, not colder than 16ºC (Giraudeau, 1992; Giraudeau and Rogers, 1994; Ziveri et al., 1995; Haidar and Thierstein, 2001; Bárcena et al., 2004). In Lisbon bay maxima of *C. leptoporus* were observed in lower temperatures (~14–15ºC). The species was always absent

during summer at this coastal station, although in previous work off Portugal it was present in offshore oceanic waters during this season and on the shelf during winter (Cachão and Moita, 2000; Cachão et al., 2000; Moita, 2001). This work reinforces these patterns and considers *C. leptoporus* as a tracer for the convergence of surface offshore subtropical waters during its colder period, i.e. through winter and spring. These data highlight that *C. leptoporus* can be regarded as a proxy of non-productive periods off the coast of Portugal and a typical component of the transition from cold to warmer water floras, which does not agree with Flores et al. (1997, 2003). These differences might be related to the observation of different morphotypes of *C. leptoporus*. Flores et al.'s (2003) conclusions refer to specimens of *C. leptoporus* with coccoliths smaller than 5 μm. In Lisbon bay our specimens include two forms, an intermediate and a larger one, based on the size of the coccosphere, but both have coccoliths larger than 5 μm (Knappertsbusch et al., 1997; Baumann and Sprengel, 2000). During winter, the two morphotypes coexisted and during spring only the intermediate one was observed. Beaufort and Heussner (2001) also observed two morphotypes with different seasonality in the Bay of Biscay, a small form associated with summer (coccolith <5 μm) and a large form during the autumn (coccolith >5 μm).

C. pelagicus appeared throughout the upwelling season (spring to early autumn) with maximum densities during spring and minima during winter (Figure 2a and 3, Table 4). This species revealed a preference for cold waters (13.6–15.9ºC) associated with moderate turbulence and was recorded between 11.5–18.4ºC and 26.9–36.0 of salinity (Figure 6). Cachão and Moita (2000) observed similar preferences for Western Iberia and suggested that *C. pelagicus* indicates the presence of an upwelling front, acting as a front tracer on the outer limits of areas where turbulence is moderate. For Lisbon bay, the species abundance can indicate the upper or lower limit of the upwelling plume rooted at Cape Roca, in relation to the Cascais site. The highest concentrations were in spring ($4x10^3$ cells l^{-1} in June 2002, 25% CA) while lower abundances occurred during the winter. 2002 was the most favourable year for this species as the upwelling season was stronger and more persistent (Figure 2a and 3, Table 4).

B. bigelowii was randomly observed during the sampling in very low concentrations and relative abundances. The maximum abundance was 600 cells l^{-1} (5% CA) in December 2004 (Figure 3, Table 2). *B. bigelowii* developed within 14.1–17.7ºC and salinities of 34.1–35.6. This coccolithophore had a sporadic and random

occurrence either in convergence or in upwelling conditions (Figure 2a and 3, Table 4) and is considered an opportunistic survivor (Tantawy, 2003; Bown and Concheyro, 2004; Thierstein et al., 2004). From 1987 until 2003 sporadic blooms were observed for the Portuguese coast (Duarte-Silva et al., 2004) always in warm (17–20ºC) and saline (35.2–36) conditions. However, the species seems to tolerate lower temperatures (14.1–17.7ºC) due to its occurrence during winter months. This work revealed that *B. bigelowii* occurred with salinities above 34 and generally associated with periods of no rain. One exception was recorded, during winter 2004, when some rain occurred although salinity was above 34.1. The ecology of *B. bigelowii* is poorly known and the present data is an additional contribution to the understanding of this species' dynamics.

All the coccolithophores which were difficult to identify under the optical microscope, but were subsequently identified with the SEM are included in the category of ''others''. This was a very heterogeneous group mainly composed by the holococcolithophores of the previously observed species as well as of disintegrated cells or free coccoliths from the genus *Syracosphaera*. Based on both the maxima of the identified heterococcolithophores and the group of ''others'', 13 samples (1–13) were chosen to be observed under the SEM (Figure 4).

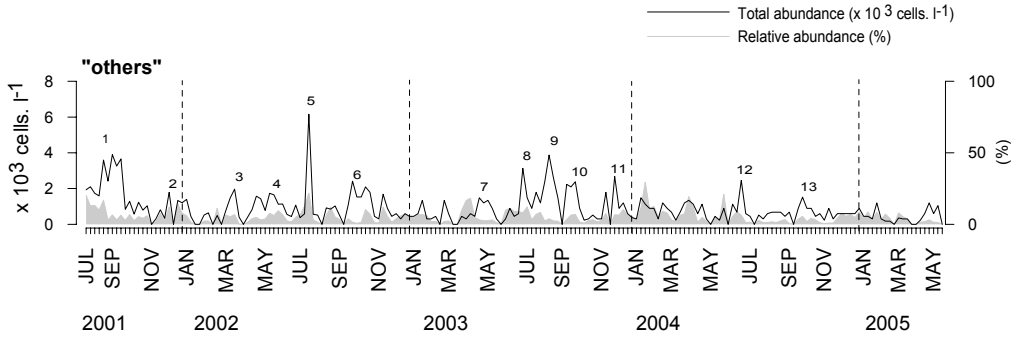

Figure 4 - Weekly distribution of the category of ''others (x 10^3 cells.l^{-1}) during the sampling period (July 2001–May 2005). Numbers from 1 to 13 represent the samples observed under SEM.

The following 10 species were identified: *Syracolithus confusus* (samples 1, 6), *Crystallolithus hyalinus* (3, 4, 5, 7), *Calyptrosphaera oblonga* (12, 13), *Crystallolithus rigidus* (2, 11), *Calyptrolithophora gracillima* (1, 6, 8, 9, 10, 12, 13), *Calyptrolithophora*

papilifera (1, 6, 8, 9, 10, 12, 13), *Corisphaera* sp. (1, 6, 8, 9, 10, 12), *Zygosphaera marsilli* (6, 8, 9, 10, 12, 13), *Syracosphaera lamina* (1, 6, 8, 9, 10, 13) and *Syracosphaera* spp.(coccoliths of different species always present). The distribution of each holococcolithophore (haploid phase) was coincident with the maxima of the correspondent heterococcolithophore (diploid phase) (Figure 3 and 4).

2001	Maxima (x 10^3 cells.l^{-1})	Relative Abundance (%)	Month	Temperature (ºC)	Salinity
E. huxleyi	31	52	September	18.6	35.5
G. oceanica	19	58	September	17.4	34.5
G. muellerae	5	14	September	17.4	34.5
G. ericsonii	3	10	September	18.8	35.2
C. mediterranea	47	56	August	18.4	34.7
S. pulchra	-	-	-	-	-
R. clavigera	2	7	October	17.5	34.4
H. carteri	43	41	September	18.8	35.2
C. leptoporus	-	-	-	-	-
C. pelagicus	-	-	-	-	-
B. bigelowii	0.5	3	December	14.6	34.2
2002					
E. huxleyi	156 75	91 44	March September	16.0 19.2	34.0 35.8
G. oceanica	33 48	86 23	August September	15.7 20.4	35.9 35.5
G. muellerae	3 13	16 12	April October	15.4 20.4	36.0 35.5
G. ericsonii	2 10	10 6	May September	16.3 19.2	35.6 35.8
C. mediterranea	1 90	3 43	April October	15.6 20.4	36.0 35.5
S. pulchra	-	-	-	-	-
R. clavigera	1.5	1	October	19.2	35.8
H. carteri	4	2	September	19.2	35.8
C. leptoporus	36 4.5	73 15	January November	14.5 16.0	35.6 35.1
C. pelagicus	4 1	25 4	June November	14.8 16.0	34.8 35.1
B. bigelowii	-	-	-	-	-

2003	Maxima (x 10^3 cells.l^{-1})	Relative Abundance (%)	Month	Temperature (ºC)	Salinity
E. huxleyi	55 73	83 67	February August	13.7 18.1	31.6 35.1
G. oceanica	19 23	18 35	August September	17.7 16.6	35.2 35.3
G. muellerae	4 9	9 14	April September	16.1 16.6	31.6 35.3
G. ericsonii	3 4	6 7	April September	16.7 16.6	32.6 35.3
C. mediterranea	4 42	9 40	April August	16.1 17.7	32.6 35.2
S. pulchra	-	-	-	-	-
R. clavigera	2	2	August	17.7	35.2
H. carteri	1	1	August	18.1	35.1
C. leptoporus	3 3	6 7	May November	15.8 14.4	35.0 33.7
C. pelagicus	0.45 3	2 6	January May	13.6 15.8	31.9 35.0
B. bigelowii	0.3	1	September	17.0	35.4

2004	Maxima (x 10^3 cells.l^{-1})	Relative Abundance (%)	Month	Temperature (ºC)	Salinity
E. huxleyi	107 81	84 81	September November	17.9 16.4	35.3 32.3
G. oceanica	29 31	62 58	June August	17.2 19.5	35.4 35.3
G. muellerae	5 5	12 4	April September	14.2 17.9	33.9 35.3
G. ericsonii	7 10	16 9	April September	14.2 17.9	33.9 35.3
C. mediterranea	0.6 30	33 61	May October	14.1 18.9	35.7 31.9
S. pulchra	26	58	August	17.7	35.4
R. clavigera	1	3	October	18.9	31.9
H. carteri	1	5	July	15.7	35.7
C. leptoporus	1.8 2	11 16	March December	14.4 14.6	35.5 35.4
C. pelagicus	0.75 0.9	10 6	January April	14.6 14.5	32.2 35.1
B. bigelowii	0.5 0.6	3 5	March December	14.4 14.6	35.6 35.4

2005	Maxima (x 10^3 cells.l^{-1})	Relative Abundance (%)	Month	Temperature (ºC)	Salinity
E. huxleyi	73	93	May	17.4	36.0
G. oceanica	21	55	May	14.8	35.8
G. muellerae	2	7	April	-	-
G. ericsonii	3	7	May	-	-
C. mediterranea	-	-	-	-	-
S. pulchra	-	-	-	-	-
R. clavigera	-	-	-	-	-
H. carteri	-	-	-	-	-
C. leptoporus	-	-	-	-	-
C. pelagicus	0.6	6	January	14.0	35.9
B. bigelowii	-	-	-	-	-

Table 4 - Correspondence between each coccolithophore maximum concentration(x10^3 cells.l^{-1}) and relative abundance (%), temperature (ºC), salinity and time of the year (July 2001–May 2005).

4.3. Principal component analysis (PCA)

A PCA was performed to summarize the observed results and help with understanding and interpreting them. The first three components explain 89.7% of the total variation in the data.

The first component (PC1) explained 34.5% of total variability within the data and was positively correlated with all species, reflecting their abundance. This is often the case (Estrada, 1984) when species are present in high concentrations (figure not shown). *E. huxleyi* was the most correlated species with PC1.

The second component (PC2) explained 23.2% of total variability within the data and opposed two groups of species that presented different interannual distributions (Figure 5). PC2 placed *H. carteri* close to *C. leptoporus* (species normally occurring in different seasons) as a result of their interannual occurrence, characterized by presenting maxima in the first year and a significant decrease during the following years (Figure 3). On the other hand, *R. clavigera* was observed every year during the summer. However, the second component also reflected some seasonal variability within the data since it separated two species that are abundant in different seasons: *R. clavigera* characteristic of the summer and *C. leptoporus* of the winter–spring. Both species reflected the convergence of warmer surface oceanic waters during the two

periods. It was expected that *R. clavigera* and *H. carteri* would be found together since they occurred in the same summer community. *H. carteri* was distributed in a wide range of temperatures, and overlapped with *C. leptoporus* at colder temperatures (Figure 6). These species as mentioned above, presented similar interannual distributions. Several works associated *H. carteri* and *C. leptoporus*. For the coast of Portugal, Cachão and Moita (2000) described the presence of both species in outer-shelf oceanic waters during summer. These species inhabit more oceanic waters but can occupy the shelf in different seasons under convergence depending on their affinities for higher (*H. carteri*—summer) or lower (*C. leptoporus*— winter) temperatures.

These seasonal short time scale differences are difficult to observe in sediment samples, cores or sediment traps that are used to obtain information on sedimentary processes and fluxes. This might be the reason why some works have found both species simultaneously, with similar seasonal trends and abundances when they occupy the same water mass (Flores et al., 2003; De Bernardi et al., 2005; Ziveri et al., 2007).These species are also found together because both are robust, solution-resistant species, whose abundance tends to increase from the water column to the sediment assemblages (Ziveri et al., 2007). In the Bay of Biscay, Beaufort and Heussner (2001) used three trap deployments to observe the existence of two morphotypes of *C. leptoporus* with different seasonalities; the large form was considered a summer species together with *H. carteri*. These data also suggested that *H. carteri* is probably associated with a more spring–summer morphotype of *C. leptoporus*.

The third component (PC3) explained 18.3% of total variability within the data and reflected the level of tolerance to upwelling intensity of *C. pelagicus*, *R. clavigera* and *H. carteri* (Figure 5). The most correlated species, *C. pelagicus*, is known to be adapted to upwelling fronts where stability is enhanced off the Iberian coast. *R. clavigera* and *H. carteri* are less tolerant to upwelling, decreasing whenever upwelling intensified, although in different seasons. Opposed to this group, but with a low correlation with PC3, is an assemblage composed of *E. huxleyi*, *G. oceanica*, *G. muellerae*, *G. ericsonii*, *C. mediterranea* and *S. pulchra* that can thrive in mild upwelling waters.

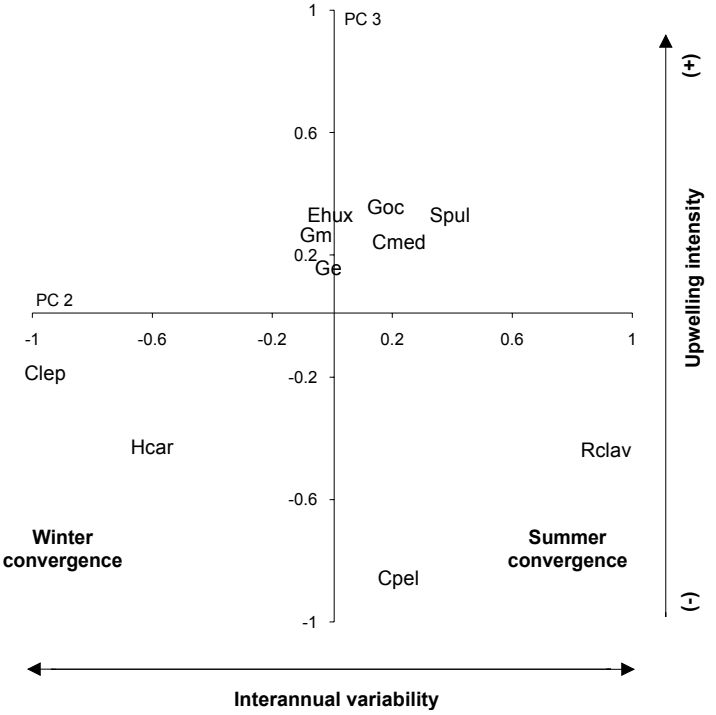

Figure 5 - Distribution of the observed coccolithophores in the space defined by the second (PC2) and third (PC3) components. PC2 evidenced: *C. leptoporus* (Clep) and *H. carteri* (Hcar) opposite to *R. clavigera* (Rclav). PC3 evidenced *E. huxleyi* (Ehux), *G. oceanica* (Goc), *G. muellerae* (Gm), *G. ericsonii* (Ge), *C. mediterranea* (Cmed), *S. pulchra* (Spul) opposite to *C. pelagicus* (Cpel).

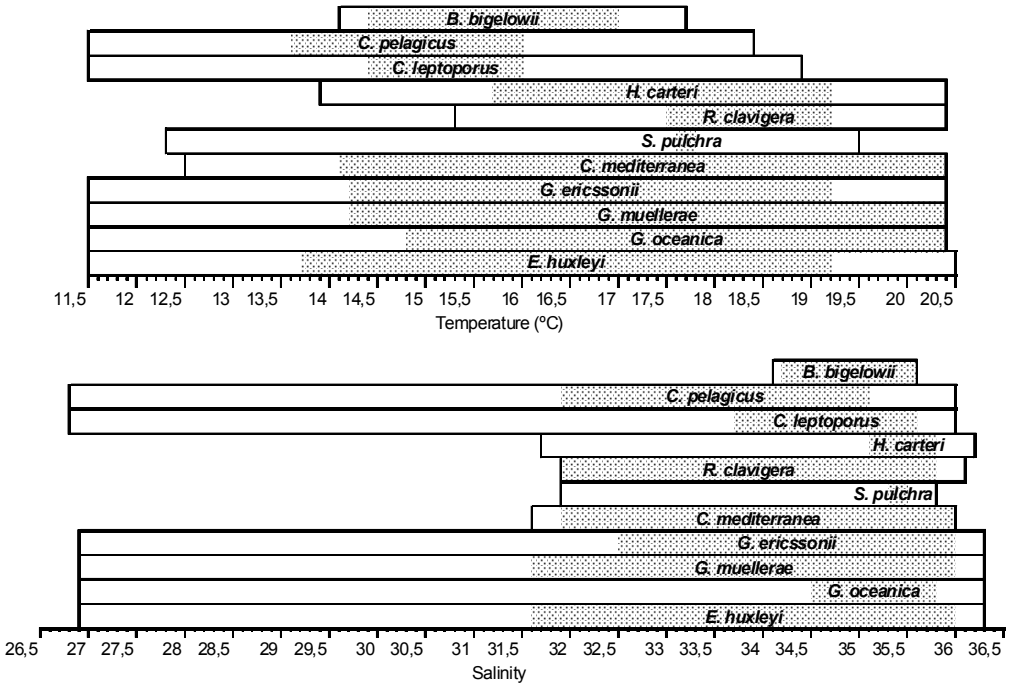

Figure 6 - Schematic representation of the distribution of the different species tolerances and preferences in relation to temperature and salinity: open squares define the species distribution; full squares represent where maxima were found.

5. CONCLUDING REMARKS

The coccolithophores observed were capable of resisting coastal processes such as turbulence and were well adapted to an environment rich in nutrients provided by both continental runoff and by upwelling. Higher abundances were, in general, associated with the productive periods of spring and summer. Most species benefited from the same favourable conditions, i.e. mature upwelled waters and low turbulent environments, but developed in different windows of temperature with mismatch maxima. As a result, a recurrent and distinct seasonal succession and interannual differences were observed. The coccolithophores distribution was both related to the seasonality, intensity and length of the upwelling–downwelling seasons and the associated temperatures and to precipitation with associated salinities. The optimum temperature range lay between 14 and 20ºC corresponding to salinities of 34–36.5 under convergence or weak upwelling conditions. The interannual increase of the upwelling persistence also seemed to be reflected in the interannual decrease of species abundance, despite the increment of ~1–1.5ºC from spring to autumn. Winter periods had a decreased ~1ºC associated with increments in precipitation, resulting in very low values of salinity linked with a strong decrease in the concentration of all species.

E. huxleyi and the *Gephyrocapsa* genus are coastal taxa that quickly respond to a decrease in turbulence during upwelling events and thus can be used as proxies for surface productivity waters, in particular during spring and summer. *E. huxleyi*, *G. muellerae* and G. ericsonii indicated the presence of colder waters associated with the beginning of the upwelling season that usually occurs during spring. In contrast, *G. oceanica* indicated productive periods during summer. Also related with upwelling, *C. pelagicus* seemed to indicate the position and displacement of the Cape Roca plume in relation to the Cascais site. *C. mediterranea*, *S. pulchra*, *H. carteri* and *R. clavigera* can be used as tracers for the convergence of subtropical warmer and saltier waters over the shelf during summer–early autumn, the transition period from upwelling to downwelling seasons. The first three species stand moderate turbulence while *R. clavigera* disappeared from the coast as soon as upwelling intensified. The convergence of oceanic waters during winter can be traced by *C. leptoporus*.

Sampling once a weak allowed exhaustive observations of coccolithophores concentrations and species diversity and of how quickly species varied significantly as

a response to upwelling pulses and coastal dynamics. The recurrence of the results also evidenced the strong seasonality of this group and the remarkable variety of preferences in oceanographic conditions. Each coccolithophore appeared associated with particular turbulence, temperature and salinity situations. Bi-weekly sampling would be adequate to reduce observation effort and still ensure the results. A larger gap between samples would result in a loss of information about biodiversity and of the interannual variability in physical and biological data as well as in difficult correlations between species and the environment. Using the present data to simulate a monthly sampling program preserved the overall distribution of species, but in lower concentrations. Relations with upwelling and temperatures were difficult to observe and species tolerance to physical parameters decreased. For instance, the number of observations of *B. bigelowii* was four times lower; *G. ericsonii* became more abundant than *G. muellerae* and *H. carteri*, and the spring distribution of *C. mediterranea* was not detected. This weekly sampling was ideal for an accurate study about the ecological preferences of coccolithophores and fundamental to observe and identify seasonal and interannual changes. Such an effort will be required to determine precise associations between species and differences between regional oceanographic regimes.

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References

Álvarez, M.C., Flores, J.A., Sierro, F.J., Diz, P., France´s, G., Pelejero, C., Grimalt, J., 2005. Millennial surface water dynamics in the Rı´a de Vigo during the last 3000 years as revealed by coccoliths and molecular biomarkers. Palaeogeography, Palaeoclimatology, Palaeoecology 218, 1–13.

Andruleit, H., 1997. Coccolithophore fluxes in the Norwegian-Greenland Sea: seasonality and assemblage alterations. Marine Micropaleontology 31, 45–64.

Andruleit, H., 2007. Status of the Java upwelling area (Indian Ocean) during the oligothrophic northern hemisphere winter monsoon season as revealed by coccolithophores. Marine Micropaleontology.

Andruleit, H., Rogalla, U., 2002. Coccolithophores in surface sediments of the Arabian Sea in relation to environmental gradients in surface waters. Marine Geology 186, 505–526.

Andruleit, H., Stager, S., Rogalla, U., Cepek, P., 2003. Living coccolithophores in the northern Arabian Sea: ecological tolerances and environmental control. Marine Micropaleontology 49, 157–181.

Andruleit, H., Rogalla, U., Stager, S., 2005. Living coccolithophores recorded during the onset of upwelling conditions off Oman in the western Arabian Sea. Journal of Nannoplankton Research 27 (1), 1–14.

Bakun, A., 1973. Coastal upwelling indices, West Coast of North America 1946–71. NOAA Technical Report NMFs SSFR-671. US Dept. of Commerce, p. 103.

Bakun, A., 1990. Global climate change and intensification of Coastal Ocean upwelling. Science 247, 198–201.

Bárcena, M.A., Flores, J.A., Sierro, F.J., Pérez-Folgado, M., Fabres, J., Calafat, A., Canals, M., 2004. Planktonic response to main oceanographic changes in the Alboran Sea (Western Mediterranean) as documented in sediment traps and surface sediments. Marine Micropaleontology 53, 423–445.

Baumann, K.-H., Sprengel, C., 2000. Morphological variations of selected coccolith species in a sediment trap north of the Canary Islands. Journal of Nannoplankton Research 22, 185–193.

Beaufort, L., Heussner, S., 2001. Seasonal dynamics of calcareous nannoplankton on West European continental margin: the Bay of Biscay. Marine Micropaleontology 3, 27–55.

Bollmann, J., 1997. Morphology and biogeography of Gephyrocapsa coccoliths in Holocene sediments. Marine Micropaleontology 29, 27–55.

Bown, P.R., Concheyro, A., 2004. Lower Cretaceous calcareous nannoplankton from the Neuque´n Basin, Argentina. Marine Micropaleontology 52 (1–4), 51–84.

Broerse, A.T.C., Ziveri, P., van Hinte, J.E., Honjo, S., 2000. Coccolithophore export production, species composition and coccolith-CaCo3 fluxes in the NE Atlantic (341N 211Wand 481N 211W). In: Particle Flux and its Preservation in Deep-Sea Sediments. Deep-Sea Research II 47 (9–11), 1877–1906.

Cabeçadas, G., Cunha, M.E., Moita, M.T., Pissarra, J., Sampayo, M.A., 1983. Red tide in Cascais bay, Portugal. Boletim do Instituto Nacional de Investigação das Pescas 10, 81-123.

Cachão, M., Moita, M.T., 2000. Coccolithus pelagicus, a productivity proxy related to a moderate fronts of western Iberia. Marine Micropaleontology 39, 131–155.

Cachão, M., Oliveira, A., Vitorino, J., 2000. Subtropical winter guests, offshore Portugal. Journal of Nannoplankton Research 22 (1), 19–26.

Colmenero-Hidalgo, E., Flores, J.A., Sierro, F.J., Bárcena, M.A., Löwemark, L., Schönfeld, J., Grimalt, J.O., 2004. Ocean surface water response to short-term climate changes

revealed by coccolithophores from the Gulf of Cadiz (NE Atlantic) and Alboran Sea (W Mediterranean). Palaeogeography, Palaeoclimatology, Palaeoecology 205, 317–336.

Cortés, M.Y., Bollmann, J., Thierstein, H.R., 2001. Coccolithophore ecology at the HOT station ALOHA, Hawaii. Deep-Sea Research II 48, 1957–1981.

De Bernardi, B., Ziveri, P., Erba, E., Thunell, R.C., 2005. Coccolithophore export production during the 1997–1998 El Niño event in Santa Barbara Basin (California). Marine Micropaleontology 55 (1–2), 107–125.

Díaz, J., García-Herrera, R., Trigo, R.M., Linares, C., Valente, M.A., Hernadéz, E., 2006. The impact of summer 2003 heat wave in Iberia: how should we measure it? International Journal of Biometeorology 50, 159–166.

Duarte-Silva, A., Palma, S., Sobrinho-Gonçalves, L., Moita, M.T., 2004. Braarudosphaera bigelowii in waters of the upwelling coast of Portugal. Journal of Nannoplankton Research 26 (2), 35.

Estrada, M., 1984. Phytoplankton composition off Galicia (North west Spain). Journal of Plankton Research 6, 417–434.

Eynaud, F., Giraudeau, J., Pichon, J.J., Pudsey, C.J., 1999. Sea-surface distribution of coccolithophores, diatoms, silicoflagellates and dinoflagellates in south Atlantic Ocean during the late austral summer 1995. Deep-Sea Research I 46, 451–482.

Fatela, F., Taborda, R., 2002. Confidence limits of species proportions in microfossil assemblages. Marine Micropaleontology 45, 169–174.

Figueiras, F.G., Labarta, U., Fernández Reiriz, M.J., 2002. Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. Hydrobiologia 484 (1–3), 121–131.

Findlay, C.S., Flores, J.A., 2000. Subtropical Front fluctuations south of Australia (451090S, 1461170E) for the last 130 ka years based on calcareous nannoplankton. Marine Micropaleontology 40, 403–416.

Findlay, C.S., Giraudeau, J., 2000. Extant calcareous nannoplankton in the Australian Sector of the Southern Ocean (austral summers 1994 and 1995). Marine Micropaleontology 40, 417–439.

Fiúza, A.F.G., 1984. Hydrology and Dynamics of the Portuguese coastal waters. Ph.D. Thesis. University of Lisbon, Lisbon, unpublished.

Fiúza, A.F.G., Macedo, M.E., Guerreiro, M.R., 1982. Climatological space and time variation of the Portuguese coastal upwelling. Oceanologica Acta 5 (1), 31–40.

Flores, J.A., Sierro, F.J., Francês, G., Vasquez, A., Zamarreño, I., 1997. The last 100,000 years in the western Mediterranean: sea surface water and frontal dynamics as revealed by coccolithophores. Marine Micropaleontology 29, 351–366.

Flores, J.A., Marino, M., Sierro, F.J., Hodell, D.A., Charles, C.D., 2003. Calcareous plankton dissolution pattern and coccolithophore assemblages during the last 600 kyr at ODP Site

1089 (Cape Basin South Atlantic): paleoceanographic implications. Palaeogeography, Palaeoclimatology, Palaeoecology 196, 409–426.

Giraudeau, J., 1992. Distribution of recent nannofossils beneath the Benguela system: Southwest African continental margin. Marine Geology 108 (2), 219–237.

Giraudeau, J., Bayley, G.W., 1995. Spatial dynamics of coccolithophore communities during an upwelling event in the Southern Benguela system. Continental Shelf Research 15, 1825–1852.

Giraudeau, J., Rogers, J., 1994. Phytoplankton biomass and sea-surface temperature estimates from sea-bed distribution of Nanofossils and Planktonic Foraminifera in the Buenguela upwelling system. Micropaleontology 40 (3), 275–285.

Giraudeau, J., Monteiro, P.M.S., Nikodemus, K., 1993. Distribution and malformation of living coccolithophores in the northern Benguela system off Namíbia. Marine Micropaleontology 22, 93–110.

Graham, W.M., Largier, J.L., 1997. Upwelling shadows as nearshore retention sites: the example of northern Monterey Bay. Continental Shelf Research 17, 509–532.

Hagino, K., Okada, H., Matsuoka, H., 2000. Spatial dynamics of coccolithophore assemblages in the Equatorial Western-Central Pacific Ocean. Marine Micropaleontology 39, 53–72.

Haidar, A.T., Thierstein, H.R., 2001. Coccolithophore dynamics off Bermuda (N. Atlantic). Deep-Sea Research II 48, 1925–1956.

Haidar, A.T., Thierstein, H.R., Deuser, D.G., 2000. Calcareous phytoplankton standing fluxes and accumulation in Holocene sediments off Bermuda (N. Atlantic). Deep-Sea Research II 47, 1907–1938.

Haynes, R., Barton, E.D., 1990. A poleward flow along the Atlantic coast of the Iberian Peninsula. Journal of Geophysical Research 95 (C7), 11425–11441.

Henriksson, A.S., 2000. Coccolithophore response to oceanographic changes in the equatorial Atlantic during the last 200,000 years. Palaeogeography, Palaeoclimatology, Palaeoecology 156, 161–173.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W., Strickland, J.D.H., 1965. Fluorimetric determination of Chlorophyll. Journal du Conseil Permanent International pour l'Exploration de la Mer, 30 (1) : 3-15.

Honjo, S., Okada, H., 1974. Community structure o coccolithophores in the photic layer of the Mid-Pacific. Micropaleontology 29, 209–230.

Kleijne, A., 1990. Distribution and malformation of extant calcareous nannoplankton in the Indonesian seas. Marine Micropaleontology 16, 293–316.

Kleijne, A., 1993. Morphology, Taxonomy and Distribution of extant coccolithophorids (calcareous nannoplankton). Ph.D. Thesis, Vrije Universiteit, Amsterdam.

Knappertsbusch, M., 1993. Geographic distribution of living and Holocene coccolithophores in the Mediterranean Sea. Marine Micropaleontology 21, 219–247.

Knappertsbusch, M., Cortes, M.Y., Thierstein, H.R., 1997. Morphologic variability of the coccolithophorid Calcidiscus leptoporus in the plankton, surface sediments and from the Early Pleistocene. Marine Micropaleontology 30, 293–317.

Margalef, R., 1978. Phytoplankton communities in upwelling areas. The example of NW Africa. Oecologica Aquatica 3, 97–132.

McGregor, H.V., Dima, M., Fisher, H.W., Mulitza, S., 2007. Rapid 20th-centuary increased in coastal upwelling off Northwest Africa. Science 315, 637–639. McIntyre, A., Bé, A.W.H., 1967. Modern coccolithophoridae of the Atlantic Ocean—I. Placoliths and Cyrtoliths. Deep-Sea Research 14, 561–597.

Mitchell-Innes, B.A., Winter, A., 1987. Coccolithophores: a major phytoplankton component in mature upwelled waters off the Cape Peninsula, South Africa in March, 1983. Marine Biology 95, 25–30.

Moita, M.T., 2001. Estrutura, variabilidade e dinâmica do Fitoplâncton na costa de Portugal Continental. Ph.D. Thesis. University of Lisbon, Lisbon, unpublished.

Moita, M.T., Oliveira, P.B., Mendes, J.C., Palma, A.S., 2003. Distribution of chlorophyll a and Gymnodinium catenatum associated with coastal upwelling plumes off central Portugal. Acta Oecologica 24, S125–S132.

Nykjaer, L., Van Camp, L., 1994. Seasonal and interannual variability of coastal upwelling along northwest Africa and Portugal from 1981 to 1991. Journal of Geophysical Research 99, 14197–14207.

Okada, H., Honjo, S., 1973. The distribution of oceanic coccolithophorids in the Pacific. Deep-Sea Research 20, 355–374.

Okada, H., McIntyre, A., 1979. Seasonal distribution of modern coccolithophores in the Western North Atlantic Ocean. Marine Biology 54, 319–328.

Rios, A.F., Pérez, F.F., Fraga, F., 1992. Water masses in the upper and middle North Atlantic Ocean east of the azores. Deep-Sea Research 39 (3/4), 645–658.

Rogalla, U., Andruleit, H., 2005. Processional forcing of coccolithophore assemblages in the northern Arabian Sea: implications for monsoonal dynamics during the last 200,000 years. Marine Geology 217, 31–48.

Roth, P.H., 1994. Distribution of coccoliths in oceanic sediments. In: Winter, A., Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press, Cambridge, pp. 199–218.

Roth, P.H., Berger, H., 1975. Distribution and dissolution of coccoliths in the South and central Pacific. In: Sliter, W.V., Bé, A.W.H., Berger, W.H. (eds.), Dissolution of Deep-Sea Carbonates. Cushman Found. Foraminiferal Research, Special Publication 13, pp. 87–113.

Samtleben, C., Baumann, K.-H., Schröder-Ritzrau, A., 1995. Distribution, composition and seasonal variation of coccolithophore communities in the Northern Atlantic. In: Flores, J.A., Sierro, F.J. (eds.), Fifth INA Conference in Salamanca Proceedings, pp. 219–235.

Santos, A.M.P., Kazmin, A.S., Peliz, A., 2005. Decadal changes in the Canary upwelling system as revealed by satellite observations: their impact on productivity. Journal of Marine Research 63 (2), 359–379.

Sprengel, C., Baumann, K.-H., Neuer, S., 2000. Seasonal and interannual variation of coccolithophore fluxes and species composition in sediment traps north of Gran Canaria (291N 151W). Marine Micropaleontology 39, 157–178.

Tantawy, A.A.A.M., 2003. Calcareous nannofossil biostratigraphy and paleoecology of the Cretaceous–Tertiary transition in the central eastern desert of Egypt. Marine Micropaleontology 47 (3–4), 323–356.

Thierstein, H.R., Cortés, M.Y., Haidar, A., 2004. Plankton community behaviour on ecological and evolucionary time-scales; when models confront evidence. In: Thierstein, H.R., Young, J.R. (eds.), Coccolithophores, from molecular Processes to Global Impact. Springer, London, pp. 455–497.

Trigo, R.M., DaCamara, C.C., 2000. Circulation weather types and their impact on the precipitation regime in Portugal. International Journal of Climatology 20, 1559–1581.

Trigo, R.M., Pozo-Vazquez, D., Osborn, T.J., Castro-Diez, Y., Gámis-Fortis, S., Esteban-Parra, M.J., 2004. North Atlantic Oscillation influence on precipitation, river flow and water resources in the Iberian Peninsula. International Journal of Climatology 24, 925–944.

Winter, A., 1985. Distribution of living coccolithophores in the California current system, Southern California borderland. Marine Micropaleontology 9, 385–393.

Winter, A., Reiss, Z., Luz, B., 1979. Distribution of living coccolithophore assemblages in the Gulf of Elat (Aqaba). Marine Micropaleontology 4, 197–223.

Winter, A., Jordan, R.W., Roth, P.H., 1994. Biogeography of living coccolithophores in ocean waters. In: Winter, A., Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press, Cambridge, pp. 161–177.

Wooster,W.S., Bakun, A.,McLain, D.R., 1976. The seasonal upwelling cycle along the eastern boundary of the North Atlantic. Journal of Marine Research 34, 131–141.

Young, J., 1994. Function of coccoliths. In: Winter, A., Siesser, W.G. (eds.), Coccolithophores. Cambridge University Press, Cambridge, pp. 63–82.

Young, J., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I., Ostergaard, J., 2003. A guide to extant coccolithophore taxonomy. Journal Nannoplankton Research (special issue 1), 123.

Ziveri, P., Thunell, R.C., 2000. Coccolithophore export production in Guaymas Basin,Gulf of California: response to climate forcing. Deep-Sea Research II 47,2073–2100.

Ziveri, P., Thunell, R., Rio, D., 1995. Export production of coccolithophores in an upwelling region: results from San Pedro Basin, Southern California Borderlands. Marine Micropaleontology 24, 335–358.

Ziveri, P., De Bernardi, B., Baumann, K-H., Stoll, H.M., Mortyn, P.G., 2007. Sinking of coccolith carbonate and potential contribution to organic carbon ballasting in the deep ocean. Deep-Sea Research II 54, 659–675.

CHAPTER 3: *Calcidiscus quadriperforatus* AND *Calcidiscus leptoporus* AS OCEANOGRAPHIC TRACERS IN LISBON BAY (PORTUGAL).

CHAPTER 3: *Calcidiscus quadriperforatus* AND *Calcidiscus leptoporus* AS OCEANOGRAPHIC TRACERS IN LISBON BAY (PORTUGAL).

Silva, A.*, Palma, S., Oliveira, P. B., Moita, M. T.

Instituto Nacional de Recursos Biológicos (INRB-IPIMAR), Av. Brasília s/n, 1449-006 Lisboa, Portugal. Tel: +351213027121, Fax: +351213015948

* Corresponding author: amsilva@ipimar.pt

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ABSTRACT

A study of *Calcidiscus* genus in Lisbon bay has revealed the presence of *C. quadriperforatus* and *C. leptoporus*. Samples were collected continuously on a weekly basis for four years (July 2001 – May 2005) and on a cruise carried out in summer 2005, covering Lisbon bay. *C. quadriperforatus* and *C. leptoporus* developed in the same range of temperature and salinity, 11.5 – 21.5ºC and 26.9 – 36.3, respectively. The two species seemed to respond independently but had a co-occurring seasonal pattern nearshore, developing in different concentrations throughout the seasons. Maxima of both species occurred in low turbulent and nutrient enriched waters, which was also favourable for the development of larger coccospheres of *C. quadriperforatus*. The concentrations of both species decreased from 2001 until 2005, due to the intensification and persistence of upwelling and rainy periods.

From late autumn until winter, mixed populations were observed nearshore, largely dominated by *C. quadriperforatus*, in colder and nutrient enriched waters, while during spring, when temperatures begin to increase, only *C. leptoporus* was observed, in mature upwelled waters. *C. quadriperforatus* was considered more opportunistic than *C. leptoporus*. The availability of nutrients seemed to have influenced the size increase of *C. quadriperforatus* coccospheres. Both species developed offshore during summer, when the shelf is occupied by colder turbulent upwelled waters. A short downwelling episode in summer 2005, associated with two counter rotating mesoscale eddies, responsible for a strong north-eastward flow of warm oceanic waters into the Bay, allowed the development of *C. quadriperforatus* nearshore. The contemporary satellite images revealed the presence and onshore displacement of these waters and *C. quadriperforatus* and *C. leptoporus* distribution highlighted these conditions. The development of each species nearshore also gave indications of local oceanographic changes during winter and spring.

Key words: Iberia upwelling system; time-series; seasonal succession; cross shelf transport; coccolithophores.

1. INTRODUCTION

Coccolithophores are sensitive indicators of surface water conditions being important markers of oceanographic changes and proxies of sea surface water-masses and temperatures, productivity and past climate changes (Kleijne, 1990; Winter et al., 1994; Beaufort and Heussner, 2001; Andruleit et al., 2003; De Bernardi et al., 2005). The group is distinctive from other phytoplankton in that at some point in their life-cycle they precipitate $CaCO_3$ in the form of calcite platelets or coccoliths, which surround the cell to form the exoskeleton which is called the coccosphere. The taxonomy of coccolithophores is primarily based on the morphology of the coccoliths and morphometric studies of species have revealed a high morphological variability that can be associated with environmental parameters, genetic variability or ecophenotype (Bollmann, 1997; Knappersbusch et al., 1997; Renaud and Klaas, 2001; Sáez, et al., 2003).

Calcidiscus leptoporus is a cosmopolitan species considered by several authors as characteristic of tropical to subtropical oligotrophic warm-water masses (McIntyre and Bé, 1967, Okada and Honjo, 1973; Winter et al., 1994), however it is capable of developing under cold and low turbulent conditions (Giraudeau, 1992; Ziveri et al., 1995; Kinkel et al., 2000, Barcena et al., 2004). This genus never reaches high abundances in surface waters in comparison to other coccolithophores and shows considerable variability in morphology. In sediments, it can dominate the coccolith assemblage (McIntyre and McIntyre, 1970) due to the advantage of being a robust, solution-resistant species (Ziveri et al., 2007). *C. leptoporus* has a monomorphic, spherical coccosphere of distinctive circular placoliths, which can vary considerably in size. The distal shield exhibit curved, smooth, overlapping elements, interspaced with sutures which curve to the left. The sutures can be traced into the clear central area, forming a central crater-shaped depression, which is closed. Size differences between the proximal and distal shields allow the placoliths to interlock tightly, forming robust coccospheres with good preservational potential in sediments. This species was previously regarded as the intermediate form of *C. leptoporus* (Knappertsbush et al., 1997) and is usually distinguished by a coccospheres with a diameter of 10-16 μm and placoliths with a diameter of 5.0-8.0 μm. *Crystallolithus rigidus* is currently recognized as the haploid/diploid motile form of *C. leptoporus* (Kleijne, 1991; Cortés, 2000, Renaud and Klass, 2001, Geisen et al., 2002).

Until recently, *Calcidiscus quadriperforatus* was regarded as the large morphotype (Knappertsbusch et al., 1997) or sub-species (Geisen et al., 2002) of *C. leptoporus*. As a result of life-cycle observations and molecular genetic analysis the taxonomy has know been revised and what was formally known as the large morphotype of *C. leptoporus* has been raised to species level (Geisen et al., 2002; Sáez et al., 2003). *C. quadriperforatus* differs from *C. leptoporus* in three main morphological features: it has coccoliths larger in size, the distal shield suture lines are more numerous and curved in appearance, and the coccoliths display an obscured central-area, which is generally infilled. Coccospheres are large in size with a diameter of > 16 μm and placoliths diameter > 8 μm. *Syracolithus quadriperforatus* is now regarded as the alternate holococcolith phase of *C. quadriperforatus* (Geisen et al., 2002).

It is also important to notice the existence of a small morphotype of *C. leptoporus* (Knappertsbush et al., 1997, Renaud et al, 2002), which was not observed in the present study, which is characterized by coccoliths with angular and serrated suture lines that can be traced into the deep conical central pore, of small size (coccolith diameter < ~5 μm). The taxonomy of the small morphotype was not changed by Geisen et al. (2002) since no cultures were available for life cycle observations and genetic analysis.

Regional studies on living coccolithophores are needed to calibrate species-specific ecological tolerances in order to use them effectively as biotic proxies and to assess the potential for paleoceanographic reconstructions in each studied area (Andruleit, 2007). For the Portuguese coast, which is an area located on the boundary between the temperate and subtropical coccolithophore biogeographic zones (McIntyre and Bé, 1967), few studies have been conducted based on water column samples. Cachão and Moita (2000) and Cachão et al. (2000), described *Calcidiscus leptoporus*, without a separation by morphotypes, as a common winter - spring coccolithophore on coastal waters. The species is usually absent nearshore during summer, when the shelf is occupied by colder turbulent upwelled waters although its presence is reported offshore by other authors (Cachão and Moita, 2000). Silva et al. (2008) considered *Calcidiscus leptoporus* as a tracer for the convergence of surface offshore subtropical waters through winter and spring and a proxy of non-productive periods off the coast of Portugal, based on weekly sampling over four years (July 2001-May 2005) in Lisbon

bay. The authors drawn attention to the existence of two morphotypes with a different seasonal pattern, only based on light microscope measurements.

The present study, based on the same time-series as Silva et al (2008), comprehensively describes the ecological preferences of the two morphotypes but now as distinct species, *C. quadriperforatus* and *C. leptoporus*, additionally supported with scanning electronic microscope (SEM) analyses. It will also address the presence of *C. quadriperforatus* nearshore during summer for the first time in four years. These winter-spring species were observed at a fixed station and also during a summer cruise covering Lisbon Bay in August 2005 which had a high spatial resolution. The study hypothesises that *C. quadriperforatus* and *C. leptoporus* can be related to physical-chemical, biological and morphological parameters (temperature, salinity, upwelling, nutrients, chlorophyll *a* and size/number of coccospheres and coccoliths) in order to trace local oceanographic changes in Lisbon bay.

2. HIDROLOGY

At west coast of Portugal upwelling generally occurs seasonally, from April to September, under northerly wind conditions. Conversely, the advection of warmer oligotrophic oceanic waters into the shelf is observed during autumn and winter, when southerly winds begin to dominate and there is an intensification of waters flowing poleward (Fiúza et al., 1982; Haynes and Barton, 1990; Peliz et al., 2005). Episodes of reverse winds can occur during both seasons. According to Rios et al. (1992), south of the Nazaré canyon, the main upwelling source is the Eastern North Atlantic Central Water sub tropical branch (ENACWst) and this influencing Lisbon bay. Discharges from the river Tagus also influence this bay, being an import source of nutrient supply, especially during winter. North of the bay, an upwelling filament rooted at cape Roca recurrently occurs during the northerly wind periods (upwelling favourable), typically extending in the south and westward direction. The structure of upwelling is however, complex at Lisbon bay. This bay represents an important coastline discontinuity being considered an upwelling shadow area where phytoplankton species can be accumulated through different retention mechanisms (Graham and Largier, 1997, Moita et al., 2003). At this coast, Moita (personal communication) identified the upwelling as the major source of seasonal and spatial variability of phytoplankton.

Cascais (Fig. 1) is located at the northern side of Lisbon bay, south of cape Roca. The phytoplankton from the sampling site is thus influenced either by upwelled waters of the Roca filament or by warmer and mature surrounding waters, depending on the intensity and persistence of the winds favourable for upwelling and the offshelf mesoscale structures which control the offshore extension and position of the upwelling filament. Weak upwelling conditions allow a larger influence of warmer and more stratified waters into the bay.

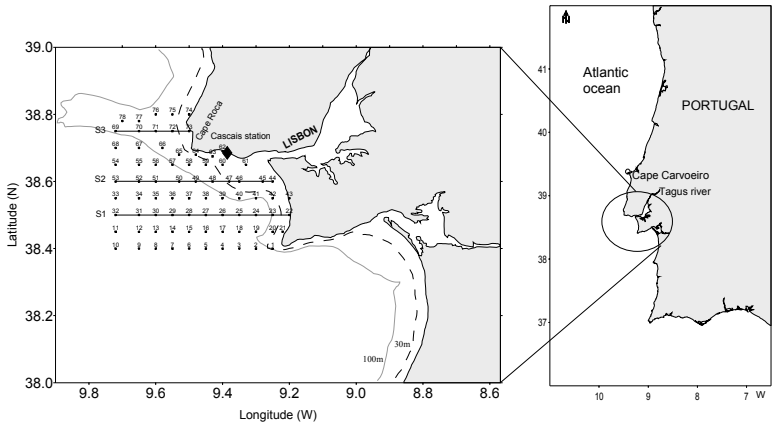

Figure 1 - Location of the Cascais sampling site 38º 41' N and 09º 24' W (♦) and of the summer cruise sampling stations covering Lisbon bay. Three sections (S) were sampled in depth (S1, S2, and S3).

3. METHODS

3.1 Sampling

Coastal sampling

Seawater samples were continuously collected on a weekly basis, from surface and bottom (5 m depth) from July 2001 to May 2005, at a fixed station located in Lisbon bay (Cascais : 38º 41' N, 09º 24' W) (Fig. 1). Sampling was carried out one hour before high tide to minimize the direct influence of Tagus estuarine waters on the area. Water samples were used for chlorophyll *a* (Chl *a*), nutrients and coccolithophore analysis. Temperature, salinity and depth were determined in situ with a Quanta CTD. Salinity

was measured using the Practical Salinity Scale. Since the surface and bottom values of environmental data did not differ significantly, both depths were averaged.

Cruise sampling

From 30 August to 1 September 2005, a survey was carried out in Lisbon bay, on board of the R.V. Noruega, with the aim of studying the dynamics of the HAB (Harmful Algal Bloom) species *Gymodinium catenatum* (Fig. 1). On 78 stations, physical data and phytoplankton samples (surface and deep chlorophyll maximum, DCM) were collected using a combined SBE911 CTD profiler and rosette sampler. In addition, three sections (S1, S2 and S3), covering the area, were sampled in depth (Fig. 1). Samples were also collected for Chl *a* and nutrient determination. Sampling depths were 0, 5, 10, 20, 30, 50, 75 m and at the DCM.

3.2 Coccolithophore analysis

Coastal samples

For coccolithophore analysis, water samples (750 or 1000 ml) were filtered through a 47 mm nitrate cellulose membranes (Whatman) with a 0.45 µm nominal pore size. A strip of the membrane was cut from the centre to the rim and slides were rendered transparent with a drop of Entellan mounting medium. Coccospheres were measured, identified and counted until at a maximum of 300 cells (Fatela and Taborda, 2002) on an area of 2.2 mm^2 of the filter with a Zeiss optical microscope under cross-polarized light at a magnification of 1250 x. Depending on the overall abundance of coccospheres in the samples, counts ranged between 1 cell, corresponding to 73 cells.l^{-1} and 300 cells equivalent to $22x10^3$ cells.l^{-1}. A scanning electron microscope (JEOL-5200) was used to verify the identifications of the coccolithophore assemblage and to observe the presence of holococcolithophores.

Cruise samples

Phytoplankton samples were preserved with hexamethylenetetramine buffered formalin to a final concentration of 2% (Throndsen, 1978). Cells were identified and enumerated in subsamples of 50 ml by the Utermöhl technique (Hasle, 1978), using a

Zeiss IM35 inverted microscope with phase contrast and bright field illumination. A magnification of 160x and 400x was used to identify and enumerate the phytoplankton assemblage with a detection limit of 40 cells.l^{-1} and 2000 cells.l^{-1}, respectively. The identification of holococcolithophores and the measurements of coccospheres and coccoliths from genus *Calcidiscus* were carried out with a scanning electron microscope (JEOL-5200). The diameter was measured in a total of 526 coccospheres and on visible coccoliths from each coccosphere. In addition the number of coccoliths per coccosphere was counted.

Classification of species from all samples followed Knappersbusch et al. (1997), Sáez et al. (2003) and Young et al. (2003).

3.3 Ancillary data

To evaluate the Chl *a* concentration, 250 ml seawater samples were filtered and pigments extracted with 90% acetone and determined on the Perkin-Elmer spectrofluorometer (Holm-Hansen et al., 1965).

The water for nutrient determination was filtered through a Millipore filter of 0.45 μm and stored at – 4ºC for subsequent analysis. Nitrites and nitrates (NO_2^- + NO_3^-) and phosphates (HPO_4^{2-}) were determined using an autoanalyser "SKALAR" according to the methods of Technicon Industrial Systems (Grasshoff, 1983). The detection limit is 0.05 μM for nitrites+nitrates and phosphates.

Daily wind data were obtained from the meteorological weather station of Cape Carvoeiro, located 50 km north of Cascais (Fig. 1). Based on the northward wind stress component a daily upwelling index was calculated (Bakun, 1973) and a running average with a window width of 7 days was determined to allow a straight line relationship between data from different time scales (weekly sampling and daily upwelling index).

To gather insight on the spatio-temporal variability of sea surface temperature (SST) and surface currents during the cruise, at the regional scale, two satellite-derived products were used: the North Atlantic Regional SST provided by the EUMETSAT Ocean and Sea Ice Satellite Application Facility (CMS, 2005), and the delayed time

Ssalto/Duacs "Up-to-date" global gridded product of sea level anomalies and geostrophic velocities (CLS, 2008).

In order to identify the external factors that may influence *Calcidiscus* species dynamics, linear regressions, as a first approximation to detect any co-variation among parameters, and principal component analysis have been performed using the software NTSYSpc version 2.02i from 1997 by Applied Biostatistics, Inc.

4. RESULTS

4.1 Interannual variability of *C. quadriperforatus* and *C. leptoporus* (July 2001 – May 2005)

During spring, upwelling was always intermittent and the onshore advection conditions decreased in frequency and strength from late summer to winter seasons. Short-periods of onshore advection were typically observed between mid-September and early December. The longest convergence period was observed during early winter 2002 (Fig. 2A). Temperature ranged between 11.5ºC (January 2003) and 20.4ºC (October 2002) (Fig. 2B). The lowest SST were observed during winter, while the highest during summer and early autumn. Summers were gradually warmer, with longer periods above 17ºC. Autumns recorded temperatures between 15 and 20 ºC with values decreasing towards winter temperatures, which ranged from 12 ºC to 15ºC. From spring to autumn seasons, SST increased ~ 1 to 1.5 ºC and winter periods became colder with a ~ 1ºC decrease associated with precipitation (data on www.inag.pt) and intensification of the SW winds conditions.

Salinity also presented patterns of seasonal variability ranging from 26.9 in January 2003 to 36.5 in October 2001, and was usually higher than 34.5 (Fig. 2C). Higher and relatively constant values were observed from spring to early autumn and lower values, from late autumn to winter.

Chlorophyll *a* presented minimum and maximum values of 0.1 $\mu g.l^{-1}$ in November 2003 and 5.24 $\mu g.l^{-1}$ in February 2005 (Fig. 2D). Spring and summer were the two most productive seasons with a raise in biomass of ~ 1 $\mu g.l^{-1}$ throughout the years.

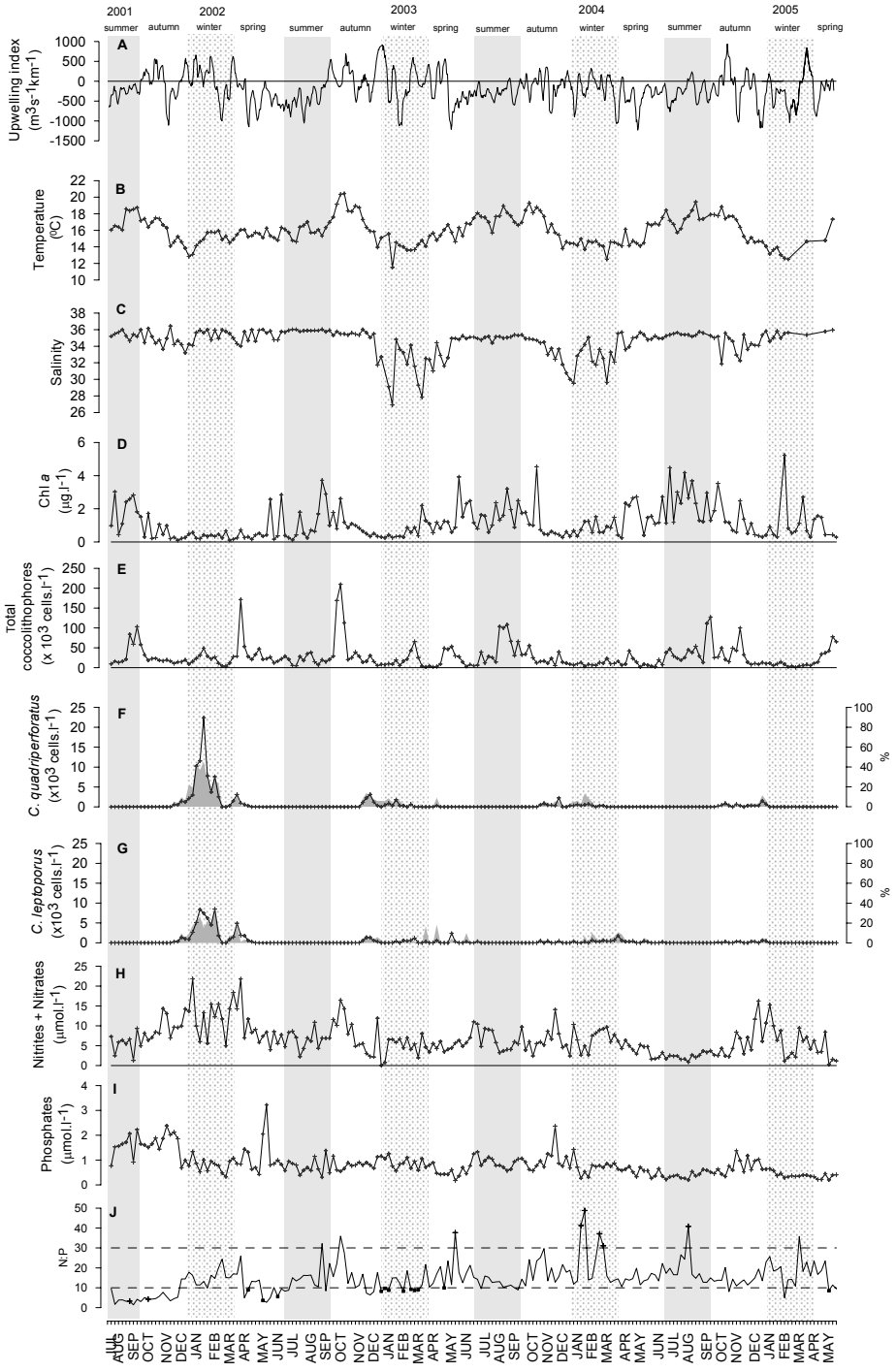

Figure 2 – Weekly distribution of A) upwelling index (negative values indicate upwelling), B) sea surface temperature, C) salinity , D) chlorophyll a, E) total coccolithophores, F) *Calcidiscus quadriperforatus*, G) *Calcidiscus leptoporus*, H) Nitrites + nitrates, I) Phosphates, during the sampling period and J) N:P ratio (July 2001 - May 2005). The shaded areas highlight the different seasons of the year, dark grey for summer and light dotted grey for winter. The dots (•) and crosses (+) indicate nitrates and phosphates limitations respectively, according to Dortch and Whitledge (1992).

During the four years, the upwelling was progressively more persistent although less intense, usually higher than -1000 $m^3 s^{-1} km^{-1}$ (Fig. 2A). From 2001 onwards, the number of days with upwelling increased (negative values in Fig. 2A) and in 2004, there were more than 55 days with upwelling conditions than in 2002. Conversely, the onshore advection periods were shorter over the years (Upwelling index >0 $m^3 s^{-1} km^{-1}$). The interannual salinity fluctuation highlighted different weather conditions since it is strongly influenced by the rainfall regime and thus by the Tagus river flow. Higher salinities, as in 2002 were associated with low precipitations and river runoff while the minor values observed subsequently decreased in agreement with the rainfall.

The longer periods of weak upwelling and the progressive increase in inter-annual SST resulted in the development of phytoplankton populations as measured by Chl *a*. The increase of phytoplankton biomass was not followed by coccolithophores populations that decreased over the 4 years (Fig. 2E). Twenty two species were identified and the optimum conditions lay between 14º and 20ºC of temperature and 34 to 36.5 of salinity, under onshore advection or weak upwelling conditions. The highest peak (210 x 10^3 cells.l^{-1}) was registered in autumn 2002, the warmest period of the study with persistent southerly winds promoting onshore advection.

The study of *Calcidiscus* genus on the time series, revealed the presence of two species: *C. quadriperforatus* (coccosphere diameter > 16 µm, coccolith diameter $\geq$ 8 µm and 12 - 26 visible coccoliths) (Fig. 3A-C) and *C. leptoporus* (coccosphere diameter 10 - 16 µm, coccolith diameter 5 - 8 µm and 12 - 15 visible coccoliths) (Fig. 3D-G). The small morphotype of *C. leptoporus* described in Knappertsbusch et al. (1997) was not observed. The seasonal pattern was similar over the four years. A decreased in the species concentrations was observed from winter 2001-2002 onwards. *C. quadriperforatus* and *C. leptoporus* occurred, in different proportions, from late autumn until spring, associated with onshore advection episodes or when the upwelling winds relaxed (Fig. 2F, G and Fig. 4). The species were found in temperatures of 11.5 - 18.9ºC and salinities of 26.9 – 36.0. *C. quadriperforatus* dominated over *C. leptoporus* during winter, while the last prevailed in the samples until early spring, related to higher SST. A maximum of *C. quadriperforatus* was observed in January 2002, 22 x 10^3 cells.l^{-1} corresponding to 46% of the coccolithophore assemblage (CA). *C. leptoporus* reached 8.5 x 10^3 cells.l^{-1} in February 2002 (31% of CA). During summer, *C. quadriperforatus* and *C. leptoporus* were never observed at this coastal station (Table 1).

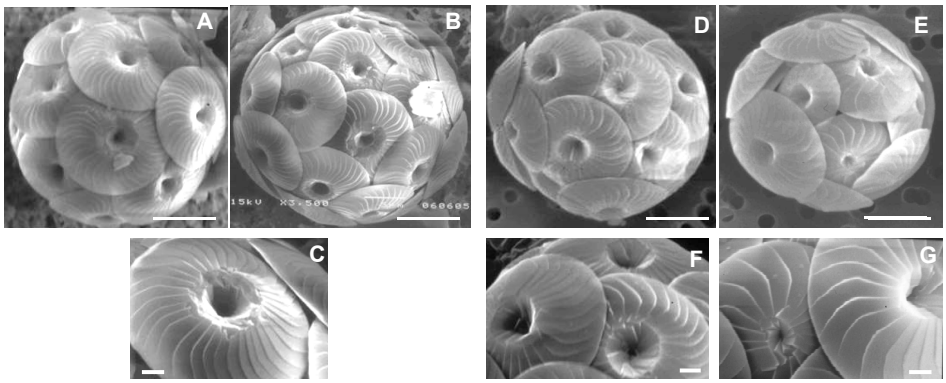

Figure 3 - *Calcidiscus quadriperforatus*: A) coccosphere, B) larger coccosphere and C) coccolith; *Calcidiscus leptoporus*: D - E) coccosphere and F - G) coccolith. Scale bar from A, B, D and E = 5 μm. Scale bar from C, F and G = 1 μm. Pictures were obtained by SEM.

Nutrient concentrations changed along the year (Fig. 2H and J), influenced by precipitation and river flow. The higher values were determined during autumn – winter and the lowest during spring and summer. Nitrites + nitrates (Fig. 2H) were most abundant during winter as in 2002. Minima and maxima were, respectively, 0.06 and 21.9 μmol l^{-1} (December 02 and December 01). Phosphates (Fig. 2I) varied between 0.2 and 3.2 μmol l^{-1} (May 03 - May 02), and were most available in 2002 and 2003. from the 197 observed samples, nitrates and phosphates were found in limited concentrations 20 times according to nutrient stoichiometry as defined by Dortch and Whitlege (1992) (Fig. 2J). These authors propose a combination between nutrient concentrations and ratios to access limitation. Limitation by nitrites + nitrates (DIN $\leq$ 1, N/P < 10 and Si:N > 1) was observed 12 times (6%), during winter and spring in 2002 and 2003. Nitrites + nitrates were always abundant in 2001 and 2004. Limited concentrations of phosphates ($PO_4^{3-} \leq 2$, N/P > 30 and Si/P > 3) occurred 8 times (4%), more than half during winter 2004. This nutrient was always available in 2001 and 2005 and limited one or two times in 2002-2003.

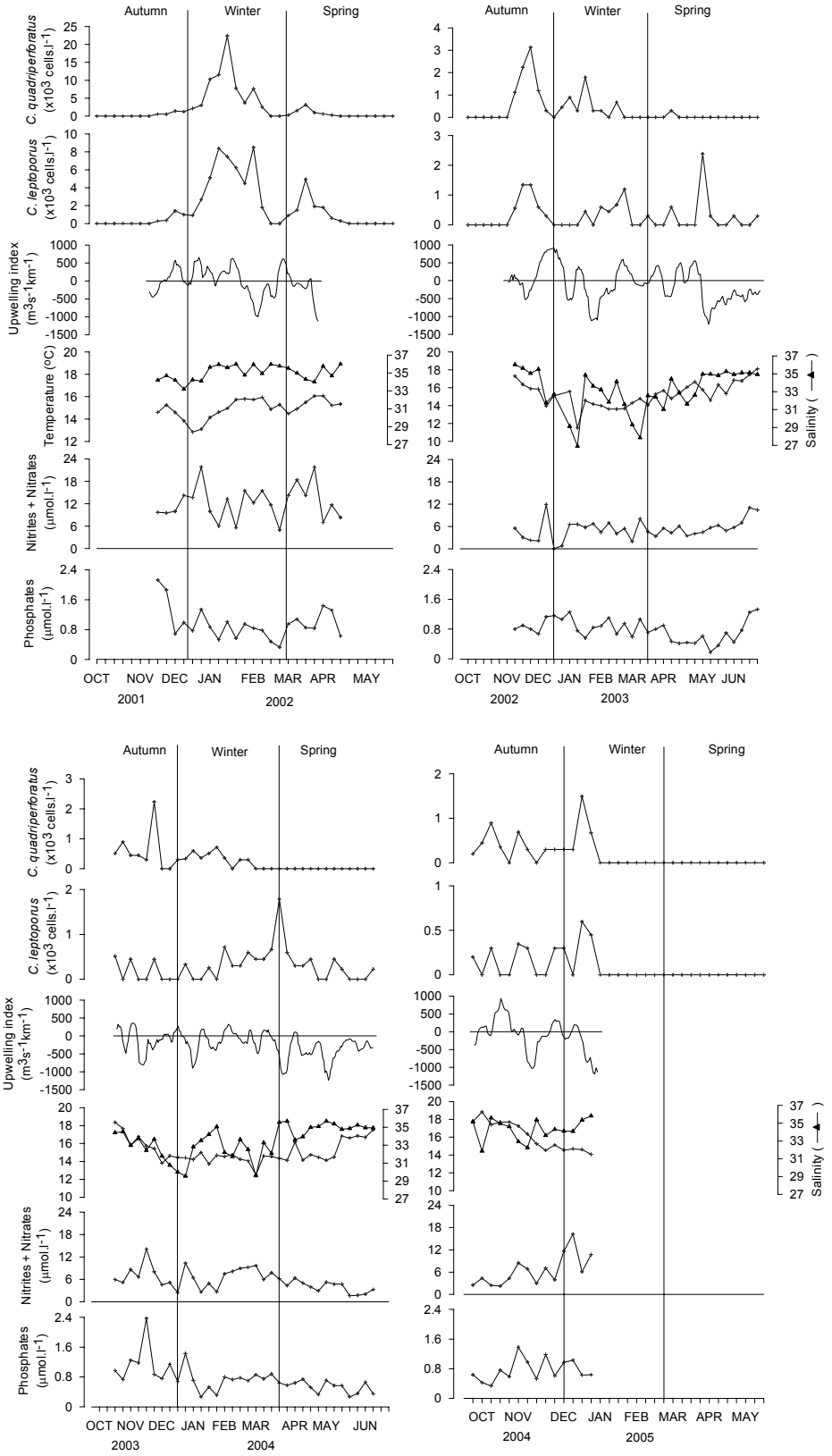

Figure 4 – Detailed weekly distribution of *Calcidiscus quadriperforatus* and *Calcidiscus leptoporus*, from October to June (2001-2005), related to upwelling index, temperature, salinity, nitrites + nitrates and phosphates.

Calcidiscus quadriperforatus

	x10^3 cells.l^{-1}	T (ºC)	S	RF (%)	Month
2001					
Summer	-	-	-	-	-
Autumn	2,1	12,9	34,3	22,7	December
Winter	-	-	-	-	-
2002					
Spring	1	16,1	34,0	0,6	March
Summer	-	-	-	-	-
Autumn	3	15,9	35,1	10,3	November
Winter	22	15,0	35,6	45,8	January
2003					
Spring	0,3	14,8	34,4	18,2	April
Summer	-	-	-	-	-
Autumn	2,2	15,4	33,7	11,2	November
Winter	1,8	14,6	34,9	18,2	January
2004					
Spring	-	-	-	-	-
Summer	-	-	-	-	-
Autumn	1,5	14,6	35,4	23,3	December
Winter	0,7	14,7	35,1	17,1	January
2005	absent until May				

Calcidiscus leptoporus

	x10^3 cells.l^{-1}	T (ºC)	S	RF (%)	Month
2001					
Summer	-	-	-	-	-
Autumn	1,4	14,6	34,3	9,6	December
Winter	-	-	-	-	-
2002					
Spring	1,9	16,1	34,0	1,1	March
Summer	-	-	-	-	-
Autumn	1,3	15,9	35,1	4,4	November
Winter	8,5	15,8	36,0	31,4	February
2003					
Spring	2,4	15,8	35,0	4,5	May
Summer	-	-	-	-	-
Autumn	0,5	18,4	34,4	3,2	October
Winter	1,2	13,7	31,6	1,8	February
2004					
Spring	0,6	14,1	35,7	8,6	March
Summer	-	-	-	-	-
Autumn	0,6	14,6	35,4	4,7	December
Winter	1,8	14,4	35,6	10,9	March
2005	absent until May				

Table 1 – Correspondence between *Calcidiscus quadriperforatus* and *Calcidiscus leptoporus* maximum concentration (x 103 cells.l-1) and temperature, salinity, relative abundance (RF%) and time of the year (Month) (July 2001 - May 2005). Dashes mean species absence.

4.2 Spatial distribution of *C. quadriperforatus* and *C. leptoporus*

C. quadriperforatus and *C. leptoporus* were observed in the samples from the cruise carried out in Lisbon Bay during summer 2005. The sampling took place after 15 days of persistent upwelling favourable winds that abated during the survey. The off-shelf surface circulation was dominated by the presence of two counter rotating mesoscale eddies, responsible for a strong north-eastward flow of warm oceanic waters into the Bay (highlighted by the white arrows in Fig. 5). This flow favoured the formation of a strong thermal front between the cold upwelled waters nearshore and the warm oceanic water and also contributed to the westward extension of the upwelling filament rooted at cape Roca.

Temperatures varied between 14.9ºC and 21ºC with the colder waters over the shelf, around cape Roca and below 40 m depth (Fig. 6). Salinities ranged from 35.9 to 36.4 with the saltier waters offshore. Tagus river influence was reflected in the lower salinity values (35.8 - 35-9) recorded just outside river mouth (Fig. 6).

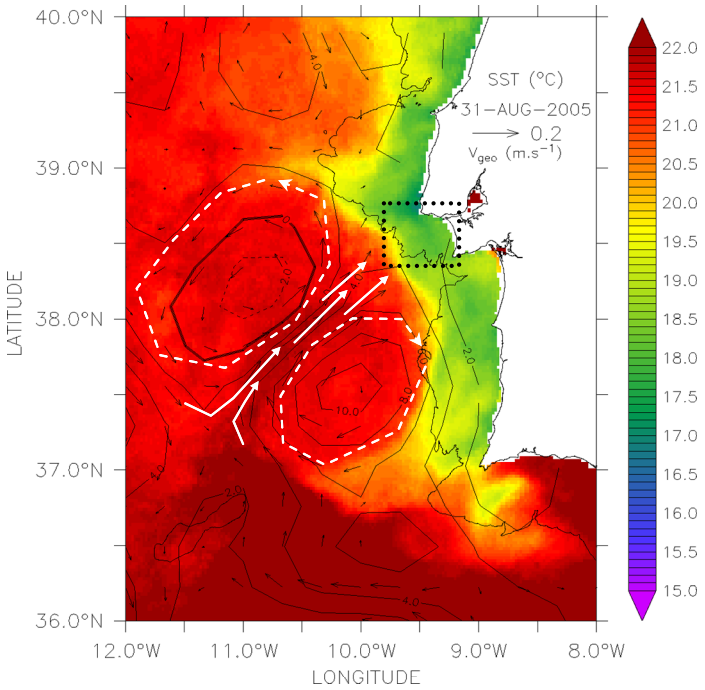

Figure 5– Satellite-derived sea surface temperature (shade), sea level anomaly (contour) and derived geostrophic velocities in 31 August 2005. The cruise sampling area is highlighted by the dashed rectangle. The presence of two counter rotating mesoscale eddies, responsible for a strong north-eastward flow of warm oceanic waters into the Bay are highlighted by the white arrows.

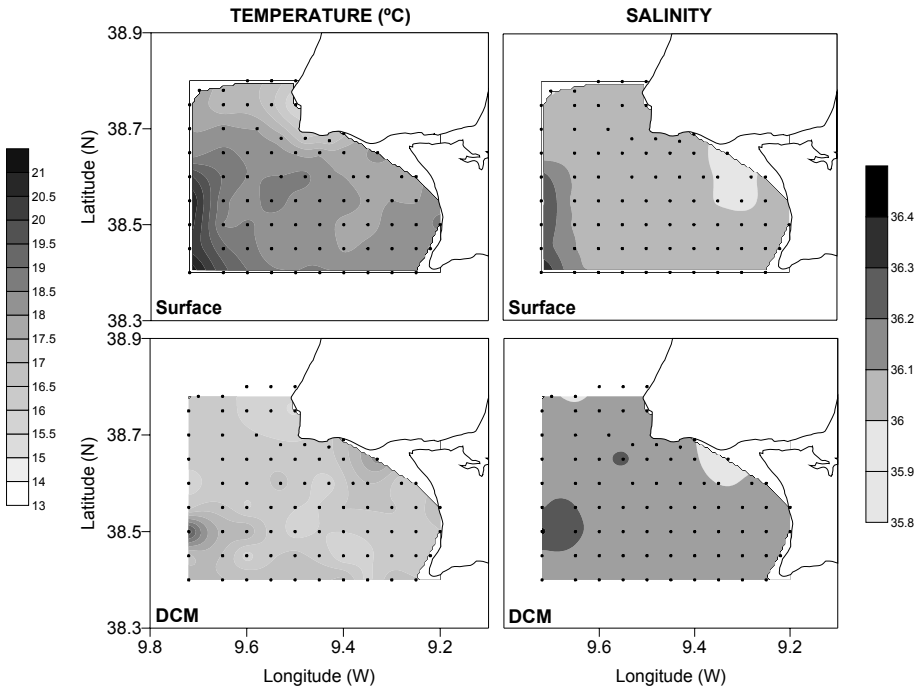

Figure 6 – Spatial distribution of temperature and salinity, surface and maxima, during the summer cruise 2005.

Chlorophyll a concentrations increased towards the coast and varied from 0.04 to 7.59 μg.l^{-1}, with the highest values nearshore and at 20-30 m depth (Fig. 7). The strong flow into the bay and the consequent displacement of the upwelling filament westwards, instead of the usual southward jet, resulted in three clear, spatially separated, phytoplankton patches. Maximum abundances were observed nearshore in surface waters and slightly decreased towards the deep chlorophyll maximum (Fig. 7).

Coccolithophores dominated the phytoplankton assemblage and broadly distributed within the intrusion of warm waters (570 x 10^4 cells. l^{-1}) in the center of the sampling area (Fig. 7). The dominant species were *Emiliania huxleyi* and *Gephyrocapsa* spp. with surface maxima close to the upwelling center and widely dispersed between surface and 25 m depth.

In lower abundances and only over the shelf, diatoms (160 x 10^4 cells. l^{-1}) and dinoflagellates (27 x 10^4 cells. l^{-1}) developed on both sides of this strong flow of warmer waters (Fig. 7).

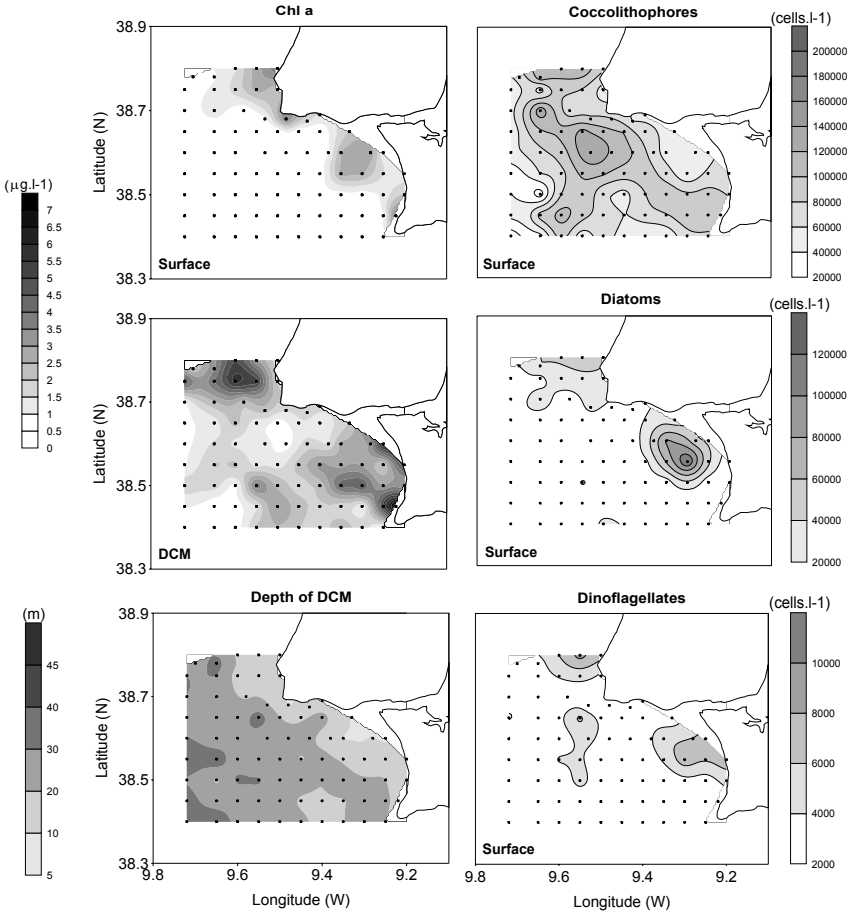

Figure 7 – Spatial distribution of surface chlorophyll a and DCM, depth of DCM and surface maxima of coccolithophores, diatoms and dinoflagellates, during the summer cruise 2005.

C. quadriperforatus and *C. leptoporus* occurred in distinct areas surrounding the upwelling filament, associated with different physical conditions (Fig. 8), the former being more abundant. For *C. quadriperforatus*, an increase in the size of the coccospheres was observed towards the coast. The larger forms had 22 - 25 μm and ~ 22 coccoliths and occurred in lower numbers nearshore in surface waters as well as broaden distributed as a concave layer at the picnocline and nutricline depths (15 - 25 m), associated with lower temperatures (14.3 – 21.5ºC) and salinities of 35.8 – 36 (Fig. 6, 8 and 9). A maximum of 2.8 x 10^3 cells.l^{-1} was observed at 25 meters depth in the bay (15.9ºC and 36.0 of salinity), representing 8% of the CA. Smaller coccospheres, with 17.5 - 22 μm and an average of 12-15 coccoliths per cell, were exclusively observed offshore in surface waters, reaching 45% of the CA with 3.5 x 10^3 cells.l^{-1} (21.5ºC and 36.4 of salinity) and were spatially separated from *C. leptoporus*. Regardless the size, all coccospheres of *C. quadriperforatus* presented coccoliths with 10 - 12 μm of diameter.

C. leptoporus was only present in surface offshore waters, in a restricted area, in salinities above 36 and temperatures higher than 19.5ºC. The maximum abundance was 1.8 x 10^3 cells.l^{-1} representing 5% of the CA (20.7ºC and 36.3 of salinity) (Fig. 6, 8 and 9). Also exclusively distributed in these offshore warmer waters was an assemblage of 25 coccolithophore species characterized by *Acanthoica quatrospinna, Anoplosolenia brasiliensis, Antosphaera fragaria, Calyptrosphaera oblonga, Calyptrolithophora papilifera, Corisphaera* sp., *Coronosphaera mediterranea, Calciosolenia murray, Calyptrolithophora gracillima Crystallolithus hyalinus, Crystallolithus rigidus, Discosphaera tubifera, Florisphaera profunda, Helicosphaera carteri, Ophyaster formosus, Ophyaster hydroideus, Rhabdosphaera clavigera, Syracolithus confuses, Syracosphaera lamina, Syracosphaera molischii, Syracosphaera pulchra, Syracosphaera* spp., *Umbelosphaera tenuis, Umbilicosphaera sibogae* and *Zygosphaera marsilli*. All species had surface maxima and distributed until 20-25 m depth with the exception of *Florisphaera profunda*, only observed at 50 m depth.

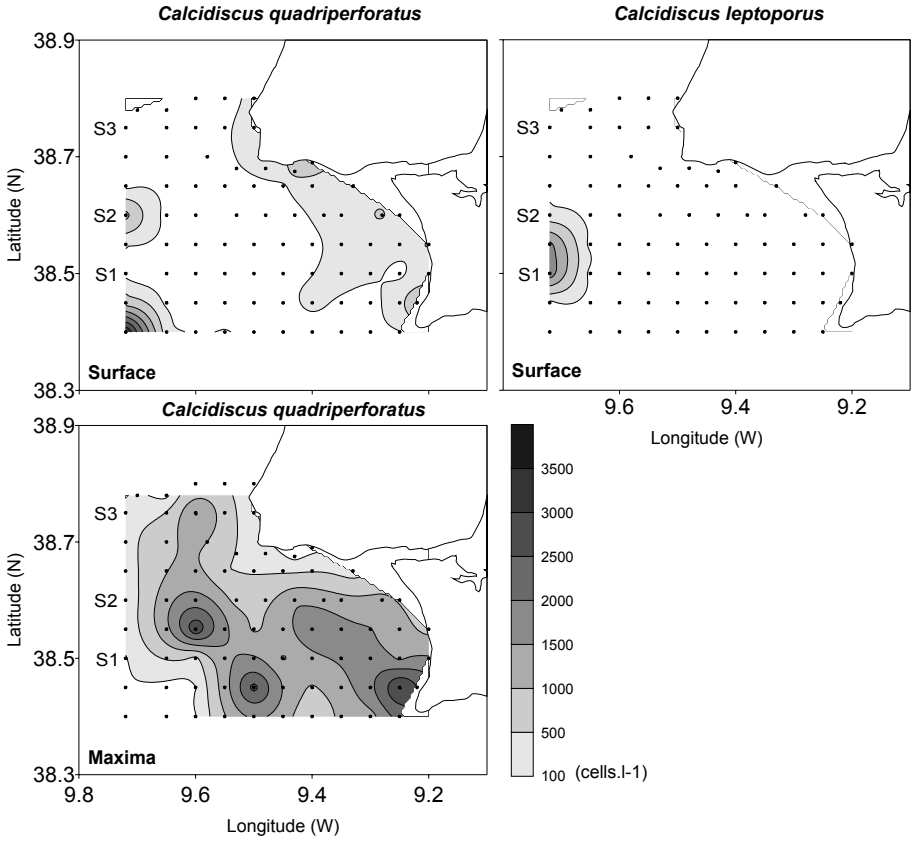

Figure 8 - Spatial distribution of *Calcidiscus leptoporus* and *Calcidiscus quadriperforatus*, surface and maxima, during the summer cruise 2005.

Nutrient concentrations increased towards the coast, especially around the upwelling center, as nitrites+nitrates distribution in section 3 (Fig. 9 - S3), as well as in depth, with maxima below 40 meters. Phytoplankton and *Calcidiscus* in particular, developed at the top of the nutricline leading to a clear nutrient depletion (Fig. 9). Phosphates varied between 0.18 and 0.72 μmol.l^{-1} and nitrites+nitrates ranged from 0.13 to 2.37 μmol.l^{-1} (Fig. 9). In this survey and according to Dortch and Whitledge's (1992) criteria, phosphates were always available while nitrites+nitrates were found in limited concentrations in several stations and usually above 20 m depth. These conditions were never coincident with maximum concentrations of *Calcidiscus* (Fig. 9).

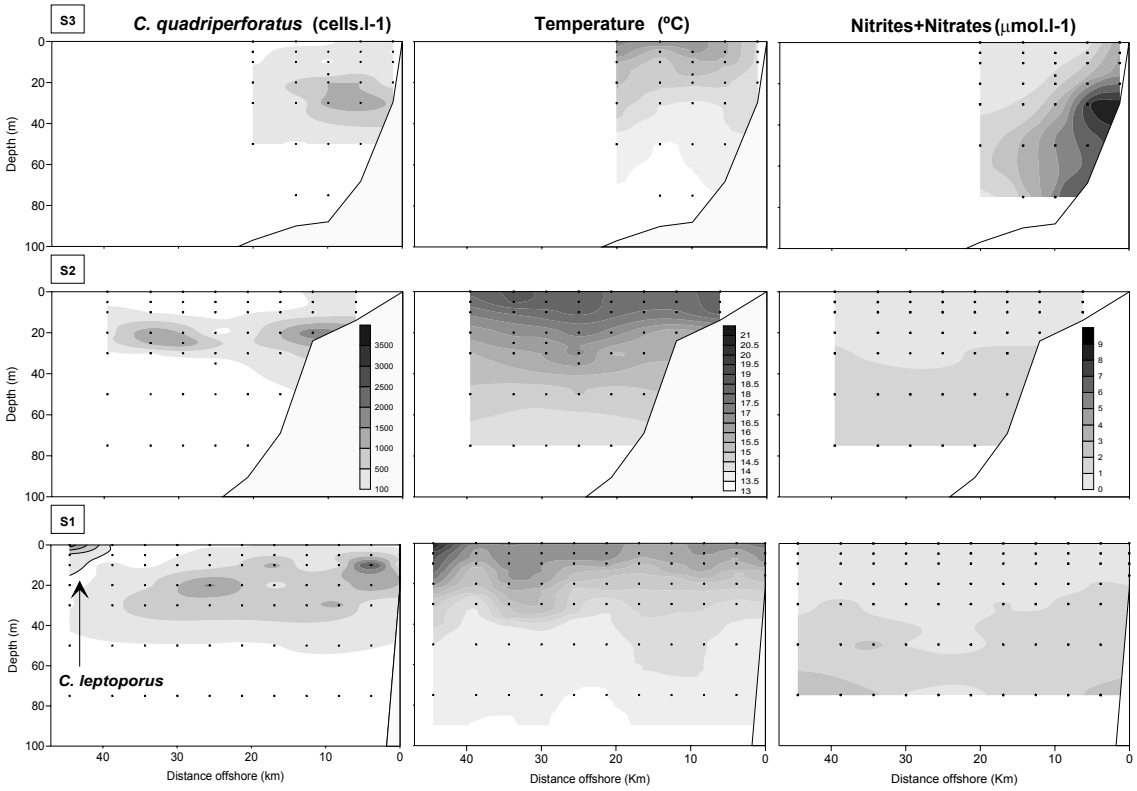

Figure 9 - Vertical distribution of *Calcidiscus quadriperforatus*, temperature and nitrites+nitrates concentrations along the three sections (S1, S2 and S3) until 75 m depth. The vertical distribution of *Calcidiscus leptoporus* along section 1 (S1) is highlighted by an arrow (↑).

The presence of *Crystallolithus rigidus* was noticed in very low abundances, coincident with the offshore distribution of *C. leptoporus* and with the winter peaks in this species (Silva et al., 2008). *Syracolithus quadriperforatus* was not observed.

4.3 Statistical analysis

Principal component analysis and linear correlations carried out with *Calcidiscus* spp. concentrations (Silva et al., 2008) showed a positive correlation with colder waters. No relevant co-variation was detected between overall abundance of *C. quadriperforatus* and *C. leptoporus* and physical (temperature, salinity), chemical (nutrients) and biological (Chl *a*, number of coccoliths, size of coccospheres and coccoliths) parameters. The most significant correlation obtained was between *C. leptoporus* from the cruise samples and salinity (n = 166, r^2 = 0.3, p < 0.01). Despite the absence of a correlation between *Calcidiscus* and salinity from coastal samples, in

winter 2003 and 2004, *Calcidiscus* was not present over the shelf during onshore advection episodes that coincided with high rainfall/river runoff (salinity below 30). The lack of significant results can probably be related with the similar environmental preferences of both species that share the same range of temperature, salinity and nutrients nearshore, during winter, and with the strong seasonality of physical-chemical parameters nearshore compared to open ocean systems. Hence, we chose to present here the raw data concerning species concentrations and physical-chemical data as they may be more useful for other authors studying coastal systems.

5. DISCUSSION

Off the coast of Portugal, the genus *Calcidiscus* is a typical component of the transition from cold to warmer water floras and was defined as a tracer for the onshore advection of oceanic waters during non-productive periods like winter (Giraudeau and Rogers, 1994; Bárcena et al., 2004, Silva et al., 2008). *Calcidiscus* spp. showed a high tolerance to low temperatures, resulting in maxima nearshore, from late autumn until early spring with major peaks during winter. This period is usually characterized by a mixture in the water column and onshore advection of oceanic warmer waters into colder shelf waters. Over the four years, the genus *Calcidiscus* decreased in abundance due to shorter and less intense onshore advection periods, to an increase in the number of days with upwelling, especially from autumn until winter and strong fluctuations in salinity caused by rainfall. In 2002, the highest concentrations of both species were observed, when the longest onshore advection period from the study was observed and nitrites+nitrates+phosphates were most available. These nutrients seemed to favour the development of these species and their decrease from 2001 onwards could be related to the lowest concentrations of *Calcidiscus* species.

From the samples studied, the two species seemed to react independently but have a co-occurring seasonal pattern nearshore, developing in different concentrations throughout the seasons. C. *quadriperforatus*, which is more abundant, had recurrent maxima during winter and can be associated with nutrient enriched waters in lower temperatures and salinities. Both species preferably developed in temperatures around 15ºC as pointed out by Ziveri et al. (2004), for the Atlantic, who observed the species at this optimum temperature but absent from higher latitudes. On the other hand, *C. leptoporus*, in lower concentrations, had maxima during winter-spring and developed in

warmer, saltier and oligotrophic waters. Both coexisted over the shelf during winter but as SST increases, *C. leptoporus* prevailed during spring. This indicates a preference for higher temperatures despite the intermittent weak upwelling conditions usual from this season.

Several studies carried out in distinct areas around the world, also revealed seasonal differences in the distribution of both *Calcidiscus* species. Two peaks of abundances and distinct distributions were also observed by Renaud and Klass (2001) off Bermuda and Renaud et al. (2002) for the NE-NW Atlantic. Off Bermuda and NW Atlantic the authors observed a spring / summer (May / July) maximum largely dominated by *C. leptoporus* associated with higher temperatures whereas both C. *quadriperforatus* and *C. leptoporus* contributed to a moderate peak during autumn/winter. The temperatures variations off Bermuda are similar to those of the NE Atlantic (Renaud et al., 2002) with overall slightly warmer and more oligotrophic conditions. For the NE Atlantic, Renaud et al. (2002) observed seasonal differences of both species. In the sampling station under subtropical influence, the spring peak was dominated by *C. leptoporus* and *C. quadriperforatus* with a dominance of the last during the onset of the spring bloom. In the sampling station under temperate conditions, there was an earlier increase of absolute abundances of both *C. leptoporus* and *C. quadriperforatus*, during winter-spring, with a major input of *C. leptoporus.*

In the South Atlantic and Southern Ocean, Boeckel et al. (2006) encountered *Calcidiscus leptoporus* in cooler waters from high-productivity environments. The species was particularly abundant south of the subtropical convergence and in the Benguela upwelling region. The authors did not differentiate the morphotypes in all of the samples but the intermediate form dominated the *Calcidiscus leptoporus* assemblages.

For the Bay of Biscay, Beaufort and Heussner (2001) observed the large morphotype (*C. quadriperforatus*) during autumn (coccolith > 5 µm) and a small morphotype associated with summer (coccolith < 5 µm) not observed in this study.

The presence of *C. leptoporus* and *C. quadriperforatus* in sediment samples cores, or sediment traps from upwelling regions should not necessarily be associated with a preference for highly productive environments and related physical conditions. In Lisbon bay, the data suggested that *Calcidiscus* species responded to nutrient availability associated with low temperatures and developed nearshore when turbulence decreased as a result of weakening northly winds during the upwelling

season (spring) or prevailing onshore advection conditions, during winter (Fig.4). During upwelling events, as in summer 2005, the genus distribution highlighted the boundary of the upwelling cores. The unusual presence of *C. quadriperforatus* nearshore during summer 2005 could be due to the occurrence of two mesoscale eddies responsible for the advection of warmer waters into the bay. This intrusion pushed the upwelling filament, with a sole westward extension (and not the usual southward jet) and was responsible for the presence of *C. quadriperforatus* nearshore, south of the filament. Most probably, the sediment record will revealed the species as an upwelling indicator or productivity proxy. The species developed over the shelf due to a displacement of oceanic waters promoted by a different orientation of the upwelling filament. These seasonal short time scale differences are difficult to observe in sediment material that is used to obtain information on sedimentary processes, fluxes and paleo-ecological inferences.

The summer cruise allowed the definition of new temperature and salinity windows, which increased from the 18.9 to 21.5ºC and from 36.0 to 36.3, respectively.

The larger coccospheres (22 - 25 μm) of *C. quadriperforatus* observed nearshore probably result from an increase in size of the smaller coccospheres (17.5 - 22 μm) once both had the same coccoliths size and morphological features. We can hypothesise that the number and the size of coccospheres were influenced by nutrient availability and low turbulence conditions in the retentive upwelling area, south of cape Roca.

6. CONCLUSIONS

The present work as allowed, for the first time, a detailed definition of the ecological preferences of *C. quadriperforatus* and *C. leptoporus* in Portuguese coastal waters.

In Lisbon bay, *C. quadriperforatus* and *C. leptoporus* were observed offshore in warmer and oligotrophic waters but nearshore the two species seemed to react independently having a co-occurring seasonal pattern but in different proportions mainly influenced by onshore advection periods or downwelling. Maxima of both species occurred in low turbulent and nutrient enriched waters, also favourable to the development of larger coccospheres of *C. quadriperforatus*. From 2001 until 2005, due

to the intensification and persistence of upwelling and rainy periods, *C. quadriperforatus* concentrations decreased ~20 times and the abundance of *C.leptoporus* was 9 times lower in 2005.

The sampling procedure allowed an accurate description of seasonality and environmental preferences of both species. Winter periods were characterized by the occurrence of mixed populations but were largely dominated by *C. quadriperforatus* while in spring only *C. leptoporus* was present related to higher SST. Being more opportunistic, *C. quadriperforatus* was associated with lower temperatures and nutrient enriched waters while *C. leptoporus* preferably develop in mature upwelled waters over the shelf during spring. The satellite images revealed the presence and onshore displacement of oceanic warmer waters and *C. quadriperforatus* and *C. leptoporus* distribution highlighted the subtropical influence of these waters nearshore. The coastal development of each species also gave indications of local oceanographic conditions.

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References

Andruleit, H., 2007. Status of the Java upwelling area (Indian Ocean) during the oligothrophic northern hemisphere winter monsoon season as revealed by coccolithophores. Marine Micropaleontology, 64: 36-51.

Andruleit, H., Stager, S., Rogalla, U., Cepek, P., 2003. Living coccolithophores in the northern Arabian Sea: ecological tolerances and environmental control. Marine Micropaleontology, 49: 157-181.

Bakun, A., 1973. Coastal upwelling indices, West Coast of North America 1946-71. NOAA Technical Report NMFs SSFR-671. U.S. Dept. of Commerce, 103.

Bárcena, M.A., Flores, J.A., Sierro, F.J., Pérez-Folgado, M., Fabres, J., Calafat, A., Canals, M., 2004. Planktonic response to main oceanographic changes in the Alboran Sea (Western Mediterranean) as documented in sediment traps and surface sediments. Marine Micropaleontology, 53: 423-445.

Beaufort, L., Heussner, S., 2001. Seasonal dynamics of calcareous nannoplankton on a West European continental margin: the Bay of Biscay. Marine Micropaleontology, 43: 27-55.

Boeckel, B., Baumann, K-H., Henrich, R., Kinkel, H., 2006. Coccolith distribution patterns in South Atlantic and Southern Ocean surface sediments in relation to environmental gradients. Deep-Sea Research I, 53: 1073-1099.

Bollmann, J., 1997. Morphology and biogeography of Gephyrocapsa coccoliths in Holocene sediments. Marine Micropaleontology, 29: 27-55.

Cachão, M., Moita, M.T., 2000. Coccolithus pelagicus, a productivity proxy related to a moderate fronts of western Iberia. Marine Micropaleontology, 39: 131-155.

Cachão, M., Oliveira, A., Vitorino, J., 2000. Subtropical winter guests, offshore Portugal. Journal of Nannoplankton Research, 22: 19-26.

CLS (Collecte Localisation Satellites), 2008. Ssalto/Duacs User Handbook : (M)SLA and (M)ADT Near-Real Time and Delayed Time Products. Ref. CLS-DOS-NT-06.034, URL: http://www.aviso. oceanobs.com/fileadmin/documents/data/ duacs/handbook_duacs.pdf.

CMS (Centre de Météorologie Spatiale) 2005. North Atlantic Regional Sea Surface Temperature Product Manual. Ref. SAF/OSI/M-F/TEC/MA/124, URL: http://www.osi-saf.org/biblio/docs/ ss1_pmnarsst_1_7.pdf.

Cortés, M.Y., 2000. Further evidence for the heterococcolith-holococcolith combination *Calcidiscus leptoporus-Crystallolithus rigidus*. Marine Micropaleontology, 39: 35-37.

De Bernardi, B., Ziveri, P., Erba, E., Thunell, R.C., 2005. Coccolithophore export production during the 1997–1998 El Niño event in Santa Barbara Basin (California). Marine Micropaleontology, 55: 107-125.

Dortch, Q., Whitledge, T.E., 1992. Does nitrogen or silicon limit phytoplankton production in the Mississipi River filament and nearby regions? Continental Shelf Research, 12: 1293-1309.

Fatela, F., Taborda, R., 2002. Confidence limits of species proportions in microfossil assemblages. Marine Micropaleontology, 45: 169-174.

Fiúza, A.F.G., Macedo, M.E., Guerreiro, M.R., 1982. Climatological space and time variation of the Portuguese coastal upwelling. Oceanologica Acta, 5: 31-40.

Geisen, M., Billard, C., Broerse, A.T.C., Cros, L., Probert., I., Young, J.R., 2002. Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation? Journal of Phycology, 37: 531-550.

Giraudeau, J., 1992.Distribution of recent nannofossils beneath the Benguela system: Southwest African continental margin. Marine Geology, 108: 219-237.

Giraudeau, J., Rogers, J., 1994. Phytoplankton biomass and sea-surface temperature estimates from sea-bed distribution of Nanofossils and Planktonic Foraminifera in the Buenguela upwelling system. Micropaleontology, 40: 275–285.

Graham, W.M., Largier, J.L., 1997. Upwelling shadows as nearshore retention sites: the example of northern Monterey Bay. Continental Shelf Research, 17: 509–532.

Grasshoff, K., 1983. Methods of seawater analysis. In: K. Grasshoff, M. Ehrhardt, K. Kremling (Editors), Verlag Chemie, New York, 419 pp.

Hasle, G.R., 1978. Phytoplankton manual: the inverted microscope method. In: A. Sournia (Editor), Monographs on oceanic methodology. Unesco, Paris, pp. 88-96.

Haynes, R, Barton, E.D., 1990. A poleward flow along the Atlantic coast of the Iberian Peninsula. Journal of Geophysics Research, 95: 11425-11441.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W., Strickland, J.D.H., 1965. Fluorimetric determination of Chlorophyll. Journal du Conseil Permanent International pour l'Exploration de la Mer, 30 (1) : 3-15.

Kinkel, H., Baumann, K-H., Cepek, M., 2000. Coccolithophores in the equatorial Atlantic ocean: response to seasonal and Late Quaternary surface water variability. Marine Micropaleontology, 39: 87-112.

Kleijne, A., 1990. Distribution and malformation of extant calcareous nannoplankton in the Indonesian seas. Marine Micropaleontology, 16: 293-316.

Kleijne, A., 1991. Holococcolithophores from the Indian Ocean, Red Sea, Mediterranean Sea and North Atlantic Ocean. Marine Micropaleontology, 17: 1-76.

Knappertsbusch, M., Cortes, M.Y., Thierstein, H.R., 1997. Morphologic variability of the coccolithophorid Calcidiscus leptoporus in the plankton, surface sediments and from the Early Pleistocene. Marine Micropaleontology, 30: 293-317.

McIntyre, A., Bé, A.W.H., 1967. Modern coccolithophoridae of Atlantic Ocean I. Placoliths and cyrcoliths. Deep-sea Research, 14: 561-597.

McIntyre, A., McIntyre, R., 1970. Coccolith concentration and differential solution in oceanic sediments. In: B.M Funnel, W.R. Reidel (Editors), Micropaleontology of the oceans. Cambridge University Press, Cambridge, pp. 253-261.

Moita, M.T., Oliveira, P.B., Mendes, J.C., Palma, A.S., 2003. Distribution of chlorophyll *a* and Gymnodinium catenatum associated with coastal upwelling filaments off central Portugal. Acta Oecologica, 24: S125-S132.

Okada, H., Honjo, S., 1973. The distribution of oceanic coccolithophorids in the Pacific. Deep Sea Research, 20: 355-374.

Peliz, A., Dubert, J., Santos, A.M.P., Oliveira, P.B., Le Cann, B., 2005. Winter upper ocean circulation in the Western Iberian Basin - Fronts, Eddies and Poleward Flows: an overview. Deep Sea Research I, 52: 621-646.

Renaud, S., Klaas, C., 2001. Seasonal variations in the morphology of the coccolithophore Calcidiscus leptoporus off Bermuda (N. Atlantic). Journal of Plankton Research, 23: 779-795.

Renaud, S., Ziveri, P., Broerse, T.C., 2002. Geographical and seasonal differences in morphology and dynamics of the coccolithophore Calcidiscus leptoporus. Marine Micropaleontology, 46: 363-385.

Rios, A.F., Pérez, F.F., Fraga, F., 1992. Water masses in the upper and middle North Atlantic Ocean east of the Azores. Deep Sea Research, 39: 645-658.

Sáez, A.G., Probet, I., Geisen, M., Quinn, P., Young, J.R., Medlin, L.K., 2003. Pseudo-cryptic speciation in coccolithophores. Proceedings of the National Academy of Sciences, 10: 7163-7168.

Silva, A., Palma, S., Moita, M.T., 2008. Coccolithophores in the upwelling waters of Portugal: four years of weekly distribution in Lisbon Bay. Continental Shelf Research, 28: 2601-2613.

Throndsen, J., 1978. Phytoplankton manual: preservation and storage. In: A. Sournia (Editor), Monographs on oceanic methodology. Unesco, Paris, pp. 69-75.

Winter, A., Jordan, R.W., Roth, P.H., 1994. Biogeography of living coccolithophores in ocean waters. In: A. Winter, W.G. Siesser (Editors) , Coccolithophores. Cambridge University Press, Cambridge, pp. 161-177.

Young, J., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I., Ostergaard, J., 2003. A guide to extant coccolithophore taxonomy. Journal of Nannoplankton Research (Special Issue 1).

Ziveri, P., De Bernardi, B., Baumann, K-H., Stoll, H.M., Mortyn, P.G., 2007. Sinking of coccolith carbonate and potential contribution to organic carbon ballasting in the deep ocean. Deep Sea Research II, 54: 659–675.

Ziveri, P., Baumann, K.H., Böckel, B., Bollmann, J., Young, J., 2004. Present day coccolithophore-biogeography in the Atlantic Ocean. In: H.Thierstein, ,J. Young (Editors), Coccolithophores: From Molecular Processes to Global Impact. Springer Verlag, 403-427

Ziveri, P., Thunell, R., Rio, D., 1995. Export production of coccolithophores in an upwelling region: Results from San Pedro Basin, Southern California Borderlands. Marine Micropaleontology, 24: 335-358.

CHAPTER 4: LONG-TERM PHYTOPLANKTON DISTRIBUTION AND COMPOSITION – FOUR YEARS OF WEEKLY SAMPLING IN LISBON BAY (PORTUGAL).

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Silva, A.*, Palma, S. and Moita, M.T

Instituto Nacional de Recursos Biológicos (INRB-IPIMAR), Av. Brasília s/n, 1449-006 Lisboa, Portugal. Tel: +351213027121, Fax: +351213015948

* Corresponding author: amsilva@ipimar.pt

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ABSTRACT

From July 2001 to May 2005, at a fixed station located in Lisbon bay (Cascais : 38º 41' N, 09º 24' W), seawater samples were collected on a weekly basis, from surface in order to full characterized the seasonal and interannual variability of the phytoplankton community in relation to physical-chemical and biological parameters (chlorophyll *a*, nutrients, temperature, salinity and wind data). With this comprehensive sampling we expect to fully describe the seasonal pattern of the major phytoplankton groups, detailing physical-chemical preferences and observe how phytoplankton communities succeed in response to environmental changes. We also associate each group maxima and oceanographic preferences with particular hydrological mesoscale structures highlighted by satellite images. Particular attention was given to the ecological niche occupied by coccolithophores in relation to diatoms and dinoflagellates. For the first time, on Portuguese coastal waters, the three groups were studied simultaneously with sampling and observation methods focus for coccolithophores.

Under prevailing upwelling conditions diatoms developed and were dominant, especially during spring, but silicates should be available. Short upwelling pulses appeared to be unfavourable for diatoms maintenance. When upwelling weakened and temperature rises due to onshore advection of warmer waters, coccolithophores dominated. This assemblage was the second most abundant during the study in particular during the short transition period from upwelling (summer) to downwelling seasons (autumn). Coccolithophores distributed in the largest range of hydrographical conditions overlaying diatoms during early spring and dinoflagellates during summer. Nitrites and nitrates seemed to favoured greater developments of this group. Dinoflagellates peaked mainly during summer and were the less abundant through the four years associated with the decrease of lasting convergence periods. Like coccolithophores, a preference for warmer waters emerged but this group seemed to have a narrow tolerance to turbulence and temperature changes.

The interannual differences observed in the phytoplankton community, in Lisbon bay, varied according to both the persistence and strength of the upwelling events and to precipitation and Tagus river flow. In 2002 the upwelling and downwelling seasons were clearly distinguished and precipitation was low. The community was dominated by diatoms and coccolithophores. The following years were characterized by longer

periods of mild upwelling, SST progressively higher and by an increased in Chl *a* concentrations. In 2004, the second higher phytoplankton concentrations were recorded and the assemblage was dominated by diatoms and, instead of coccolithophores, by dinoflagellates, with the two most expressive peaks of the study. The year of 2003 was particularly characterized by longer periods of intense precipitation and strong fluctuations in salinity and lower temperatures. Phytoplankton maxima were observed later in the year and attended in very low numbers despite the availability of nutrients.

Associated with the seasonal variation it was possible to identify short succession cycles dependent from coastal upwelling events. Intermittent and weak pulses allowed the coexistence of species from the different succession stages but peaks were not simultaneous.

Keywords: phytoplankton succession, time series, diatoms, coccolithophores, dinoflagellates, upwelling

1. INTRODUCTION

At the west coast of Portugal upwelling generally occurs seasonally, from April to September, under northerlies, while onshore advection of oceanic waters is observed during autumn and winter, when southerly winds begin to dominate and there is an intensification of waters flowing poleward (Fiúza et al., 1982; Haynes and Barton, 1990; Peliz et al., 2005). Episodes of reverse winds can occur during both seasons. Lisbon bay is either influenced by the subtropical branch of the Eastern North Atlantic Central Water (ENACWst) as by Tagus river discharges, an import nutrient source especially during winter. North of the bay, an upwelling filament rooted at cape Roca recurrently occurs during the northerly wind periods (upwelling favourable), typically extending to the south and westward direction. However, at Lisbon bay the structure of upwelling is complex since it represents an important coastline discontinuity. This region is considered an upwelling shadow area where phytoplankton species can be accumulated through different retention mechanisms (Graham and Largier, 1997, Moita et al., 2003; Oliveira et al., 2008). Moita (2001), for this coast, identified upwelling as the major source of seasonal and spatial variability of phytoplankton. Primary production depends from light, temperature, nutrients and oligoelements and in upwelling systems is defined practically by the external energy made available (Margalef, 1978a). In shelf waters, phytoplankton production is primarily controlled by the interaction of water masses supplying different levels of nutrients to the euphotic zone (Ciotti et al., 1995) and by the alterations of the water column stability (Laubscher et al., 1993; Brandini et al., 2000). Thus, the development of a certain size structure of the phytoplankton community depends on the physical-chemical characteristics of the environment (Kiørboe, 1993).

The sampling site, Cascais (Figure 1), is located at the northern side of Lisbon bay and south of cape Roca. Here, phytoplankton is either influenced by upwelled waters of the Roca filament or by warmer and mature surrounding waters. The prevailing condition depends on the intensity and persistence of upwelling favourable winds and on the offshelf mesoscale structures controlling the offshore extension and position of the upwelling filament. Weak upwelling conditions allow a larger influence of warmer and stratified waters into the bay.

The main goal of this study, based on a long term and high resolution sampling, was a full characterization of the phytoplankton community in relation to physical –

chemical parameters as upwelling, SST, salinity, chlorophyll a and various nutrients. Seasonal and interannual differences from major phytoplankton groups will be highlighted and community composition described. Particular attention will be given to the ecological niche of the coccolithophore assemblage, described in detailed in Silva et al., (2008), in relation to the diatoms and dinoflagellates.

2. MATERIALS AND METHODS

2.1 Surveyed area and sampling strategy

From July 2001 to May 2005, at a fixed station located in Lisbon bay (Cascais : 38º 41' N, 09º 24' W) (Figure 1), seawater samples were collected on a weekly basis, from surface, one hour before high tide to minimize the direct influence of Tagus estuarine waters on the area. The surface samples were used for chlorophyll *a*, the fraction of chlorophyll *a* less than 20 μm, phytoplankton composition and nutrient determination. Temperature, salinity and depth were determined *in situ* with a Quanta CTD.

Daily wind data were obtained from the meteorological station of Cape Carvoeiro, located 50 km north of Cascais (Figure 1).

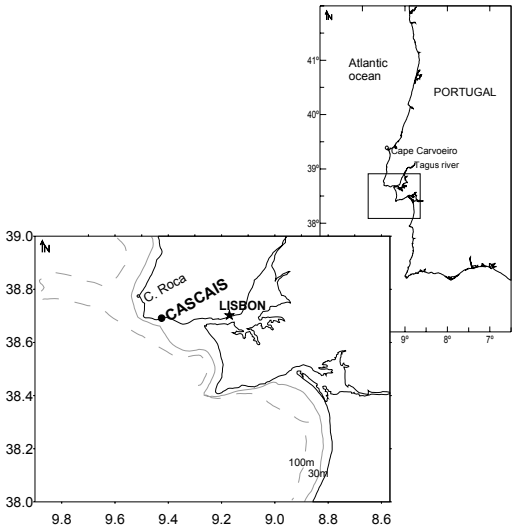

Figure 1 - Location of the Cascais sampling site 38º41'N and 09º24'W (●).

2.2 Chlorophyll *a* and nutrient analyses

To evaluate the chlorophyll *a* concentration, 250 ml seawater samples were filtered and for chlorophyll *a* < 20 μm, the same volume was filtered through a 20 μm net placed on top of the filter (Whatman, 47mm nitrate cellulose membrane with a 0.45 μm nominal pore size). Pigments were extracted with 90% acetone and determined on the Perkin-Elmer spectrofluorometer (Holm-Hansen et al., 1965).

The water for nutrient determination was filtered through a Millipore filter of 0.45 μm and stored at – 4ºC for subsequent analysis. Ammonia (NH_4^+), nitrites and nitrates (NO_2^- + NO_3^-), phosphates (HPO_4^{2-}) and silicates ($Si(OH)_4$) were determined using an autoanalyser "SKALAR" according to the methods of *Technicon Industrial Systems* (Grasshoff, 1983). The detection limit is 0.2 μM for ammonia and silicates and 0.05 μM for nitrites+nitrates and phosphates.

2.3 Phytoplankton analyses

Phytoplankton samples were preserved with hexamethylenetetramine buffered formalin to a final concentration of 2% (Throndsen, 1978). The species composition of the phytoplankton community were identified and enumerate in subsamples of 50 ml by the Utermöhl technique (Hasle, 1978), using a Zeiss IM35 inverted microscope with phase contrast and bright field illumination. A magnification of 160x and 400x was used to identify and enumerate the phytoplankton assemblage with a detection limit of 40 cells.l^{-1} and 2000 cells.l^{-1}, respectively. When possible, the cells were identified to species level according to Hasle and Syvertsen (1996) and Dodge (1982). Coccolithophores were separately identified and counted from water samples (750 or 1000 ml) filtered through a 47mm nitrate cellulose membranes (Whatman) with a 0.45 μm nominal pore size. A strip of the filter was cut from the centre to the rim and slides were rendered transparent with a drop of Entellan mounting medium. Coccospheres were identified and counted until at a maximum of 300 cells of all taxa per sample (Fatela and Taborda, 2002) on an area of 2.2 mm^2 of the filter with a Zeiss optical microscope under cross-polarized light, at a magnification of 1250 x. Depending on the overall abundance of coccospheres in the samples, counts ranged between 1 cell, corresponding to 73 cells.l^{-1} and 300 cells equivalent to 22x10^3 cells.l^-A scanning

electron microscope (JEOL-5200) was used to complete the identifications of the coccolithophore assemblage. Species were identified following Young et al. (2003).

2.4 Data analysis

Based on the values of northward wind stress component a daily upwelling index was calculated (Bakun, 1973). A running average, with a window width of 7 days, was determined to allow a straight relation between data from different time scales (weekly sampling and daily upwelling index).

In order to describe the associations between phytoplankton species, a Principal Component Analysis (PCA) was performed using the software NTSYSpc version 2.02i from 1997 by Applied Biostatistics, Inc. The analysis was carried out with the species that occurred at least in 20% of the samples during the four years. From the 209 species identified, 106 were excluded due to a relative low frequency.

A linear regression has been performed, between the overall abundance of phytoplankton groups and the environmental factors. No significant co-variation was detected among parameters. The highest correlation obtained was 0.2 ($n = 197$, $p < 0.05$) between coccolithophores and temperature, 0.3 ($p<0.01$) for diatoms versus Chl *a* and 0.4 ($n = 197$, $p < 0.01$) for total phytoplankton and Chl *a*.

The satellite-derived sea surface temperature (SST) maps were extracted from EUMETSAT's Ocean and Sea Ice Satellite Application Facility "Regional SST" product, available at 2-km resolution (CMS, 2005)

3. RESULTS

3.1 Hidrography and nutrients

During the four years, the upwelling became more persistent although less intense, usually higher than -1000 $m^3 s^{-1} km^{-1}$ (Figure 2a) and the number of days per year with upwelling increased (negative values in Figure 2a). In 2004, there were more 55 days with upwelling conditions than in 2002. Conversely, the onshore advection

periods were shorter over the years (Upwelling index > 0 $m^3 s^{-1} km^{-1}$). During spring, upwelling was always intermittent and the onshore advection conditions decreased in frequency and strength from late summer to winter seasons. Short-periods of onshore advection were typically observed between mid-September and early December (Figure 2a).

Temperature ranged between 11.5ºC and 20.4ºC, observed in January 2003 and October 2002 (Figure 2b) and varied from seasonal to interannual time scales. The lowest SSTs were observed from late autumn until spring, while the highest during summer and early autumn. From spring to autumn seasons, SST increased ~ 1 – 1.5ºC. From 2001 to 2005, summers were gradually warmer, with longer periods above 17ºC. Autumns had temperatures varying between 15 – 20ºC, with values decreasing towards winter temperatures that ranged from 12ºC to 15ºC. Winter periods became colder with a ~ 1ºC decrease associated with precipitation (data on www.inag.pt and Silva et al. 2008) and intensification of the SW winds conditions.

Salinity also presented patterns of seasonal and interannual variability ranging from 26.9 in January 2003 to 36.5 in October 2001, and was usually higher than 34.5 (Figure 2c). Higher and relatively constant values were observed from spring to early–autumn and lower values, from late-autumn to winter coincident with an intensification of precipitation and associated runoff.

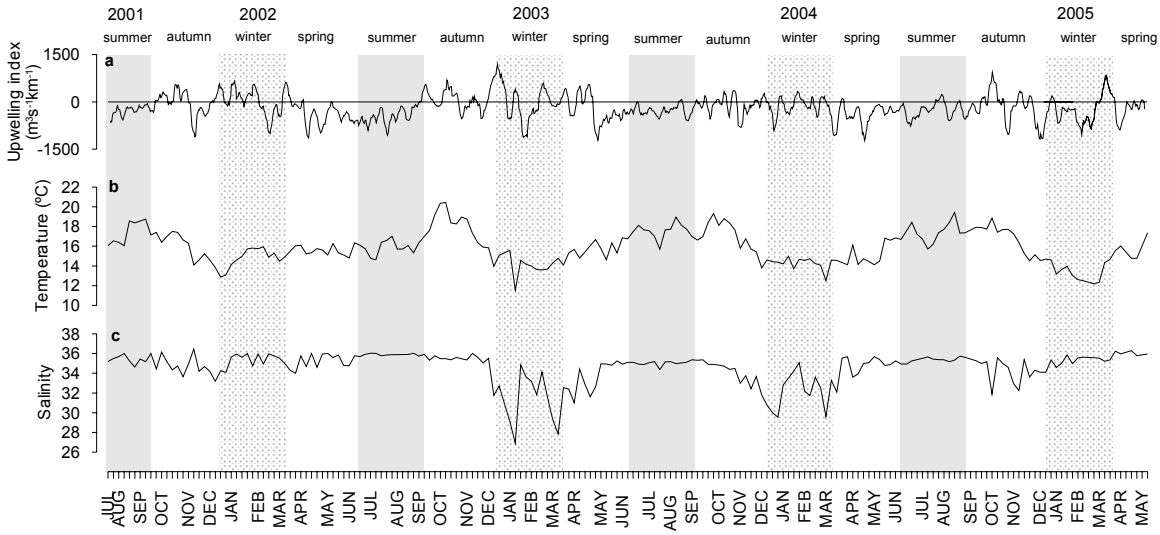

Figure 2 – Weekly distribution of upwelling index (a), temperature (b) and salinity (c), from July 2001 to May 2005.

Average nutrient concentrations per season and year are summarised in Table 1. Nutrient concentrations changed along the year (Figure 3), with the higher values recorded during autumn – early spring and the lowest during late spring – summer. The measured phosphates (Figure 3a) varied between 0.2 – 3.2 μmol l^{-1} (May 03 - May 02), and were most available in half 2001, 2002 and 2003. As silicates are considered (Figure 3b), 2002 and 2003 presented the highest concentrations especially from autumn to spring while summer had minor concentrations. This nutrient ranged between 0.1 – 29.7 μmol l^{-1} (July 02 - December 03). Regarding nitrites + nitrates (Figure 3c), the highest concentrations were determined in 2002 being most abundant during autumn - winter. Minima and maxima were 0.06 and 21.9 μmol l^{-1} (December 02 and December 01). Ammonia (Figure 3d) values were between 0.3 – 21.0 μmol l-1 (February 05 - January 04) and autumn 2003 presented the highest concentrations followed by winter 2004.

(μmol.l^{-1})	2001	2002	2003	2004	2005
Phosphates					
Winter	-	0,8	0,9	0,7	0,4
Spring	-	1,1	0,6	0,5	0,3
Summer	1,6	0,8	0,9	0,4	-
Autumn	3,0	0,8	1,0	0,8	-
Total	**4,6**	**3,5**	**3,4**	**2,4**	**0,7**
Silicates					
Winter	-	6,6	16,3	11,1	2,5
Spring	-	8,4	6,1	4,3	1,2
Summer	3,6	7,4	1,7	3,2	-
Autumn	13,9	8,2	8,8	5,6	-
Total	**17,5**	**30,6**	**32,9**	**24,2**	**3,7**
Nitrites+nitrates					
Winter	-	12,6	5,2	7,0	6,4
Spring	-	8,5	5,5	3,8	4,0
Summer	5,5	7,0	6,5	2,5	-
Autumn	10,0	7,3	6,0	5,8	-
Total	**15,5**	**35,4**	**23,2**	**19,1**	**10,4**
Ammonia					
Winter	-	2,7	5,3	8,3	1,5
Spring	-	4,7	4,2	3,6	1,6
Summer	3,1	4,1	4,8	4,7	-
Autumn	4,2	4,6	11,7	4,4	-
Total	**7,3**	**16,1**	**26,0**	**21,0**	**3,1**

Table 1 – Seasonal average and interannual variability of phosphates, silicates, nitrites + nitrates and ammonia. Grey squares indicate that sampling occurred during ~ 2.5 months.

Concerning nutrient stoichiometry and according to Dortch and Whitlege (1992), from the 197 observed samples, limitations values were found 44 times. These two authors propose a combination between nutrient concentrations and ratios to access limitation. Limited concentrations of phosphates ($PO_4^{3-} \leq 2$, N/P > 30 and Si/P > 3) occurred 8 times (4%), more than half during winter 2004 (Figure 3a, e). This nutrient was always available in 2001 and 2005 and limited one or two times in 2002-2003. Nitrates limitation (DIN ≤ 1, N/P < 10 and Si:N > 1) was observed 12 times (6%), during winter and spring in 2002 and 2003. Nitrites + nitrates were always abundant in 2001 and 2004 (Figure 3c, e). Limitation by silicates ($SiO_4^{4-} < 2$, Si/N < 1, Si/P < 3) was recorded 24 times (12%) through summer seasons (8 x in 2003) and winter-spring 2005 (8 x) (Figure 3b, f). Silicates were in general available during 2001 and 2002.

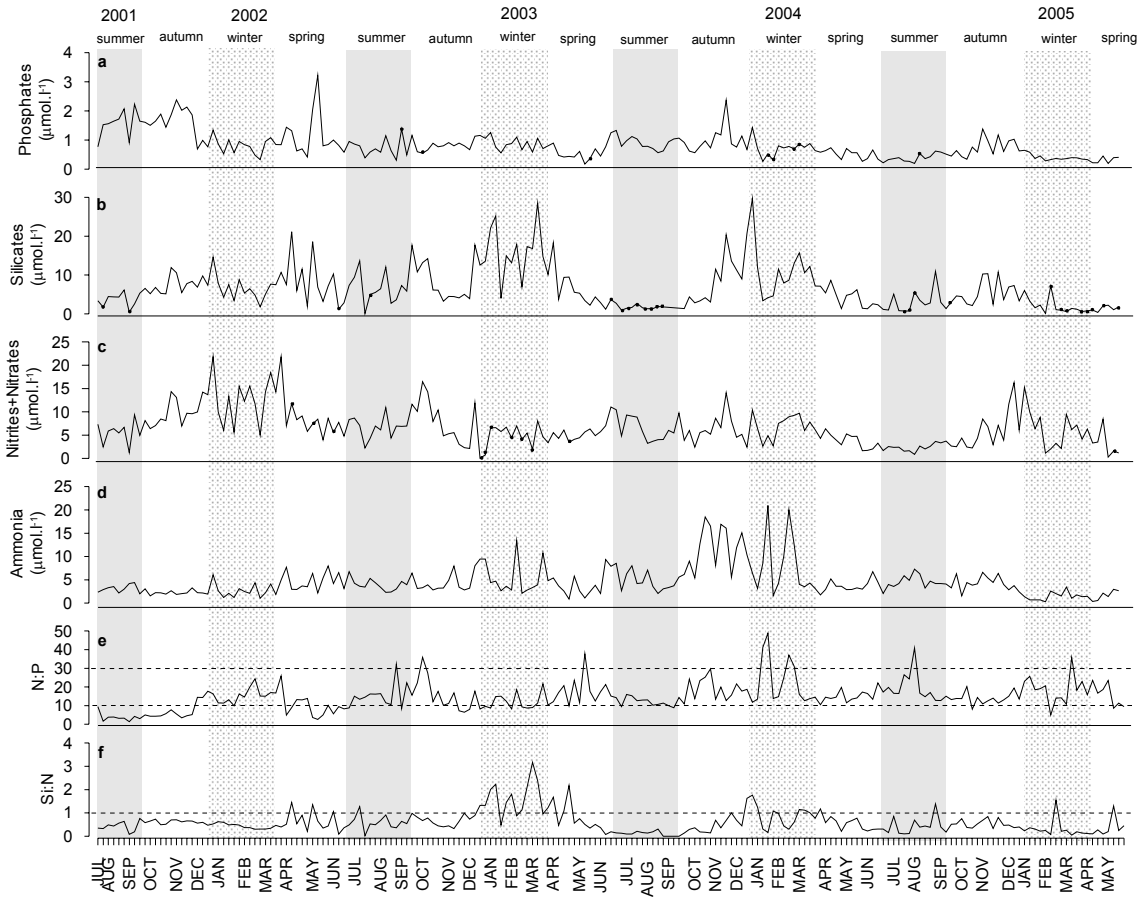

Figure 3 – Weekly concentrations of phosphates (a), silicates (b), nitrites + nitrates (c), ammonia (d), N:P ratios (e) and Si:N ratios (f) from July 2001 to May 2005. The dots (•) indicate nutrient limitation, according to Dortch and Whitledge (1992).

3.2 Chlorophyll *a* and phytoplankton distribution

The development of the phytoplankton community can be measured by chlorophyll *a* (Chl *a*) that presented a minimum and maximum values of 0.1 μg.l^{-1} (November 2001 and March 2005) and 5.3 μg.l^{-1} (February 2005) respectively (Figure 4a). In average and only comparing entire years, the lowest Chl *a* concentrations were observed in 2002 while in 2004 they were the highest, especially during spring and summer (Table 2). The lower concentrations were usually during winter however, Chl *a* values increased in this season since 2002 onwards (Table 2).

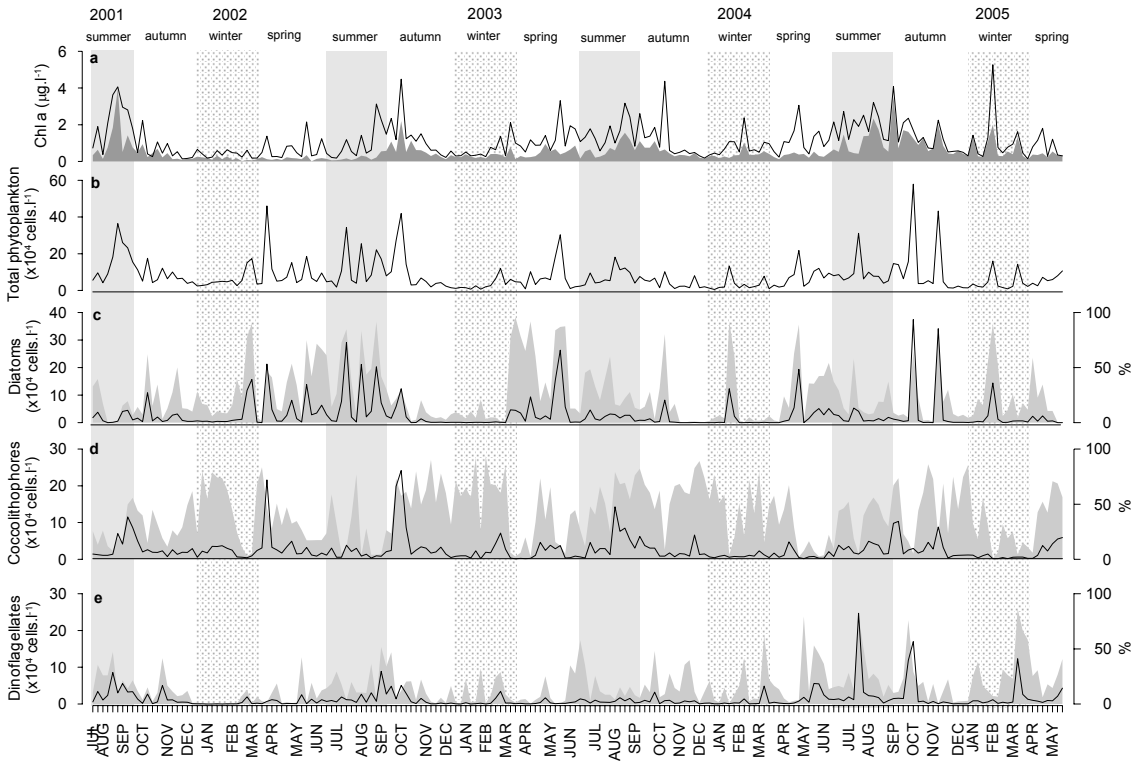

Figure 4 - Weekly distribution of Chl *a* (line) and Chl *a* below 20 μm (shadow) (a), total phytoplankton (b), diatoms (c), coccolithophores (d) and dinoflagellates (e), from July 2001 to May 2005.

The fraction of Chl *a* less than 20 μm varied from 0.01 μg.l^{-1} in May 2002 to 4.0 μg.l^{-1} in August 2001 (Figure 4a) and includes a phytoplankton community composed by small coccolithophores, small-sized phytoplankton as cryptophytes, chlorophytes,

prasinophytes, cyanobacteria and other not identified small algae. This fraction represented 50% of the Chl *a* measured foremost contributing to the pool of Chl *a* especially during summer-autumn seasons (Table 2). During winter and spring the average concentrations of Chl *a* < 20 μm were lower. Despite the similar concentrations, differences were found in the contributions to total Chl *a*, during spring Chl *a* < 20 μm never exceed 40% of total Chl *a* while during winter varied between 40-60% of total biomass. Maximum concentrations of phytoplankton less than 20 μm occurred during 2004 as expressed by this fraction of Chl *a* especially during summer 2004 (1.3 μg l^{-1}, 62% of total Chl *a*) and autumn, (0.90 μg l^{-1}, 90% of total Chl *a*).

	2001	**2002**	**2003**	**2004**	**2005**
Chl a (μg.l^{-1})					
Winter	-	0,4	0,7	0,9	1,2
Spring	-	0,7	1,3	1,4	0,7
Summer	2,3	1,1	1,6	2,1	-
Autumn	0,6	1,1	0,9	1,0	-
Total	**2,9**	**3,3**	**4,5**	**5,4**	**1,9**
Chl a < 20 μm (μg.l^{-1})					
Winter	-	0,2	0,3	0,4	0,7
Spring	-	0,2	0,5	0,5	0,3
Summer	1,2	0,3	0,8	1,3	-
Autumn	0,3	0,6	0,4	0,9	-
Total	**1,5**	**1,3**	**2,0**	**3,1**	**1,0**
Total phytoplankton ($x10^4$ cells.l^{-1})					
Winter	-	78	44	40	51
Spring	-	150	98	107	49
Summer	150	160	101	120	-
Autumn	97	120	47	150	-
Total	**247**	**508**	**290**	**417**	**100**

Table 2 – Seasonal average and interannual variability of Chl *a* and Chl *a* < 20 μm and total phytoplankton. Grey squares indicate that sampling occurred during half season ~ 2.5 months.

The phytoplankton community was mainly composed by diatoms, dinoflagellates and coccolithophores representing more than 90% of cell counts. The remaining assemblage account less than 10% of total phytoplankton and was composed by

several algal groups randomly observed. This assemblage will not be described forwards but it is assumed to contribute to Chl *a* values.

The distribution of each group maxima along time was related to particular physical-chemical preferences (Figure 4b-e). The highest concentrations were observed in 2002 and 2004 mainly from spring to autumn (160 $x10^4$ cells.l^{-1} in summer 2002) while the lowest were during winter seasons as 40 $x10^4$ cells.l^{-1} in winter 2004 (Figure 4b and Table 2).

Diatoms (Figure 4c) represented the most abundant biomass source with several maxima from spring to early autumn and lower concentrations through late autumn to winter seasons (Table 3). During spring the group usually represented more than 80% of the phytoplankton assemblage. In 2002, diatoms were particularly abundant, with high concentrations during summer and in October 2004 it was observed the highest peak of the sampling period, 38 $x10^4$ cells.l^{-1} (65% of total phytoplankton). The community was composed by 104 identified taxa dominated by *Chaetoceros* spp., *Thalassiosira* spp., *Pseudo-nitzschia* spp., *Skeletonema costatum*, *Asterionelopsis glaciallis*, *Leptocylindrus danicus*, *Detonula pumila*, *Guinardia delicatula*, *Guinardia* spp., *Thalassionema nitzschioides* and *Cylindrotheca closterium* (Appendix 1).

($x10^4$ cells.l^{-1})	2001	2002	2003	2004	2005
Diatoms					
Winter	-	34	10	15	21
Spring	-	70	68	50	8
Summer	17	99	26	19	-
Autumn	24	23	11	76	-
Total	**41**	**226**	**115**	**160**	**29**
Coccolithophores					
Winter	-	28	23	11	7
Spring	-	49	22	20	24
Summer	38	22	63	48	-
Autumn	28	73	24	34	-
Total	**66**	**172**	**132**	**113**	**31**
Dinoflagellates					
Winter	-	4	6	8	18
Spring	-	9	6	22	14
Summer	31	29	10	42	-
Autumn	12	13	9	45	-
Total	**43**	**55**	**31**	**117**	**32**

Table 3 – Seasonal and interannual variability of total diatoms, coccolithophores and dinoflagellates. Grey squares indicate that sampling occurred during ~ 2.5 months.

The satellite image from 13-18 July 2002 clearly shows the presence of colder waters (<15ºC) around cape Roca, defining an upwelling filament with a southward orientation (highlighted by white arrows in Figure 5a). The phytoplankton assemblage from day 18 was dominated by diatoms (85% of phytoplankton assemblage) being *Pseudo-nitzschia* spp. the main genus observed (Table 4). Coccolithophores and dinoflagellates presented very low concentrations (11% and 3% of phytoplankton assemblage, respectively) with a reduced number of species (2 species each).

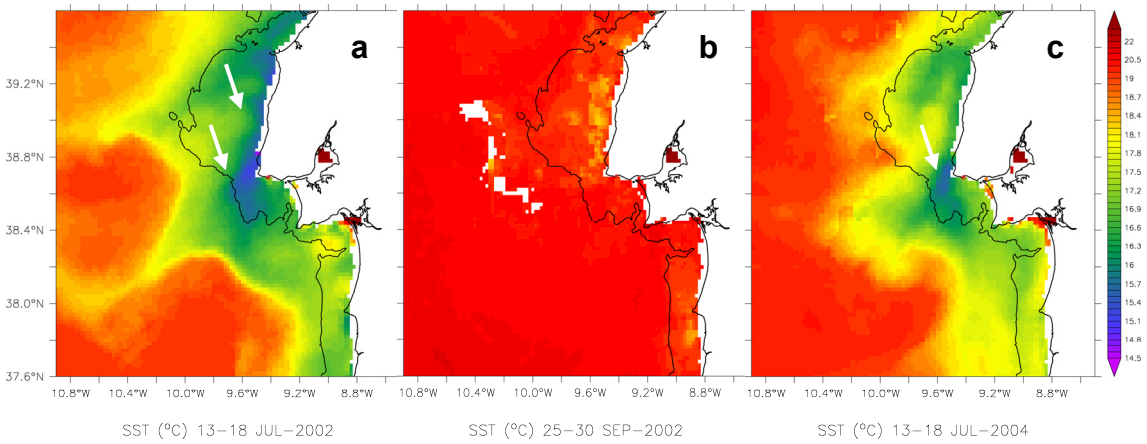

Figure 5 - Six-day average sea surface temperature (SST) derived from satellite data, previous to selected events of maxima concentration of diatoms (a), coccolithophores (b) and dinoflagellates (c). White arrows indicated the location of the upwelling filament.

Coccolithophores (Figure 4d) reached major abundances in 2002 in particular during autumn when the highest concentration was observed (24 $x10^4$ $cells.l^{-1}$ in October 2002, 58% of total phytoplankton). In 2003 this group also reached great concentrations especially during summer. On the other hand coccolithophores recorded minor developments during winter seasons (Table 3) however represented >90% of the phytoplankton assemblage. Twenty two species were identified, being the assemblage dominated by *Emiliania huxleyi*, *Gephyrocapsa oceanica*, *Coronosphaera mediterranea*, *Calcidiscus quadriperforatus*, *Calcidiscus leptoporus*, *Gephyrocapsa muellerae*, *Helicosphaera carteri*, *Syracosphaera pulchra* and *Gephyrocapsa ericsonii* (Appendix 1). The SST distribution (Figure 5b) at the end of September 2002 shows that the prevailing downwelling conditions led to the onshore advection of the warm surface waters capping the remnants of the upwelled water at the end of the season. The coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica* dominated the

phytoplankton assemblage (Table 4) and dinoflagellates attended in very low numbers (12% of total phytoplankton) but with a higher species diversity (9 species present).

Date **Physical conditions**	**18 July 2002** **upwelling**	**1 October 2002** **downwelling**	**19 July 2004** **weak upwelling**
Diatoms	29	12	4
Dinoflagellates	1	5	25
Coccolithophores	4	24	1,5
Dominant phytoplankton species (cell number)	*Pseudo-nitzschia* spp. (16)	*E. huxleyi* (9) *Gephyrocapsa* spp. (9)	*S.* cf. *trochoidea* (24)
Chl *a*	1,8	2,61	2,33
Chl *a* < 20 µm	0,16	1,2	0,49
Satellite image - Figure	5a	5b	5c

Table 4 – Maxima ($x10^4$ cells.l^{-1}) of diatoms, coccolithophores and dinoflagellates, respective dominant species and Chl *a* plus Chl *a* < 20 µm concentrations (µg.l^{-1}) during the period highlighted by the satellite images.

Dinoflagellates (Figure 4e), the less abundant group, developed during summer - autumn and maximum abundances were recurrently observed during 2004. The lowest concentrations were during winter and spring seasons (Table 3). A peak (25 x 10^4 cells.l^{-1}) occurred in July 2004 representing 79% of total phytoplankton. From the 83 taxa identified the community was dominated by a permanent development of *Scripsiella* cf. *trochoidea* and in lower numbers by the species *Ceratium fusus*, *Prorocentrum micans*, *Ceratium furca* and several others included in the genus *Ceratium* spp., *Prorocentrum* spp., *Protoperidinium* spp., *Dinophysis* spp. and *Gymnodinium* spp. (Appendix 1). When the peak was observed, the satellite data from 13-18 July 2004, showed the prevailing mild upwelling conditions and the presence of colder waters (<15ºC) just in a small core around cape Roca (highlighted by a white arrow in Figure 5c). *S.* cf. *trochoidea* dominated 95% of the dinoflagellate community only represented by four species (Table 4). Diatoms and coccolithophores, in lower numbers, were observed with six identified species each (13% and 5% of total phytoplankton, respectively).

3.3 Interannual variability

Throughout the years, chlorophyll *a* increased 0.76 $\mu g.l^{-1}$ and reflected the major trends in phytoplankton development. In contrast with the following years, 2002, was characterized by the upwelling and downwelling seasons clearly distinguished and by low precipitation, as a consequence the highest phytoplankton concentrations were recorded. The community was dominated by diatoms under upwelling conditions and coccolithophores when onshore advection prevailed. Maxima of both groups interspersed with silicates and nitrates peaks, respectively, and were a possible reason to its subsequent reduction. Nutrients were always available, a feature explained by the coastal location of the sampling site and changes in concentrations, sometimes until limit values, was probably due to phytoplankton maxima or precipitation and runoff. The following years were characterized by longer periods of mild upwelling and in 2004, the next high concentrations were observed. However the assemblage was dominated by diatoms and, instead of coccolithophores, by dinoflagellates with two short and expressive peaks. The comparison between the SST distributions of July 2002 and 2004 (Figure 5a, c) shows that, despite the similar patterns of the cold upwelled water along the coast, there is a difference of more than 1ºC in the offshore temperatures, being higher in 2004, with a 15-20 day lag between the two years (P.B. Oliveira, Personal communication).

Between these two years, 2003 was particularly characterized by the longest periods of intense precipitation and strong fluctuations in salinity and lower temperatures. Phytoplankton maxima were observed later in the year and attended in very low numbers. Silicates were largely available, probably from a riverine origin, and as salinity and temperatures begin to rise the depletion of this nutrient was coincident with a diatom peak.

The interannual salinity fluctuation highlighted different weather conditions since it is strongly influenced by the rainfall regime and thus by the Tagus river flow (data from Silva et al., 2008). Higher salinities, as in 2002, were associated with low precipitations and river runoff while the lowest values observed afterwards decreased in agreement with the rainfall and associated runoff

3.4 Principal component analysis

A PCA was performed to summarise the observed results and the first 3 components explain 37% of the total variation in the data.

The first component (PC1) explained 15% of total variability within the data and was positively correlated with all species. This component highlighted the total phytoplankton abundance. This relation is pointed out in Figure 6 by the distribution of PC1 scores with total phytoplankton. PC1 separated two assemblages: the first correlated with the higher values of the component, included species with a regular attendance during summer, such as *Gymnodinium* spp., *Diplopsalis* spp., *C. mediterranea*, *Prorocentrum* spp., *G. oceanica*, *P. diabolum* and *S. costatum* among others (Table 5).

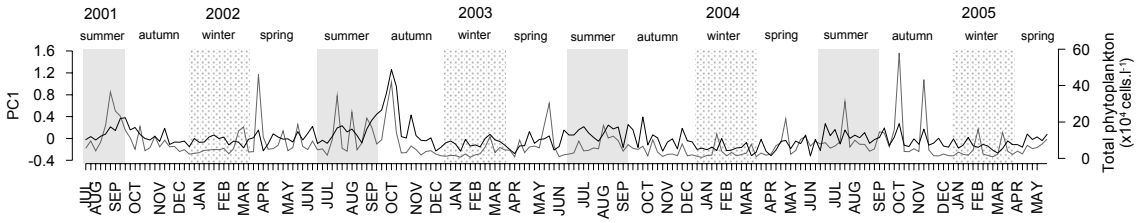

Figure 6 - Seasonal and interannual distribution of PC1 scores (black line) in relation to total phytoplankton (grey line).

The second assemblage, correlated with lower values of PC1, developed during spring, when the second highest concentrations were observed, and was composed by *Guinardia* spp., *L. danicus*, *T. nitzschioides*, *D. pumila*, *Odontela* spp. and *Chaetoceros* spp. which attended as short peaks in this season, dominating the assemblage.

The PC2 and PC3 explained each one 11% of total variability within the data. PC2 can be related to turbulence (positive values) and stratification (negative values); PC3 axis separated cold (positive values) and warm (negative values) waters (Figure 7 and Table 5). The distribution of taxonomic groups in the PC2 and PC3 axis allowed the definition of three distinct assemblages. One group, distributed in the positive scores of PC2, was mainly composed by diatoms. The species within this group were chain forming diatoms as *Thalassionema nitzshioides*, *Odontella* spp., *Chaetoceros* spp., *Guinardia* spp., *Pseudo-nithzschia* spp., *G. delicatula*, *L. danicus*, *S. costatum*, *C.*

closterium and *D. pumila* (Table 5). Some of these, like *Odontella* spp., *Diploneis* spp. or *Licmophora* spp., were never responsible by major diatom peaks but were recurrently observed in low numbers having a high global frequency in the samples.

Associated with the negative scores of PC2, was another group dominated by dinoflagellates, with a preference for stratified conditions in warmer waters. This assemblage was composed by *C. furca*, *C. fusus*, *Ceratium* spp., *Gymnodinium* spp., *D. acuminata*. It is interesting to notice the detachment of *D. acuminata* from the other dinoflagellates. This species appeared highly correlated with stratification but associated with colder waters.

Between the two assemblages and detached by PC3, there is a third cluster, composed mainly by coccolithophores and some dinoflagellates. The coccolithophore assemblage, distributed in the positive axis of PC3, was represented by *Calcidiscus* spp., in colder waters, *G. muelleare*, *G. ericsonii*, *E. huxleyi*, *G. oceanica* and *C. mediterranea*. This assemblage developed preferably under mild turbulent conditions caused by weak upwelling or onshore advection. As for dinoflagellates, an assemblage composed by *Gonyaulax* spp., *P. steinii*, *P. bipes*, *P. diabolum, S.* cf. *trochoidea* and *Gyrodinium* spp. were negatively correlated with PC3, with a preference for warmer waters.

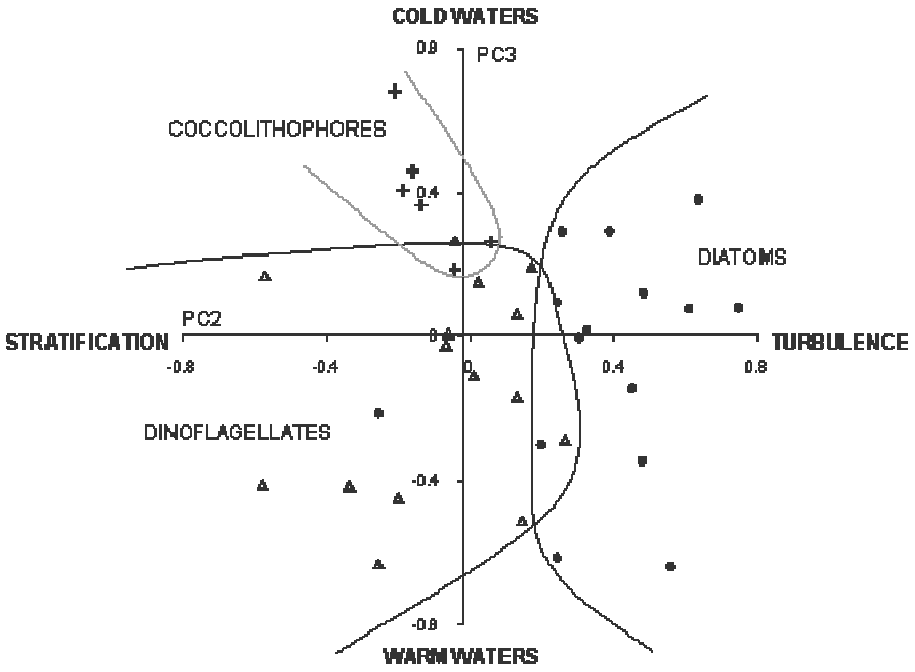

Figure 7 – Distribution of diatoms (•), coccolithophores (+) and dinoflagellates (empty ▲) in the space defined by the second (PC2) and third (PC3) components.

Species		PC1	Species		PC2	Species		PC3
Gymnodinium spp.	△	0,73	*T.nitzschioides*	●	0,75	*Calcidiscus* spp.	+	0,69
Diplopsalis sp.	△	0,69	*Odontela* spp.	●	0,63	*G.muellerae*	+	0,46
C.mediterranea	+	0,67	*Chaetoceros* spp.	●	0,61	*G.ericsonii*	+	0,41
Prorocentrum spp.	△	0,65	*Guinardia* spp.	●	0,56	*Odontela* spp.	●	0,38
G.oceanica	+	0,58	*Gramatophora* spp.	●	0,48	*E.huxleyi*	+	0,37
P.diabolum	△	0,58	*Pseudo-nitzschia* spp.	●	0,48	*Thalassiosira* spp.	●	0,29
S.costatm	●	0,56	*G.delicatula*	●	0,45	*L.danicus*	●	0,29
Gyrodinium spp.	△	0,53	*L.danicus*	●	0,39	*C.mediterranea*	+	0,27
G.ericsonii	+	0,52	*S.costatm*	●	0,33	*Gonyaulax* spp.	△	0,27
D. acuminata	△	0,51	*C.closterium*	●	0,31	*P.diabolum*	△	0,19
P. steinii	△	0,50	*D.pumila*	●	0,30	*G.oceanica*	+	0,19
G.muellerae	+	0,49	*S. cf trochoidea*	△	0,26	*D. acuminata*	△	0,17
C.furca	△	0,48	*Thalassiosira* spp.	●	0,25	*P. steinii*	△	0,15
Gonyaulax spp.	△	0,38	*Diploneis spp.*	●	0,24	*Gramatophora* spp.	●	0,12
Thalassiosira spp.	●	0,37	*Rhizosolenia* spp.	●	0,24	*C.closterium*	●	0,10
P.bipes	△	0,34	*Licmophora sp.*	●	0,20	*Diploneis spp.*	●	0,10
C.closterium	●	0,31	*P.diabolum*	△	0,17	*T.nitzschioides*	●	0,08
E.huxleyi	+	0,28	*Gyrodinium* spp.	△	0,14	*Chaetoceros* spp.	●	0,08
Protoperidinium spp.	△	0,27	*P.bipes*	△	0,13	*P.bipes*	△	0,06
C.fusus	△	0,26	*Prorocentrum* spp.	△	0,13	*S.costatm*	●	0,02
Gramatophora spp.	●	0,24	*C.mediterranea*	+	0,06	*D.caudata*	△	0,01
Rhizosolenia spp.	●	0,23	*P. steinii*	△	0,02	*D.pumila*	●	0,00
Licmophora sp.	●	0,22	*Diplopsalis* sp.	△	0,01	*Protoperidinium* spp.	△	-0,02
S. cf trochoidea	△	0,21	*G.oceanica*	+	-0,04	*Diplopsalis* sp.	△	-0,11
P.sulcata	●	0,20	*Gonyaulax* spp.	△	-0,05	*G.delicatula*	●	-0,14
Calcidiscus spp.	+	0,19	*D.caudata*	△	-0,06	*Prorocentrum* spp.	△	-0,17
Diploneis spp.	●	0,18	*Protoperidinium* spp.	△	-0,07	*P.sulcata*	●	-0,21
G.delicatula	●	0,17	*E.huxleyi*	+	-0,13	*S. cf trochoidea*	△	-0,29
Pseudo-nitzschia spp.	●	0,14	*G.muellerae*	+	-0,16	*Licmophora* sp.	●	-0,30
D.caudata	△	0,13	*G.ericsonii*	+	-0,18	*Pseudo-nitzschia* spp.	●	-0,35
Ceratium spp.	△	0,13	*Gymnodinium* spp.	△	-0,20	*Ceratium spp.*	△	-0,41
Chaetoceros spp.	●	0,04	*Calcidiscus* spp.	+	-0,21	*C.furca*	△	-0,42
Odontela spp.	●	0,01	*P.sulcata*	●	-0,25	*Gymnodinium* spp.	△	-0,45
D.pumila	●	-0,01	*C.fusus*	△	-0,26	*Gyrodinium* spp.	△	-0,51
T.nitzschioides	●	-0,03	*C.furca*	△	-0,34	*Rhizosolenia* spp.	●	-0,61
L.danicus	●	-0,09	*D. acuminata*	△	-0,57	*C.fusus*	△	-0,63
Guinardia spp.	●	-0,09	*Ceratium* spp.	△	-0,58	*Guinardia* spp.	●	-0,64

Table 5 – Scores of phytoplankton species defined by the projection of PC1, PC2 and PC3. Diatoms (•), Coccolithophores (+) and dinoflagellates (empty ▲).

4. DISCUSSION

On Lisbon bay the annual phytoplankton succession was characterized by a seasonal cycle typical of a temperate area and by short succession cycles influenced by the intensity and persistence of upwelling and downwelling events, changes in temperature and salinity and nutrient availability. The persistence of upwelling, a trend also observed by Santos et al. (2005) for the Iberian margin and McGregor et al. (2007) for the NW Africa, can be one of the keys to explain the increased in biomass (Chl *a*) from 2001 onwards. From a general point view, the distribution patterns and species succession of diatoms, coccolithophores and dinoflagellates occurred along a gradient of decaying turbulence and nutrient availability as previously described by several authors (Margalef, 1978a; Margalef, 1978b and Estrada and Blasco, 1985 for the NW Africa; Hutchings et al., 1995; Venrick, 1998 for the California current; Lassiter et al., 2006 for the north California).

During spring, diatoms were the most abundant group, most favoured by the progressively increase of weak upwelling conditions, exploiting periods of higher nutrient availability. The high concentrations of silicates, during this season, especially after rainy periods, coincided with the first major peaks recurrently observed through February months, the transition from winter to spring conditions. Sometimes diatoms blooms caused silicates reduction until limitation values, influencing the subsequent low cell numbers. This group dominated the phytoplankton assemblage, contributing almost exclusively to Chl *a*. Changes in species composition were related to turbulence. Short upwelling pulses as observed from 2003 onwards appeared to be unfavourable for diatoms maintenance and consequent lower concentrations over the years. This group distributed in the positive axis of PC2 reflecting its preference for turbulent waters in a wide range of temperatures as evidenced by PC3 (Figure 7).

The coccolithophore *Emiliania huxleyi* also developed during spring, reaching concentrations closer to diatoms but was apparently favoured by the high concentrations in nitrites+nitrates. Numerous field studies have demonstrated that *E. huxleyi* exhibits a competitive advantage over other phytoplankton in areas of high nitrate and/or low inorganic phosphate (Balch et al., 1991; Fernández et al., 1993; Iglesias-Rodriguez et al., 2002; Tyrrel and Merico, 2004; Mohan et al., 2008). The species is known to synthesise the enzyme alkaline phosphatase which is used to assimilate dissolved organic phosphates and therefore imparts an advantage when

inorganic phosphate is limiting (Kuenzler and Perras, 1965). The presented data do not suggest a relation between silicates and the development of this species, however being a species with r-selected "characteristics" it is capable to dominate in eutrophic environments such as upwelling regions, river mouths, spring blooms and oceanographic fronts as long as there is sufficient dissolved silicates (Tyrrel and Merico, 2004). *E. huxleyi* is considered a coastal taxon with an opportunistic behaviour and can be used as a proxy for highly productive environments generated by upwelling (Bárcena et al., 2004, Rogalla and Andruleit, 2005, Silva et al., 2008). The specie most contributed to coccolithophores maxima during spring and with diatoms, explained the high Chl *a* values during this season.

During summer it was observed a development of the dinoflagellate assemblage yet the group was the less abundant throughout the four years probably influenced by shorter water stratified periods and an intensification of upwelling conditions. We can also hypothesize a competitive disadvantage in relation to coccolithophores. The favourable hydrographic conditions for dinoflagellates development are exemplified in Figure 5b. The group was not the dominant but a high number of species was present. However, dinoflagellates maximum concentration was observed during July 2004 under mild upwelling but only *S.* cf. *trochoidea* was able to dominate (Figure 5c, Table 5). The PC analysis (Figure 7) join dinoflagellate species in the third quadrant reflecting their preference by both stratified waters (negative values of PC2) and high temperatures (negative values of PC3).

At the end of summer, there was also a significant development of a phytoplankton assemblage with less than 20 μm that can be related to an increase in small flagellates (unpublished results) associated with maximum stratification like observed in Biscay Gulf by Estrada (1982).

As observed for the NW Iberia by Figueiras et al. (2002), autumn was regarded, as a short transition period, of about one month, from upwelling to downwelling seasons. During this period, coastal turbulence is usually reduced but nutrients provided by upwelling are still available. These conditions are characteristic of mature upwelled waters (Giraudeau et al., 1993; Kleijne, 1993; Ziveri et al., 1995) where coccolithophores are most favoured. The satellite image from October 2002 when maximum was observed (Figure 5b) reinforces the role of coccolithophores as tracers for the confluence of warmer offshore waters due to downwelling, as pointed out by Silva et al. (2008). The group was the second most abundant during the study however

the shorter and less intense convergence periods seemed associated with a decrease throughout the sampling. Coccolithophores distributed in the largest range of hydrographical conditions overlaying diatoms during early spring and dinoflagellates during summer. For this reason and as emphasized by the PC analysis, coccolithophores were placed between the other two groups (Figure 7). In accordance with Margalef's model, Young (1994) additionally related the ecological distribution of coccolithophores to their morphology, and found the lowest diversity and abundances both in strongly eutrophic environments and in extreme oligotrophic conditions, while the highest were in intermediate conditions.

The winter season was usually characterized by onshore advection of oceanic waters, nutrient availability and lower SST. Increments in precipitation and in Tagus river flow (Silva et al., 2008) influenced the low salinity values and coincided with the main shifts in nutrient concentrations. It was also noticed a decrease of all phytoplankton groups. The lower salinities seemed responsible for the absence of diatoms, since silicates were highly available, while a decrease in nitrates can also explain the low numbers of coccolithophores and dinoflagellates. The winter season is also characterized by shorter light periods. Since light is essential for biological productivity, the high nutrient concentrations cannot be fully utilized by phytoplankton due to lower light availability. Dinoflagellates, in particular, evidenced a narrow tolerance to winter conditions while the coccolithophores, *Calcidiscus quadriperforatus* and *Calcidiscus leptoporus*, dominated the phytoplankton assemblage. This genus was associated with colder waters typical from the winter period as shown by its positive correlation with PC3 (Figure 7).

Besides the seasonal variation of phytoplankton it was possible to identify short succession cycles dependent from coastal upwelling events. Along a template of r versus K growth strategies, diatoms (r-selected) exploited well-mixed, turbulent, nutrient-rich conditions, while dinoflagellates (K-selected) dominated in stable, stratified waters with low nutrient regimes (Brand, 1994). Margalef (1978a) divided the phytoplankton succession associated with upwelling in three stages. In Lisbon bay under upwelling conditions the phytoplankton community was first dominated by small sized chain-forming and colonial diatoms within the genus *Chaetoceros* and *Thalassiosira* and by *A. glacialis,* and *L. danicus*. After the upwelling peak the second stage was characterized by larger cells like *Pseudo-nitzschia* spp., *D. pumila* and *C. pelagica* and third, during relaxation periods, by *Dinophysis*, *Peridinium* and *Ceratium* and other dinoflagellates. A small reduction in upwelling intensity especially during

summer allows the development *S.* cf. *trochoidea*, a neritic and estuarine species. This species was capable of a fast response when northlies weaken and was always present as the most abundant dinoflagellate in samples. *S.* cf. *trochoidea* can be considered a specie typical from a third stage of succession. The satellite image (Figure 5c) show the hydrographic conditions favouring the development of the mono-specific bloom of *S.* cf. *trochoidea* that is not representative of the whole dinoflagellates preferences. The hypothesis of a local development of *S.* cf. *trochoidea* can be pointed out once this specie was always present in the samples and dominated the dinoflagellate assemblage. Ribeiro and Amorim (2008) observed in sediments from Lisbon bay, a high percentage of cysts of *S.* cf. *trochoidea* and other species of the genus *Scripsiella*.

In Lisbon bay, the coccolithophore species changed according with the seasonal distribution of the group (Silva et al., 2008) The placolith-bearing species were shown to dominate assemblages in nutrient rich, turbulent environments such as upwelling areas, coastal and shelf seas, and constituted the main bloom-forming coccolithophores. These were recognised as early succession r-selected species like *E. huxleyi* (Okada and Honjo, 1973; Honjo and Okada, 1974; Honjo, 1977) and *Gephyrocapsa* spp. (Silva et al., 2008). The remaining coccolithophores included species which rarely dominate assemblages and showing a tendency towards weak K selection.

The interannual variability of phytoplankton was influenced both by the intensity and persistent of upwelling events and precipitation, responsible for the decreased in salinity. Persistent upwelling conditions, allowed the development of mid stage species, as *D. pumila* and *C. pelagica*. These larger diatoms had a superior Chl *a* content which result in elevated chlorophyll values, not always associated with higher cell counts, as in 2004. The opposite situation was recorded in 2002, when higher cell counts and lower Chl *a* concentrations were observed and were associated with small chain diatoms and coccolithophores.

In 2004 the minor development of coccolithophores coincided with the lowest concentrations of nitrites and nitrates, during summer-early autumn, when this group usually reached maxima, as well as with an overall decrease in phosphates during this year in particular. Low silicates and N:P ratios between 6-11 were pointed out by Tyrell and Merico (2004) as favourable conditions for coccolithophore development; however, the present data shows 2004 as the year when silicates were lower but N:P ratios were

>11. Moreover, nitrate plays an important role in calcification (Baumann et al., 2005) and thus essential for coccolithophores growth, that certainly could be influenced by low concentrations of this nutrient. The coccolithophore *S. pulchra* in particular, appeared for the first time during this year, in June 2004 onwards, dominating the coccolithophore community, under mild upwelling conditions. The species was associated with low nitrates, a significant decreased in phosphates (N:P > 11) and limited silicate (Si:N < 1). An interesting finding was the presence of offshore waters warmer than in July 2002.

Also, *D. acuminata*, a DSP (Diarrhetic Shellfish Poisoning) producer and one of the first dinoflagellates to attend each year during late spring – early summer, usually in low concentrations, reached a maximum of 4 x 10^3 cells.l^{-1} in 2003. According to Palma et al. (1997), *D. acuminata* is associated with colder and less salty waters with maxima observed further north in the Portuguese coast. These preferences could explain the detachment of this *Dinophysis* from the summer dinoflagellate assemblage (Figure 7), negative correlated with semi-axis of PC2 (stratification) but slightly positively correlated with PC3 (cold waters).

It is also important to highlight that phytoplankton community and its seasonal variability was not only dependent from physical-chemical process, but also represent the interaction of species specific net growth rates, combining variability of specific growth and loss rates (Domingues et al., 2005). For that reason, losses such as grazing, viral lysis and autolysis, could also explain part of phytoplankton succession and should be evaluated in future studies.

5. CONCLUSIONS

This comprehensive sampling strengthened the seasonal pattern of the major phytoplankton groups, detailing physical-chemical preferences and showing how phytoplankton communities succeed in response to environmental changes. The seasonal and interannual variation of the phytoplankton community reflected the influenced of persistent upwelling conditions, during spring and summer, of prevailing onshore advection conditions during autumn and of precipitation/runoff during winter (Figure 8). The presence of upwelling species (e.g. *Pseudo*-nitzschia spp., *D. pumila*) or oceanic coccolithophores revealed the influence of waters with different

hydrographic features. Coccolithophores appeared as the most tolerant group, with species thrivering in a remarkable variety of oceanographic conditions between diatoms upwelling favourable and dinoflagellates thermal stratified affinities. Distinctive nutrient proportions coincided with the development of this group however we are inclined to suggest a greater influence of nitrates changes.

Finally, particular attention should be given when interpreting Chl *a* values. Similar concentrations were found to be related with differences in floral composition and cell counts, indicating the importance of microscope identifications in the interpretation of peaks. The Chl *a* associated with the phytoplankton fraction < 20μm present a great contribution for total Chl *a* particularly, during summer. Our results also showed that the mono- or multi-specific composition of a group peak is dependent of different environmental conditions.

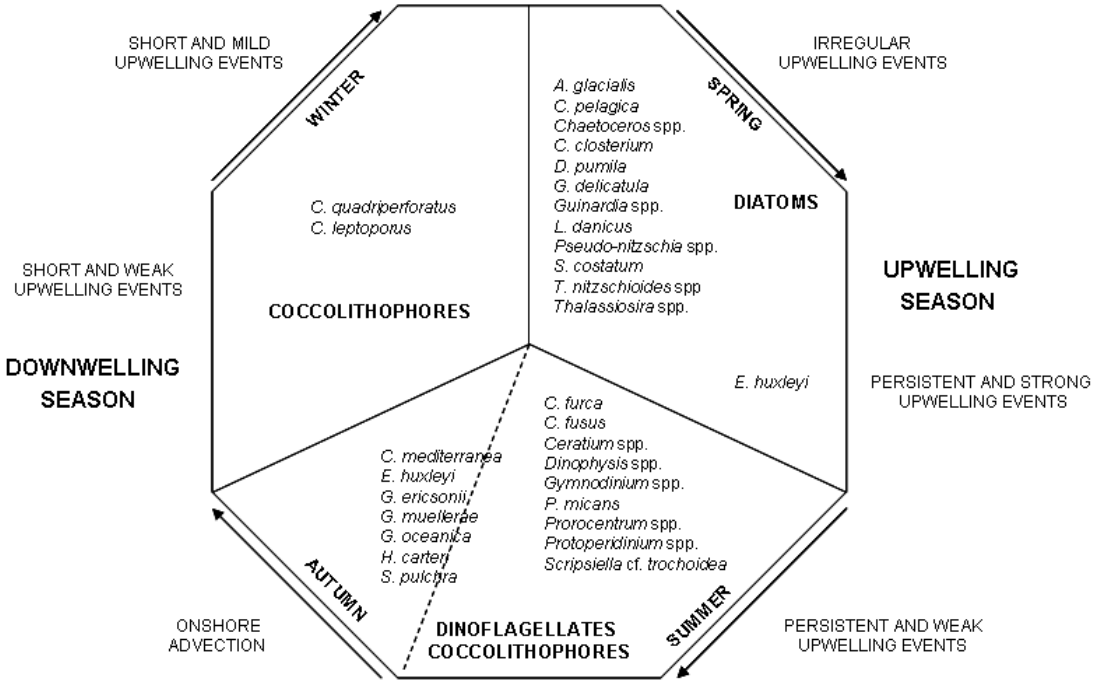

Figure 8 – Schematic representation of the seasonal distribution of the major phytoplankton groups and dominant species in relation to upwelling and downwelling seasons.

Appendix 1 - List of phytoplankton species observed during the sampling separated in three major groups, diatoms, dinoflagellates and coccolithophores.

DIATOMS
Acinoptychus senarius
Acnanthes spp.
Amphiprora spp.
Amphora spp.
Asterionellopsis glacialis
Asteromphalus flabellatus
Asteromphalus sarcophagus
Asteromphalus spp.
Auricula spp.
Bacillaria paxillifera
Bacteriastrum delicatulum
Bacteriastrum furcatum
Bacteriastrum hyalinum
Bacteriastrum spp.
Biddulphia alternans
Biddulphia pulchella
Biddulphia spp.
Centric diatoms
Cerasterias cetauroides
Cerataulina pelagica
Chaetoceros curvisetus
Chaetoceros danicus
Chaetoceros decipiens
Chaetoceros lorenzianus
Chaetoceros pseudocur/curv.
Chaetoceros rostratus
Chaetoceros socialis
Chaetoceros spp.
Cocconeis spp.
Corethron criophilum
Coscinodiscus marginatus
Coscinodiscus radiatus
Coscinodiscus spp.
Cylindrotheca closterium
Dactyliosolen fragilissimus
Dactyliosolen phuketensis
Detonula pumila
Diploneis bombus
Diploneis sp.
Ditylum brightwellii
Eucampia cornuta
Eucampia longicornis
Eucampia spp.
Eucampia zoodiacus
Grammatophora marina
Grammatophora spp.
Guinardia cf. delicatula
Guinardia cf. striata
Guinardia flaccida
Guinardia spp.
Hemiaulus membranaceus
Hemiaulus sinensis
Hemiaulus spp.
Lauderia annulata
Leptocylindrus danicus
Leptocylindrus mediterraneus
Leptocylindrus minimus
Leptocylindrus spp.
Licmophora sp.
Lithodesmio undulatum
Melosira distans
Melosira granulata
Melosira spp.
Meuniera membranacea
Navicula complanata
Navicula spp.
Nitzschia longissima
Odontela mobiliensis
Odontela spp.
Odontella longicornis
Paralia sulcata
Pennate diatoms
Pleurosigma spp.
Podosira spp.
Podosira stelliger
Proboscia alata
Pseudo-nitzschia spp.
Rhabdonema adriaticum
Rhizosolenia hebetata
Rhizosolenia imbricata
Rhizosolenia setigera
Rhizosolenia spp.
Rhizosolenia styliformis
Rhoicosigma spp.
Skeletonema costatum
Skeletonema sp.
Stephanopyxis palmeriana
Stephanopyxis spp.
Stephanopyxis turris
Streptotheca thamensis
Striatella unipunctata
Surirella spp.
Synedra spp.
Thalassionema bacilare
Thalassionema fraunfeldii
Thalassionema nitzschioides
Thalassionema spp.
Thalassiosira anguste-lineata
Thalassiosira eccentrica
Thalassiosira rotula
Thalassiosira spp.
Thalassiosira subtilis
Thalassiotrix spp.
Trachyneis aspera

DINOFLAGELLATES
Alexandrium spp.
Amphidoma caudatum
Ceratium candelabrum
Ceratium furca
Ceratium fusus
Ceratium gibberum
Ceratium horridum
Ceratium kofoidii
Ceratium lineatum
Ceratium macroceros
Ceratium massiliense
Ceratium minutum
Ceratium spp.
Ceratium symetricum
Ceratium teres
Ceratium tripos
Corythodinium spp.
Dinoflagelados
Dinophysis acuta
Dinophysis caudata
Dinophysis cf. acuminata
Dinophysis dens
Dinophysis diegensis
Dinophysis fortii
Dinophysis rotundata
Dinophysis skagii
Dinophysis spp.
Diplopsalis
Dissodinium asymmetricum
Erytropsodinium spp.
Gonyaulax diacantha
Gonyaulax diegensis
Gonyaulax spp.
Gymnodinium catenatum
Gymnodinium impudicum
Gymnodinium spp.
Gyrodinium fusiforme
Gyrodinium spp.
Histioneis spp.
Lingulodinium polyedricum
Mesoporus perforatus
Micracanthadinium spp.
Noctiluca sintilans
Oxyrris spp.
Oxytoxum spp.
Phalacroma rotundata
Podolampas palmipes
Preperidinium spp.
Pronoctiluca spinifera
Prorocentrum compressum
Prorocentrum gracile
Prorocentrum lima
Prorocentrum micans
Prorocentrum minimum
Prorocentrum scuttelum
Prorocentrum spp.
Prorocentrum triestinum
Protoceratium sp.
Protoceratium spinulosum
Protoperidinium aciculiferum
Protoperidinium bipes
Protoperidinium breve
Protoperidinium conicum
Protoperidinium crassipes
Protoperidinium depressum
Protoperidinium diabolum
Protoperidinium divergens
Protoperidinium globolum
Protoperidinium leonis
Protoperidinium murraay
Protoperidinium oceanicum
Protoperidinium pellucidum
Protoperidinium pentagonum
Protoperidinium quinquecorne
Protoperidinium spp.
Protoperidinium steinii
Pyrocystis elegans
Pyrocystis lunula
Pyrocystis spp.
Scripsiella cf. trochoidea
Thoracosphaera heimii
Torodinium robustum
Triadinium polyedricum

COCCOLITHOPHORES
Calcidiscus leptoporus
Calcidiscus quadriperforatus
Calyptrolithophora gracillima
Calyptrolithophora papilifera
Calyptrosphaera oblonga
Corisphaera sp.
Coronosphaera mediterranea
Crystallolithus hyalinus
Crystallolithus rigidus
Emiliania huxleyi
Gephyrocapsa ericsonii
Gephyrocapsa muellerae
Gephyrocapsa oceanica
Helicosphaera carteri
Syracolithus confusus
Syracosphaera lamina
Syracosphaera pulchra
Syracosphaera spp.
Zygosphaera marsilli

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References

Baumann, K-H., Andruleit, H., Bockel, B., Geisen, M. and Kinkel, H., 2005. The significance of extant coccolithophores as indicators of ocean water masses, surface water temperature, and palaeoproductivity: a review. Palaeontologische Zeitschrift, 79 (1): 93-112.

Bakun, A., 1973. Coastal upwelling indices, West Coast of North America 1946-71. NOAA Technical Report NMFs SSFR-671. U.S. Dept. of Commerce.

Balch, W.M., Holligan, P.M., Ackleson, S.G. and Voss, K.J., 1991. Biological and optical properties of mesoscale coccolithophore blooms in the Gulf of Maine. Liminology and Oceanography, 36 (4): 629-643.

Bárcena, M.A., Flores, J.A., Sierro, F.J., Pérez-Folgado, M., Fabres, J., Calafat, A., Canals, M., 2004. Planktonic response to main oceanographic changes in the Alboran Sea (Western Mediterranean) as documented in sediment traps and surface sediments. Marine Micropaleontology, 53: 423-445.

Brand, L.E., 1994. Physiological ecology of marine coccolithophores. In: Winter, A., Siesser, W.G. (Eds.) Coccolithophores. Cambridge University Press, pp. 39-49.

Brandini, F.P., Boltovskoy, D., Piola, A., Kocmur, S., Röttgers, R., Abreu, P.C., Lopes, R.M., 2000. Multiannual trends in fronts and distribution of nutrients and chlorophyll in the southwestern Atlantic (30–62ºS). Deep-Sea Research Part I, 47**:** 1015–1033.

Ciotti, A.M., Odebrecht, C., Fillmann, G., Möller Jr., O.O., 1995. Freshwater outflow and subtropical convergence influence on phytoplankton biomass on the southern Brazilian continental shelf. Continental Shelf Research, 15 (14): 1737–1756.

CMS (Centre de Météorologie Spatiale), 2005. North Atlantic regional sea surface temperature product manual. Météo-France Satellite Meteorology Center, Lannion, Brittany, France. SAF/OSI/MF/TEC/MA/ 124. http://www.osi saf.org/biblio/docs/ss1_pmnarsst_1_7.pdf.

Dodge, J.D., 1982. Marine Dinoflagellates of the British Isles. Her Majesty's Stationary Office, London.

Domingues, R.B., Barbosa, A, Galvão, H., 2005. Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia) Estuarine Coastal and Shelf Science, 64 (2-3): 249-260.

Dortch, Q., Whitledge, T.E., 1992. Does nitrogen or silicon limit phytoplankton production in the Mississipi River filament and nearby regions? Continental Shelf Research, 12: 1293-1309.

Estrada, M., 1982. Ciclo annual del fitoplâncton en la zona costera frente a Punta Entada (Golfo de Viscaya). Investigacion Pesquera, 46 (3): 469.491.

Estrada, M., Blasco, D., 1985. Phytoplankton assemblages in coastal upwelling areas. In: Bas, C., Margalef, R., Rubias, P. (eds.) International Symposium of Upwelling of W Africa, Instituto de Investigaciones Pesqueras, Barcelona, 1, pp. 379–402.

Fatela, F., Taborda, R., 2002. Confidence limits of species proportions in microfossil assemblages. Marine Micropaleontology 45: 169-174.

Fernández, E., Boyd, P., Holligan, P.M., Harbour, D.S., 1993. Production of organic and inorganic carbon within a large scale coccolithophore bloom in the northeast Atlantic Ocean Marine Ecology Progress Series, 97: 271-285.

Figueiras, F.G., Labarta, U., Fernández Reiriz, M.J., 2002. Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. Hydrobiologia, 484 (1-3): 121-131.

Fiúza, A.F.G., Macedo, M.E., Guerreiro, M.R., 1982. Climatological space and time variation of the Portuguese coastal upwelling. Oceanologica Acta, 5 (1): 31-40.

Giraudeau, J., Monteiro, P.M.S., Nikodemus, K., 1993. Distribution and malformation of living coccolithophores in the northern Benguela system off Namíbia. Marine Micropaleontology, 22: 93-110.

Giraudeau, J., Rogers, J., 1994. Phytoplankton biomass and sea-surface temperature estimates from sea-bed distribution of Nanofossils and Planktonic Foraminifera in the Buenguela upwelling system. Micropaleontology, 40 (3): 275- 285.

Graham, W.M., Largier, J. L., 1997. Upwelling shadows as nearshore retention sites: the example of northern Monterey Bay. Continental Shelf Research, 17: 509-532

Grasshoff, K., 1983. Methods of seawater analysis. In: K. Grasshoff, M. Ehrhardt, K. Kremling (Eds.), Verlag Chemie, New York, 419 pp.

Hasle, G.R., 1978. Phytoplankton manual: the inverted microscope method. In: Sournia, A. (Ed.), Monographs on oceanic methodology. Unesco, Paris, pp. 88-96.

Hasle, G.R., Syvertsen, E.E., 1996. Marine diatoms. In: Tomas, C.R. (Ed.), Identifying Marine Diatoms and Dinoflagellates. Academic Press, Inc., London, pp. 5–385.

Haynes, R, Barton, E.D., 1990. A poleward flow along the Atlantic coast of the Iberian Peninsula. Journal of Geophysics Research, 95: 11425-11441.

Honjo, S. 1977. Biogeography and provincialism of living Coccolithophorids in the Pacific Ocean. In: Ramsey, A.T.S. (Ed), Oceanic Micropaleontology. Academic Press, London, pp. 951-972.

Honjo, S. and Okada, H. 1974. Community structure of coccolithophores in the photic layer of the Mid-pacific. Micropaleontology, 29 (2): 209-230.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W., Strickland, J.D.H., 1965. Fluorimetric determination of Chlorophyll. Journal du Conseil Permanent International pour l'Exploration de la Mer, 30 (1) : 3-15.

Hutchings, L., Pitcher, G.C., Probyn, T.A., Bailey, G.W., 1995. The chemical and biological consequences of coastal upwelling. In: Summerhayes, C.P., Emeis, K.-C., Angel, M.V., Smith, R.L., Zeitschel, B. (Eds.) Upwelling in the Oceans; Modern Processes and Ancient Records, Wiley, New York, pp. 65–81.

Iglesias-Rodriguez, M.D., Brown, C.W., Doney, S.C., Kleypas, J.A., Kolber, D., Kolber, Z., Hayes, P.K., Falkowski, P.G., 2002. Representing key phytoplankton functional groups in ocean carbon cycle models. Global Biogeochemical Cycles, 16 (4): 1100.

Kiørboe, T., 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Advanced Marine Biology, 29: 1–73.

Kleijne, A., 1993. Morphology, Taxonomy and Distribution of extant coccolithophorids (calcareous nannoplankton). Ph.D Thesis, Vrije Universiteit Amsterdam.

Kuenzler, E.J., Perras, J.P., 1965. Phosphatase of marine algae. Biological Bulletin, 128: 271-284.

Lassiter, A.M., Wilkerson, F.P, Dugdale, R.C., Hogue, V.E., 2006. Phytoplankton assemblages in the CoOP-WEST coastal upwelling area. Deep Sea Research II, 53: 3063-3077.

Laubscher, R.K., Perissinoto, R., McQuaid, C.D., 1993. Phytoplankton production and biomass at frontal zones in the Atlantic sector of the Southern Ocean, Polar Biology, 13 (7): 471–481.

Margalef, R., 1978a. Life-forms of phytoplankton as survival alternatives in an unstable environment, Oceanologia Acta, 1 (4): 493–509.

Margalef, R., 1978b. Phytoplankton communities in upwelling areas. The example of NW Africa. Oecologica Aquatica, 3: 97-132.

McGregor, H.V., Dima, M., Fisher, H.W., Mulitza, S., 2007. Rapid 20th-Centuary Increased in Coastal Upwelling off Northwest Africa. Science, 315: 637-639.

Mohan, R., Mergulhão, L.P., Guptha, M.V.S., Rajakumar, A.; Thamban, M., AnilKumar, N.;, Sudhakar, M. Ravindra, R., 2008. Ecology of coccolithophores in the Indian sector of the Southern Ocean. Marine Micropaleontology, 67: 30–45.

Moita, M.T., 2001. Structure, variability and dynamics of phytoplankton from the Portuguese continental coast. Ph.D. Thesis. University of Lisbon, Lisbon, unpublished.

Moita, M.T., Oliveira, P.B., Mendes, J.C., Palma, A.S., 2003. Distribution of chlorophyll a and Gymnodinium catenatum associated with coastal upwelling plumes off central Portugal. Acta Oecologica, 24: S125-S132.

Okada, H. and Honjo, S. 1973. The distribution of oceanic coccolithophorids in the Pacific. Deep Sea Research, 20: 355-374.

Oliveira, P., Nolasco, R., Dubert, J., Moita, T., Peliz, A., 2008. Surface temperature, chlorophyll and advection patterns during a summer upwelling event off central Portugal. Continental Shelf Research,. doi:10.1016/j.csr.2008.08.004.

Palma, A.S., Vilarinho, M.G, Moita, M.T., 1997. Interannual trends in the longshore variation of Dinophysis off Portuguese coast. Harmfull Algae. B. Reguera, B., J. Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Xunta de Galicia and Intergovernmental Oceanographic Comissiono f Unesco, Santiago de Compostela, Spain, pp. 124-127

Peliz, A., Dubert, J., Santos, A.M.P., Oliveira, P.B., Le Cann, B., 2005. Winter upper ocean circulation in the Western Iberian Basin - Fronts, Eddies and Poleward Flows: an overview. Deep Sea Research I, 52: 621-646.

Ribeiro, S., Amorim, A., 2008. Environmental drivers of temporal succession in recent dinoflagellate cyst assemblages from a coastal site in the North-East Atlantic (Lisbon Bay, Portugal). Marine Micropaleontology doi:10.1016/j.marmicro.2008.01.013

Rogalla, U., Andruleit, H., 2005. Processional forcing of coccolithophore assemblages in the northern Arabian Sea: Implications for monsoonal dynamics during the last 200,000 years. Marine Geology 217, 31-48.

Santos, A.M.P, Kazmin, A.S., Peliz, A., 2005. Decadal changes in the Canary upwelling system as revealed by satellite observations: Their impact on productivity. Journal of Marine Research 63 (2), 359-379.

Silva, A., Palma, S., Moita, M.T., 2008. Coccolithophores in the upwelling waters of Portugal: four years of weekly distribution in Lisbon Bay. Continental Shelf Research, 28: 2601-2613.

Throndsen, J., 1978. Phytoplankton manual: preservation and storage. In: Sournia, A. (Ed.), Monographs on oceanic methodology. Unesco, Paris, pp. 69-75.

Tyrrel, T. and Merico, A., 2004. *Emiliania huxleyi*: bloom observations and the conditions that induce them. In: Thierstein, H.R., Young, J.R. (Eds.), Coccolithophores, from molecular processes to global impact. Springer-Verlag Heidelberg, Germany, pp. 75-98.

Venrick, E.L., 1998. Spring in the California Current: the distribution of phytoplankton species, April 1993 and April 1998. Marine Ecology Progress Series, 167: 73-88.

Young, J., 1994. Function of coccoliths. In: Winter, A., Siesser, W.G. (Eds.), Coccolithophores. Cambridge University Press, Cambridge, pp. 63–82.

Young, J., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I., Ostergaard, J., 2003. A guide to extant coccolithophore taxonomy. Journal Nannoplankton Research, Special Issue, 1: 123.

Ziveri, P., Thunell, R., Rio, D., 1995. Export production of coccolithophores in an upwelling region: Results from San Pedro Basin, Southern California Borderlands. Marine Micropaleontology, 24: 335-358.

Ziveri, P., Thunell, R.C., 2000. Coccolithophore export production in Guaymas Basin, Gulf of California: response to climate forcing. Deep-Sea Research II, 47: 2073-2100.

CHAPTER 5: SHORT-TIME SCALE VARIATION OF PHYTOPLANKTON SUCCESSION IN LISBON BAY (PORTUGAL) AS REVEALED BY MICROSCOPY CELL COUNTS AND HPLC PIGMENT ANALYSIS

CHAPTER 5: SHORT-TIME SCALE VARIATION OF PHYTOPLANKTON SUCCESSION IN LISBON BAY (PORTUGAL) AS REVEALED BY MICROSCOPY CELL COUNTS AND HPLC PIGMENT ANALYSIS

Silva, A [a,*], Mendes, C. R.[b], Palma S [a]., Brotas, V. [b]

[a] Instituto Nacional de Recursos Biológicos (INRB-IPIMAR), Av. Brasília s/n,1449-006 Lisboa, Portugal. Tel: +351213027121, Fax: +351213015948

[b] Instituto de Oceanografia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

* Corresponding author: amsilva@ipimar.pt

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ABSTRACT

The phytoplankton distribution and composition in Lisbon bay was studied, at a short time scale based on a weekly sampling, during one year (April 2004 – May 2005), using microscopic examination and pigment analysis with high-performance liquid chromatography (HPLC). This work is a contribution to the knowledge on species succession and ecology of coastal communities. The frequency of the sampling permitted monitoring peak blooming and decaying, a process which frequently occurred within 1 –2 weeks.

Cell counts determined that the classes Dinophyceae, Bacillariophyceae and Prymnesiophyceae dominated the assemblages. Maxima abundances and diversity of phytoplankton were observed from spring to autumn. HPLC analysis reflected the major seasonal variations observed by the cell counts and in addition detected the presence of four small sized phytoplankton classes that were not identified by microscopy. Phytoplankton counts were essential to identify the main contributing species to total chlorophyll a. Fucoxantin, peridinin and 190-hexanoyloxyfucoxanthin appeared as good indicators for diatoms, dinoflagellates and coccolithophores, respectively, with synchronized seasonal variations and significant positive correlations.

Keywords: Coastal phytoplankton succession; weekly sampling; HPLC; biomarker pigments.

1. INTRODUCTION

Phytoplankton studies are crucial in studies of marine ecosystems as they play an important role in the structure and efficiency of the food web and thus contribute for the understanding of the organization and dynamics of these ecosystems. In classical studies, phytoplankton composition and abundance (cells l^{-1}) are determined from fixed samples observed under microscopy (Hasle, 1978). This technique allows a characterization to species level of the phytoplankton community. However, many species are difficult to identify and quantify by microscopy, because, in addition to their reduced size, are often fragile and not readily survive the various routine fixative and counting procedures used to enumerate cell abundances (Mackey et al., 1998; Havskum et al., 2004). An alternative method of characterizing phytoplankton relies on high performance liquid chromatography (HPLC) pigment analysis, which can provide complementary data to the direct cell counts. HPLC is used for estimating the quantitative contribution of phytoplankton groups to chlorophyll *a* (Chl *a*) using photosynthetic marker pigments (Gieskes and Kraay, 1983; Schlüter and Havskum, 1997; Ediger et al., 2006). Examples of carotenoid biomarkers for single algal class are alloxanthin for cryptophytes, prasinoxanthin for prasinophytes, peridinin for dinoflagellates and 19'-hexanoyloxyfucoxanthin for prymnesiophyceans. Less specific biomarkers are fucoxanthin for diatoms (also present in chrysophytes and prymnesiophyceans) and zeaxanthin for cyanobacteria (also present in green algae) (Jeffrey and Vesk, 1997).

As many algal classes share pigments, a reliable interpretation of the data derived from pigment analysis should be supported by cell counts (Mackey et al., 1996; Jeffrey et al., 1999; Irigoien et al., 2004). The sole use of pigment signatures without a concurrent microscopic verification can sometimes be misleading (Millie et al., 1993). Thus a combination of both approaches has been recommended (Hallegraeff, 1981; Jeffrey and Hallegraeff, 1987), despite the tendency to rely mostly on pigment chemotaxonomy using HPLC analysis mainly because of shorter analysis time (Barlow et al., 1993; Peeken, 1997).

In the present study, the seasonal variability of the phytoplankton community in Lisbon bay will be described based on a weekly sampling. The major phytoplankton groups will be compared using the chemotaxonomic approach based on HPLC pigment analysis and cell counting by inverted microscopy. Cell counts are expected to

corroborate the pigments identifications and variability and thus validate the use of marker pigments as indicators of the major phytoplankton groups. We intent to reinforce the utility and reliability of the HPLC as a monitoring tool for evaluating rapid and large scale changes in phytoplankton community.

2. MATERIALS AND METHODS

2.1. Study site

Phytoplankton composition and abundance was weekly studied at a fixed station in Cascais (located at 38º 41' N and 09º 24' W) (Figure 1) during one year (April 2004 – May 2005). Surface seawater samples were collected with a Nansen bottle one hour before high tide, to avoid the direct influence of estuarine waters on the area.

The water for nutrient determination was filtered through a Millipore filter of 0.45 μm and stored at -4ºC for subsequent analysis. Ammonia (NH^4_{4+}), nitrites and nitrates (NO^2_{2-} + NO^3_{3-}), phosphates (PO^4_{3-}) and silicates ($Si(OH)_4$) were determined using an autoanalyser ''SKALAR'' according to the methods of Technicon Industrial Systems (Grasshoff, 1983). The detection limit is 0.2 μM for ammonia and silicates and 0.05 μM for nitrites + nitrates and phosphates.

Temperature and salinity were determined in situ with a Quanta CTD. Data from Tagus flow were obtained from the ''Water National Institute'' in a public database (http://www.inag.pt) and a weekly average was calculated before each sampling date.

2.2. HPLC pigment analysis

Surface seawater samples (5 l) were filtered onto a Whatman GF/F filter (0.7 μm nominal pore size and 47 mm diameter), under vacuum pressure lower than 500 mbA. The filters were kept frozen at -80ºC before extraction. Photosynthetic pigments were extracted with 3 ml of 95% cold-buffered methanol (2% ammonium acetate) for 30 min at -20ºC, in the dark. Samples were sonicated for 30s in the beginning of the extraction period. The samples were centrifuged at 3000 rpm for 15 min, at 4ºC. Extracts were

filtered (Millipore membrane filters, 0.2 μm nominal pore size) immediately before injection in the HPLC to remove cell and filter debris. Each sample was diluted in 10% water (HPLC-grade), to prevent distortion of early eluting peaks (Zapata and Garrido, 1991). Pigment extracts were analyzed using a Shimadzu HPLC comprised of a solvent delivery module (LC-10ADVP) with system controller (SCL-10AVP) and a photodiode array (SPD-M10ADVP). The chromatographic separation of pigments was achieved using a C_8 column for reverse phase chromatography (Symmetry; 15 cm long; 4.6 mm diameter; 3.5 μm particles). The mobile phase used was: A = methanol:acetonitrile:aqueous pyridine solution (0.25 M pyridine, pH adjusted to 5.0 with acetic acid) in the proportions 50:25:25 (v/v/v), and B = acetonitrile:acetone (80:20 v/v). The solvent gradient followed Zapata et al. (2000) with a flow rate of 1 ml min^{-1}, an injection volume of 100 μl with duration of 40 min. Pigments were identified from absorbance spectra plus retention times and concentrations calculated from the signals in the photodiode array detector. Calibration of the HPLC peaks was performed using commercial standards, namely, chlorophyll *a* and chlorophyll *b* standards from Sigma, chlorophyll c_2, chlorophyll c_3, peridinin, fucoxanthin, diadinoxanthin, diatoxanthin, 19'-hexanoyloxyfucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, alloxanthin, 19'-butanoyloxyfucoxanthin and zeaxanthin standards from the DHI (Institute for Water and Environment, Denmark).

2.3. Phytoplankton microscopic identification

Phytoplankton samples were preserved with hexamethylenetetramine buffered formalin to a final concentration of 2% (Throndsen, 1978). Subsamples of 50 ml were allowed to settle for 36 h (Margalef, 1969 in Hasle, 1978). Cells were identified and counted by the Utermöhl technique using a Zeiss IM35 inverted microscope with phase contrast and bright field illumination (Hasle, 1978). A magnification of 160x and 400x was used to identify and enumerate the phytoplankton assemblage with a detection limit of 40 cells l^{-1} and 2000 cells l^{-1}, respectively. When possible, the cells were identified to species level according to Hasle and Syvertsen (1996) and Dodge (1982). Small-sized phytoplankton with morphological features difficult to recognize were placed into the category of ''others''. This group would likely include different algal classes: criptophyceae, chlorophyceae, prasinophyceae, cyanobacteria and other not identified small algae. Coccolithophores were separately identified following Young et

al. (2003) and counted, from an area of 2.2 mm^2 of a nitrate cellulose membrane (Whatman, 47 mm with a 0.45 μm nominal pore size) at a maximum of 300 cells (Fatela and Taborda, 2002) with a Zeiss optical microscope under cross-polarized light at a magnification of 1250x.

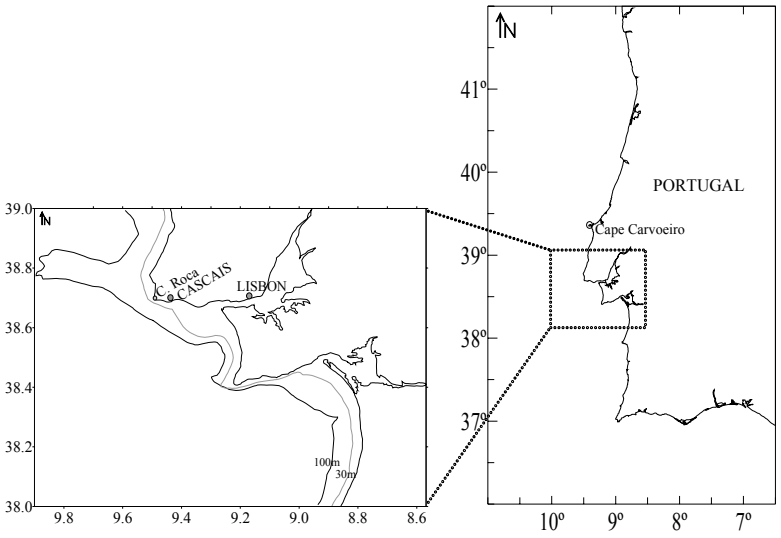

Figure 1 - Location of the sampling site 38º41'N and 09º24'W. - Cascais bay.

3. RESULTS

3.1. Hydrographic data

Sea surface temperature (Figure 2) was characterized by minima and maxima values of 12.2ºC and 20.5ºC recorded in February 2005 and August 2004. The lower values were observed from mid-December 2004 until Mars 2005 while during the rest of the year temperatures were always above 14ºC.

Surface salinity (Figure 2) was measured using the Practical Salinity Scale and remained constant (34.5 – 35.5) through the year, except during autumn 2004 when the lowest salinities (31.7) were observed, coincident with rainy periods. Tagus river flow (Figure 2) also showed a major increase during this period, reaching 263 $m^3\ s^{-1}$ in November 2004. The lower runoff values (21 $m^3\ s^{-1}$) were recorded during summer

2004 and from winter 2005 until the end of the sampling. Winter 2005 corresponded to a drought period, reflected in the low river flow values registered, well below average annual value of 400 $m^3 s^{-1}$.

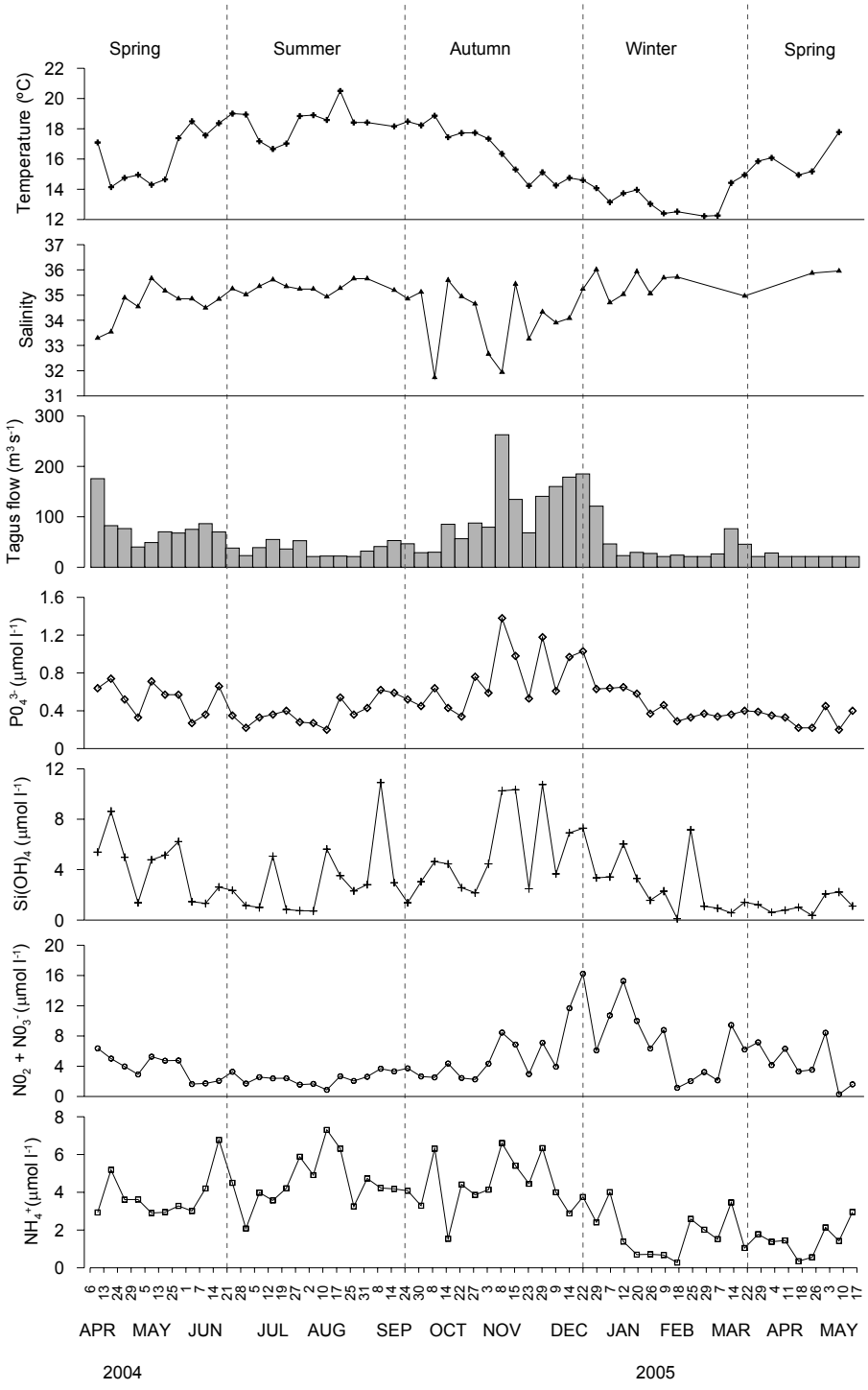

Figure 2 - Weekly distribution of sea surface temperature, surface salinity, Tagus flow, phosphates, silicates, nitrites þ nitrates and ammonia, during the sampling period (April 2004 –May 2005). The day of each sampling is represented on the abscissa axis.

Nutrient concentrations (Figure 2) changed along the year. The measured phosphate varied between 0.20 and 1.38 μmol l^{-1} mol l-1 (August 04 – November 04), silicates ranged between 0.11 and 10.91 μmol l^{-1} mol l^{-1} (February 05 – August 04), nitrate + nitrite between 0.29 and 16.23 μmol l^{-1} (May 05 – December 04) and ammonia values were between 0.28 and 7.30 μmol l^{-1} (February 05 – August 04). Phosphates and nitrite + nitrate had minimum values during spring and summer and maxima during autumn – winter. Positive significant correlations were found between Tagus runoff and phosphates (r^2 = 0.6, p < 0.001) as well as with silicates (r^2 = 0.3, p < 0.05). Ammonia values were generally lower during 2005, in accordance to reduced runoff.

Concerning nutrient stoichiometry, from the 57 sampling occasions, it was observed that half of N:P ratios were lower than 16 (during spring and autumn 2004) whilst 95% of the Si:N values were lower than 1 (Figure 3).

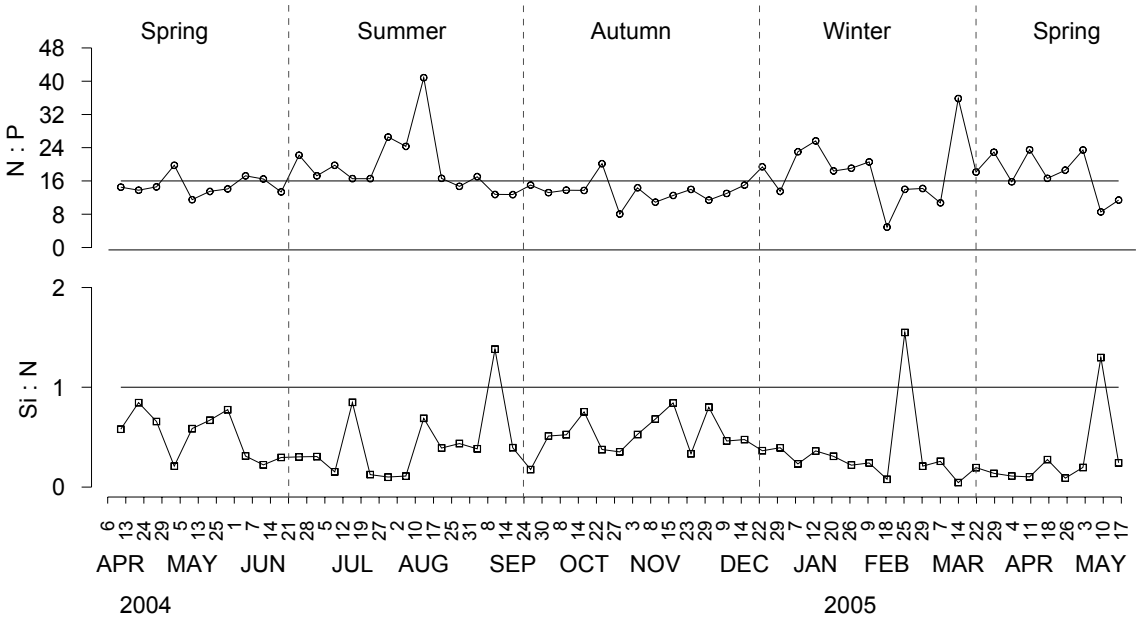

Figure 3 - N:P and Si:N ratios during the sampling period (April 2004 – May 2005). The day of each sampling is represented on the absciss axis.

3.2. Seasonal succession: HPLC pigment analysis versus species quantification

Chromatographic analysis revealed the presence of a wide range of pigments, exhibiting a clear temporal variability. Chlorophyll *a* and fucoxanthin (a proxy for

diatoms) were the two most abundant pigments, present in all samples. Relatively high concentrations of two other accessory pigments were also observed: peridinin and 19'-hexanoyloxyfucoxanthin, which are the major carotenoids of dinoflagellates and prymnesiophyceans, respectively. The only prymnesiophyceans identified by microscopy were the coccolithophores. In addition to these pigments, chlorophyll *b*, chlorophyll c_1 + c_2 and c_3, diadinoxanthin, diatoxanthin, violaxanthin, neoxanthin, zeaxanthin, prasinoxanthin, 19'-butanoyloxyfucoxanthin and alloxanthin concentrations also were quantified (Table 1).

Pigments	Concentration (µg l^{-1})	%	Occurrence
Chlorophyll *a*	0.260 (0.005 – 0.916)	49,1	A proxy of total algae biomass
Chlorophyll c_1, c_2	0.199 (0.000 – 2.546)	37,5	Diatoms, prymnesiophytes, crysophytes, dinoflagellates
Chlorophyll c_3	0.040 (0.000 – 0.233)	7,5	Crysophytes, prymnesiophytes
Chlorophyll *b*	0.031 (0.000 – 0.118)	5,8	Chlorophytes, euglenophytes, prasinophytes
Total chlorophylls	**0.530 (0.005 – 3.813)**	**100**	
Fucoxanthin	0.349 (0.021 – 3.142)	54,4	Diatoms, prymnesiophytes, crysophytes
Peridinin	0.121 (0.000 – 2.341)	18,8	Dinoflagellates
Diadinoxanthin	0.081 (0.000 – 0.995)	12,6	Diatoms, prymnesiophytes, crysophytes, dinoflagellates
19'-hexanoyloxyfucoxanthin	0.024 (0.000 – 0.113)	3,7	Prymnesiophytes
Alloxanthin	0.024 (0.000 – 0.171)	3,7	Cryptophytes
Violaxanthin	0.016 (0.000 – 0.496)	2,5	Chlorophytes, prasinophytes
Prasinoxanthin	0.008 (0.000 – 0.055)	1,2	Prasinophytes
Diatoxanthin	0.008 (0.000 – 0.094)	1,2	Diatoms, prymnesiophytes, crysophytes, dinoflagellates
Neoxanthin	0.005 (0.000 – 0.077)	0,8	Chlorophytes, prasinophytes
Zeaxanthin	0.004 (0.000 – 0.037)	0,6	Cyanobacteria, chlorophytes
19'-butanoyloxyfucoxanthin	0.002 (0.000 – 0.035)	0,3	Crysophytes, prymnesiophytes
Total carotenoids	**0.642 (0.021 – 7.556)**	**100**	

Table 1 - HPLC photopigments concentration registered (annual average and range) and their associated phytoplankton classes (Jeffrey et al. 1997; Gibb et al. 2001).

The abundance of phytoplankton classes contributing to total Chl *a* can be estimated from the concentrations of biomarker pigments using a Chemical Taxonomy software, known as Chemtax (Mackey et al., 1996). This chemotaxonomic approach was attempted but it did not provide any additional relevant information than the simple regression analysis between cell counts of a given class and its most characteristic pigment (Figure 4 and 5 represent the statistical correlations obtained), hence we chose to present the raw data concerning pigment concentrations, as we found to be more useful for other authors studying coastal systems. The index of phytoplankton biomass, Chl *a*, evidenced a good correlation with cell counts (r^2 = 0.37; p < 0.01; Figure 4). The seasonal variation of Chl *a* was coincident with the seasonality of total phytoplankton (Figure 6) with maxima occurring through all seasons and reflecting the

highest concentrations of the dinoflagellates, diatoms and coccolithophores. Additionally, the major Chl *a* peaks matched the peaks of diatoms. The highest Chl *a* value observed was 0.916 μg l^{-1} in October 2004.

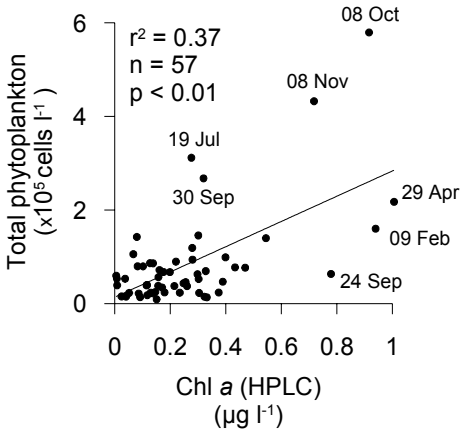

Figure 4 - Total phytoplankton measured by the Utermöhl technique in relation to chlorophyll *a* measured by HPLC.

The most abundant pigment detected, fucoxanthin, evidenced a very good correlation with diatoms cell counting (r^2 = 0.81; p < 0.01; Figure 5a). The seasonal variation of this carotenoid was coincident with the diatoms distribution along the year, with maximum values of 3.142 μg l^{-1} in October 2004 and 1.116 μg l^{-1} in February 2005 (Figure 6). Significant correlation (p < 0.01) was found between the concentration of peridinin and the density of dinoflagellates (r^2 = 0.54; Figure 5b) as well as between coccolithophores abundance and 19'-hexanoyloxyfucoxanthin (r^2 = 0.56; Figure 5c). The annual variation of these two carotenoids accompanied dinoflagellates and coccolithophores seasonality, respectively (Figure 6). The carotenoid 19'-butanoyloxyfucoxanthin, a trace pigment in some chrysophytes and prymnesiophytes (but a major pigment in *Phaeocystis*), according to Jeffrey et al. (1997), occurred only four times (Table 1), without any relation to cell countings from these two divisions. Peridinin reached a maximum concentration of 2.341 μg l^{-1} in October 2004 (Figure 6). Maxima of 19'-hexanoyloxyfucoxanthin were during summer and autumn with values of 0.11 μg l^{-1} in September and October 2004 (Figure 6).

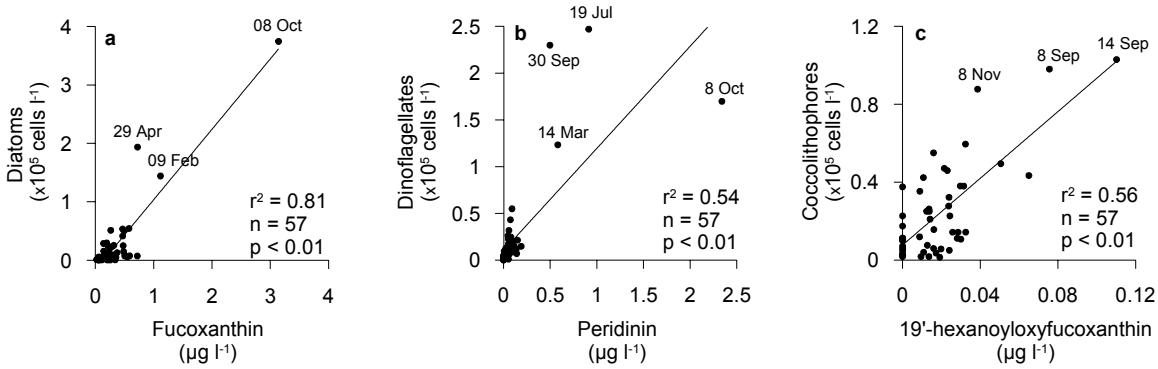

Figure 5 - Relationship between (a) fucoxanthin concentration and diatoms density, (b) peridinin and dinoflagellates density and (c) 19'-hexanoyloxyfucoxanthin and coccolithophores density.

Prasinoxanthin was present in lower concentrations throughout the year with a maximum abundance of 0.05 µg l^{-1} in October 2004 (Figure 7). This carotenoid is exclusive of prasinophytes, a group not identified under the microscope during the sampling period. Another phytoplankton group not recognized during cell counts was the cryptophytes however relevant concentrations of alloxanthin (exclusive pigment of this group) were detected by HPLC with maxima during summer and autumn (0.171 µg l^{-1} in September 2004; Figure 7). Several minor pigments were also detected by chromatography, such as Chl *b*, zeaxanthin, violaxanthin and neoxanthin, which we considered as representing an assembly of euglenophytes, chlorophytes and cyanobacteria. Cells from the last two divisions were not identified by microscopy. This set of pigments had maximum concentrations during summer and autumn (0.647 µg l^{-1} in October 2004; Figure 7).

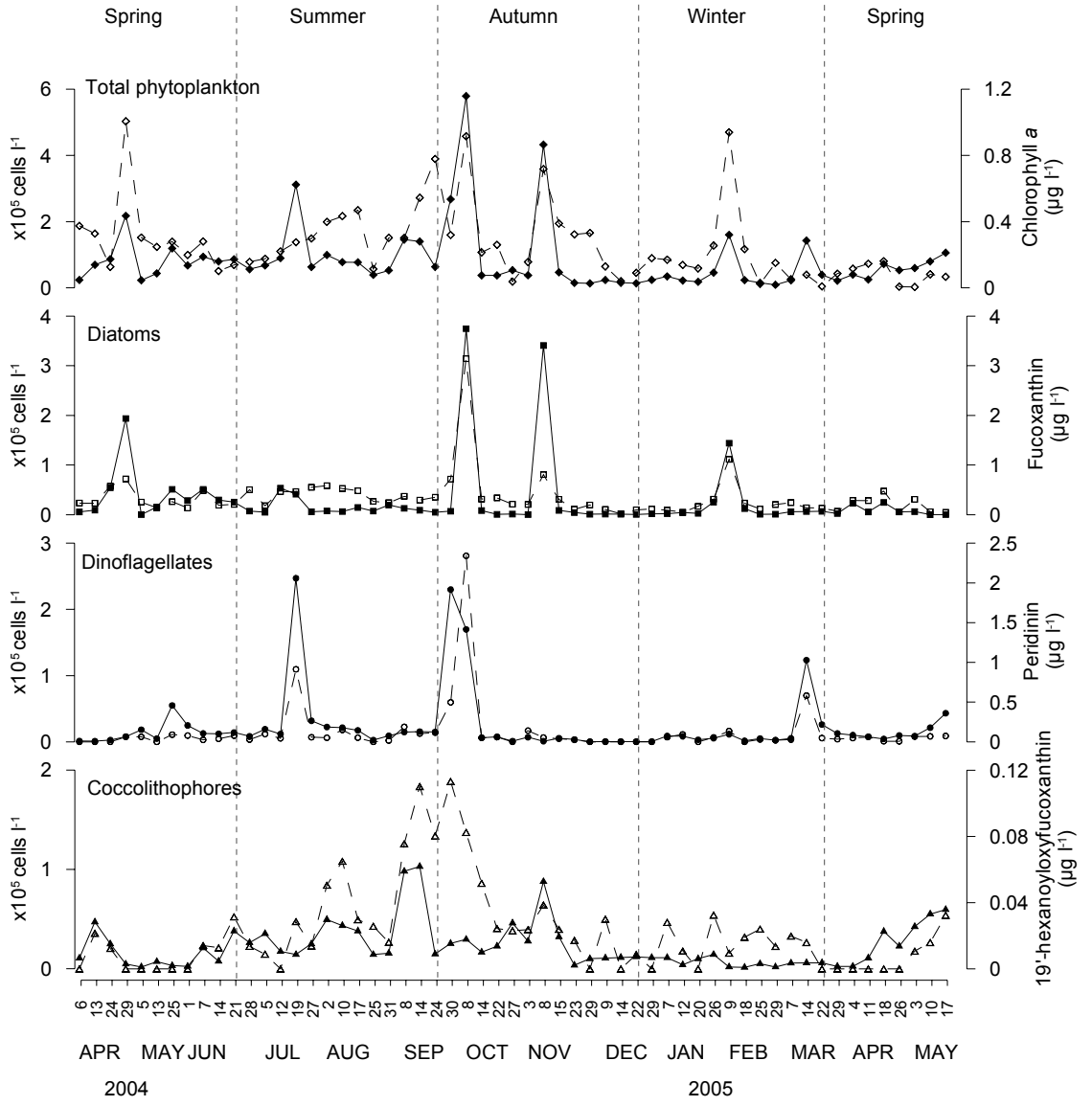

Figure 6 - Weekly surface distribution of total phytoplankton and chlorophyll a and of the dominant phytoplankton groups with the respective marker pigments, during the sampling period (April 2004 – May 2005). Diatoms and fucoxanthin, dinoflagellates and peridinin, coccolithophores and 19'-hexanoyloxyfucoxanthin. Cell counts and pigments are represented by solid and dotted lines, respectively. The day of each sampling is represented on the absciss axis.

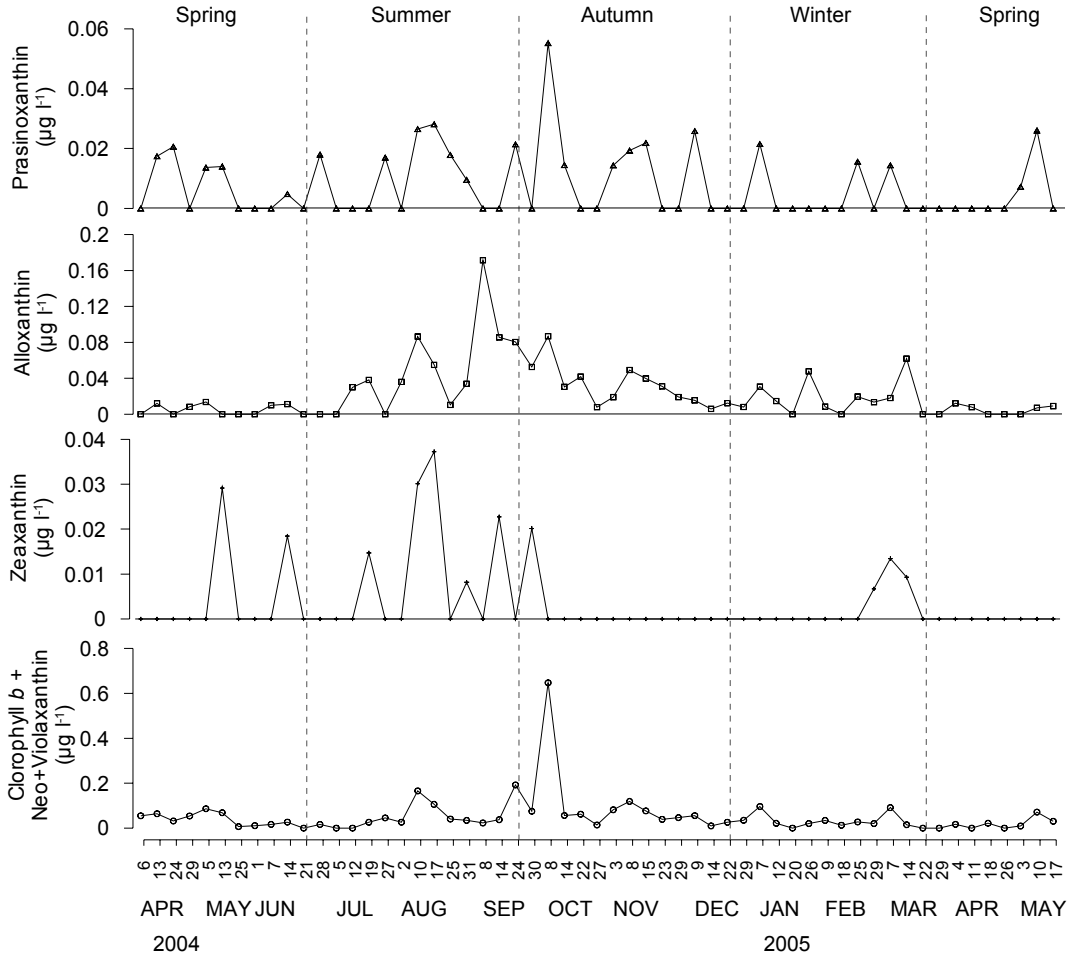

Figure 7 - Weekly surface distribution of marker pigments during the sampling period (April 2004 – May 2005): prasinoxanthin, alloxanthin, zeaxanthin and chlorophyll *b* + neoxanthin + violaxanthin. The day of each sampling is represented on the absciss axis.

3.3. Phytoplankton species composition

The 129 phytoplankton species observed were grouped into four classes and one extra group with different contributions to total abundance: 43% of dinophyceae (dinoflagellates), 41% of bacillariophyceae (diatoms), 9% of prymnesiophyceae (coccolithophores), 2% of euglenophyceae and 5% of the extra group designated as ‘‘others’’ (not identified small algae). A species richness index (SR) was determined, as it is the simplest measure of diversity, representing the total number of different species in a given area (Kevin and Spicer, 2004). It ranged from 11 to 44 species identified per

sample. Species diversity increased from spring to summer, attaining its maximum, and decreased towards the winter to values three times lower (Table 2).

The majority of the taxa were dinoflagellates, with 56 identified species. *Protoperidinium*, with 11 species and *Ceratium* with 10 species, were the two most represented genus, followed by *Dinophysis* and *Prorocetrum* with 6 species each. Nonetheless, the dinoflagellate *Scripsiella* cf. *trochoidea* was the dominant species from this group, being responsible for all the four maximum values. Dinoflagellates contribution to total biomass ranged between 0.2 and 86% (Table 2) reaching a maximum abundance of 2.5 x 10^5 cells l^{-1} in July 2004 (Figure 6).

Diatoms were the second largest group with 53 identified species. The most representative diatom genera were *Thalassiosira* and *Guinardia* both with 4 species identified, but the major abundances belonged to chain forming species like *Thalassiosira* spp., *Chaetoceros* spp., *Asterionelopsis glacialis*, *Skeletonema costatum*, *Pseudo-nitzschia* spp., *Detonula pumila*, *Lauderia annulata* and *Leptocylindrus danicus*. The contribution of diatoms to total abundance varied between 0.3 and 90% (Table 2) and this group reached a maximum concentration of 3.7 x 10^5 cells l^{-1} in October 2004 (Figure 6), achieving 65% of phytoplankton abundance. From the four maxima recorded (Figure 6) just the peak observed in April 2004 was dominated by *Pseudo-nitzschia* spp. (Table 2), the others were mainly composed by *Thalassiosira* spp. (October and November 2004 and February 2005).

Seven species of coccolithophores were identified, *Emiliania huxleyi* and *Gephyrocapsa* spp. being the main components of this community with a regular occurrence throughout the study. The contribution of coccolithophores to total biomass was between 1 and 86% (Table 2) and a maximum abundance of 1 x 10^5 cells l^{-1} was observed in September 2004 (Figure 6) constituted by *E. huxleyi*. From the end of July 2004 until mid August 2004, *Syracosphaera pulchra* dominated the coccolithophore assemblage reaching 0.3 x 10^5 cells l^{-1} in August 2004 (Table 2).

The class of euglenophyceae reached a maximum concentration of 5 x 10^3 cells l^{-1} in April 2005 and the category of ‘‘others’’ achieved 3 x 10^4 cells l^{-1} in June 2004 (Table 2).

			Phytoplankton groups (%)						
	Day	UI	Diat.	Dino.	Cocc.	Eugl.	Others	SR	Dominant phytoplankton species
APR	6	101	23,9	5,0	45,1	0,2	25,9	24	*Pseudonitzschia* spp.; *Emiliania huxleyi*
2004	13	-514	13,5	1,5	**67,7**	0,9	16,4	31	*Pseudonitzschia* spp.; *Emiliania huxleyi*; *Gephyrocapsa* spp.
	24	-196	62,8	3,0	28,9	0,5	4,9	27	*Pseudonitzschia* spp.; *Emiliania huxleyi*; *Gephyrocapsa* spp.
	29	-412	**89,0**	3,6	2,2	0,2	5,0	32	*Pseudonitzschia* spp.; *Chaetoceros* spp.
MAY	5	-1231	1,6	**79,6**	7,8	2,4	8,7	16	*Scripsiella* cf. *trochoidea*
	13	-419	35,7	11,2	16,4	7,1	29,6	30	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	25	-151	43,0	46,0	2,6	0,3	8,1	32	*Detonula pumila*; *Scripsiella* cf. *trochoidea*
JUN	1	-82	42,5	36,5	3,5	2,5	15,0	33	*Detonula pumila*; *Scripsiella* cf. *trochoidea*
	7	-422	54,5	13,5	22,4	0,0	9,6	27	*Detonula pumila*; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	14	-228	37,3	15,2	9,5	0,2	37,8	34	*Detonula pumila*; *Scripsiella* cf. *trochoidea*
	21	-329	29,5	16,4	44,1	0,1	9,9	44	*Detonula pumila*; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	28	-246	13,1	14,9	**46,4**	0,1	25,6	40	*Thalassiosira* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
JUL	5	-202	7,5	**28,2**	52,1	0,0	12,2	43	*Ceratium fusus*; *Ceratium furca*; *Gephyrocapsa* spp.
	12	-685	59,5	13,0	19,4	0,2	7,8	30	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	19	-543	13,3	79,3	4,6	0,2	2,7	43	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*
	27	-291	9,5	50,4	39,8	0,0	0,3	38	*Scripsiella* cf. *trochoidea*; *Syracopshaera pulchra*
AUG	2	-213	7,7	22,7	**50,0**	0,0	19,7	38	*Scripsiella* cf. *trochoidea*; *Syracopshaera pulchra*
	10	-268	8,4	27,5	**56,0**	0,0	8,1	38	*Ceratium fusus*; *Syracopshaera pulchra*
	17	-65	19,1	22,4	49,5	0,3	8,7	38	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	25	194	18,8	6,5	36,5	0,0	38,2	30	*Pseudonitzschia* spp.; *Gephyrocapsa* spp.; *Syracopshaera pulchra*
	31	-521	37,2	17,2	29,7	0,2	15,7	29	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
SEP	8	-451	8,9	10,1	67,3	0,8	12,8	34	*Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.; *Emiliania huxleyi*
	14	-25	6,5	11,0	**73,6**	0,1	8,7	30	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	24	-293	7,9	23,6	23,3	1,6	43,5	29	*Protoperidinium* spp.;*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	30	-217	2,7	**85,9**	9,5	0,4	1,6	28	*Scripsiella* cf. *trochoidea*
OCT	8	-129	**64,7**	29,3	5,1	0,2	0,7	31	*Thalassiosira* spp.; *Skeletonema costatum*; *Scripsiella* cf. *trochoidea*
	14	-65	22,3	16,4	44,2	0,3	16,7	35	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	22	157	2,0	20,1	60,5	0,9	16,5	24	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	27	593	2,5	2,0	86,3	1,3	8,0	21	*Emiliania huxleyi*; *Gephyrocapsa* spp.
NOV	3	611	1,1	18,2	72,9	2,3	5,6	17	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	8	-2	**78,9**	0,2	**20,3**	0,1	0,5	21	*Thalassiosira* spp.; *Chaetoceros* spp.; *Emiliania huxleyi*
	15	90	18,9	11,6	68,7	0,6	0,2	20	*Thalassiosira* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	23	-1020	30,1	22,6	23,8	9,6	13,8	21	*Chaetoceros* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	29	-128	8,0	1,2	74,9	0,0	15,9	15	*Gephyrocapsa* spp.
DEC	9	292	5,6	2,2	45,6	3,4	43,2	15	*Emiliania huxleyi*; *Gephyrocapsa* spp.
	14	-183	7,4	2,7	73,7	0,8	15,4	17	*Emiliania huxleyi*; *Gephyrocapsa* spp.
	22	192	4,8	3,0	85,5	4,5	2,1	12	*Emiliania huxleyi*; *Gephyrocapsa* spp.
	29	-859	7,5	1,5	46,6	2,3	42,1	17	*Emiliania huxleyi*; *Gephyrocapsa* spp.
JAN	7	-1127	6,2	25,5	32,2	12,1	24,0	21	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
2005	12	-296	21,2	38,3	18,3	3,8	18,3	17	*Thalassiosira* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	20	190	15,8	16,3	55,8	0,7	11,4	21	*Paralia sulcata*; *Protoperidinium* spp.; *Gephyrocapsa* spp.
	26	-744	55,1	11,9	31,1	1,6	0,3	26	*Thalassiosira* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
FEB	9	-178	**90,1**	7,1	1,1	0,2	1,4	28	*Thalassiosira* spp.; *Asterionellopsis glacialis*
	18	-245	52,5	6,6	6,1	0,9	33,9	18	*Asterionellopsis glacialis*
	25	-849	6,2	32,0	32,4	13,4	16,0	19	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	29	-677	11,6	26,2	19,2	0,0	43,0	15	*Thalassiosira* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
MAR	7	-461	25,9	23,1	24,9	6,4	19,6	31	*Lauderia annulata*; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	14	-714	4,6	**86,4**	4,2	0,5	4,3	31	*Scripsiella* cf. *trochoidea*
	22	2	17,7	66,4	15,2	0,5	0,2	15	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	29	419	11,8	58,4	11,0	0,4	18,4	17	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
APR	4	551	58,9	25,4	6,0	9,7	0,0	20	*Pseudonitzschia* spp.; *Scripsiella* cf. *trochoidea*
	11	170	22,8	30,8	42,8	2,4	1,3	21	*Leptocylindrus danicus*; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
	18	-780	34,7	6,1	52,3	0,2	6,7	20	*Leptocylindrus danicus*; *Gephyrocapsa* spp.
	26	-544	10,7	17,9	42,3	9,7	19,3	29	*Chaetoceros* spp.; *Scripsiella* cf. *trochoidea*; *Gephyrocapsa* spp.
MAY	3	-98	10,6	14,4	70,9	0,6	3,4	26	*Chaetoceros* spp.; *Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	10	38	0,3	26,6	68,9	1,4	2,7	23	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*
	17	-195	0,4	40,7	**56,3**	0,6	2,0	20	*Scripsiella* cf. *trochoidea*; *Emiliania huxleyi*

Table 2 - Weekly phytoplankton relative distribution (%), species richness (SR) and dominant phytoplankton species. Diat. – diatoms; Dino. – dinoflagellates; Cocc. – coccolithophores; Eugl. – euglenophytes

4. DISCUSSION

Dinoflagellates, diatoms and coccolithophores dominated the phytoplankton assemblage in terms of abundance and community dynamics as showed both by microscopic observations and pigment analysis. Maxima concentrations of total phytoplankton were observed in autumn, although short-time peaks were registered throughout all seasons.

The correlations obtained between biomarker pigment concentration and the corresponding taxon specific cell number, constitute interesting results and are a relevant contribution of the present paper to coastal phytoplankton studies. The microscopic analysis showed that the outliers of these correlations (Figure 4) were coincident with maximum concentrations (cells l^{-1}) of each phytoplankton group, evidencing the need of microscopic observations to fully characterize peak events. Specifically, diatoms presented three outliers, which corresponded to peaks of chain forming species such as: *Pseudo-nitzschia* spp., *Chaetoceros* spp., *Thalassiosira* spp., *Skeletonema costatum* and *Asterionellopsis glacialis*. The four maxima abundances of dinoflagellates were coincident with the outliers present in the correlation and were constituted by *Scripsiella* cf. *trochoidea*. Finally, the same picture was found for coccolithophores: the three outliers corresponded to maxima of *Emiliania huxleyi* (Figure 3, Table 2). Therefore, the variations between the three main phytoplankton groups, dinoflagellates, diatoms and coccolithophores were reflected by the peridinin, fucoxanthin, and 19'-hexanoyloxyfucoxanthin concentrations, respectively, although the last two can not be considered truly fingerprint pigments as they are present in other phytoplankton classes. However, the good agreement between fucoxanthin and diatoms concentration (r^2 = 0.81) indicate that this group is the most important carrier of this pigment for our samples. Hence, in spite of being present also in haptophytes (Jeffrey and Vesk, 1997), fucoxanthin can be used to trace diatoms, providing a solid proxy for monitoring seasonal variations, in this region. As far as coccolithophores are concerned, the positive correlation (p < 0.01) between the concentrations of 19'-hexanoyloxyfucoxanthin and the density of coccolithophores (r^2 = 0.54) but not with fucoxanthin, point out this carotenoid as biomarker of coccolithophores in our waters. The same conclusion was achieved by Ediger et al. (2006), who found a good correlation between *Emiliania huxleyi* and 19'-hexanoyloxyfucoxanthin, but not with

fucoxanthin. Furthermore, Stolte et al. (2000), indicate 19'-hexanoyloxyfucoxanthin as the major light harvesting carotenoid in all Atlantic strains for this species.

The seasonal distribution of total phytoplankton biomass was generally higher in spring and summer however it did not evidence the typical pattern of temperate phytoplankton seasonal evolution. The relevant biomass peaks were registered in autumn, with a major bloom of diatoms, dinoflagellates, prasinophytes and other chlorophyll *b* containing groups on 8 October 2004, followed by a second one in 8 November, dominated by diatoms and coccolithophores. In both occasions, salinity attained its minimum values due to heavy rainfall.

Phytoplankton growth is dependent on light and nutrients availability. In Cascais Bay, nutrients seem to be mostly from riverine origin, the transport of silicates and phosphates from Tagus estuary was clearly proved by the correlations obtained between each of these nutrients and runoff. For dissolved inorganic nitrogen, a statistical valid correlation was not found however, ammonia values diminished considerable on drier year 2005, whereas nitrates + nitrites increase in December/January as a response to the higher runoff in November/December. In order to assess nutrient limitation, the obtained results were discussed following Dortch and Whitledge (1992). Phosphates were only limiting on 10 August 2004 (with $PO_4^{3-} \leq 0.2$, N/P > 30 and Si/P > 3), where the community was dominated by the coccolithophore *Syracosphaera pulchra* and the dinoflagellate *Ceratium fusus*. Nitrates were limiting during a major bloom of the diatom *Asterionellopsis glacialis* at 18 February 2005, as DIN was 1 μmol l^{-1}, N:P ratio 5 (< 10), and Si:N lower than 1 (0.08). Availability of silicates clearly diminished from February 2005 onwards, most probably due to decreased river flow, potentially limiting conditions, with $SiO_4^{4-} < 2$ μmol l^{-1}, Si:N < 1 and Si:P < 3, occurred a dozen times, in summer 2004 and spring 2005, however, according to the authors op cit, caution must be applied when discussing silicate limitation in marine environments. Tagus river flow seems to be a strong influence on phytoplankton temporal distribution however the action of upwelling waters in this region can not be discarded. The other phytoplankton groups not identified under the microscope as well as euglenophytes seemed to prefer more stable situations, especially during summer, when the higher abundances were recorded. Gameiro et al. (2007), registered within Tagus estuary, higher abundances of euglenophytes during this season.

5. CONCLUSIONS

The pigments detected under the HPLC showed a good correlation with phytoplankton identifications with maxima ($\mu g\ l^{-1}$) coincident with the higher phytoplankton cell counts. Fucoxantin, peridinin and 19'-hexanoyloxyfucoxanthin appeared as good indicators, for diatoms, dinoflagellates and coccolithophores, respectively, with synchronized seasonal variations and significant positive correlations. Furthermore, the chemotaxonomic analysis had the capacity of quantifying concentrations of biomarker pigments and recognizing the presence of phytoplankton taxa that were difficult to identify and enumerate by microscopy such as cryptophytes, prasinophytes, chlorophytes and cyanobacteria. These groups face problems mainly concerned with their small size making the HPLC approach an accurate tool to access and describe the total phytoplankton biomass. The pigment methodology was a helpful and faster way of analyze larger changes of the phytoplankton community with relatively much less effort compared to microscopic studies. However, these studies revealed changes within phytoplankton groups and allowed us to recognize small scale variations on species succession and an accurate characterization of total biomass and species composition. Microscopic analyses are crucial to an exact assignment of marker pigments to phytoplankton taxa and thus permit a reliable study of phytoplankton community structure and dynamics.

Acknowledgments

This work was supported by projects QCAIII- POPesca MARE ''Caracterização ecológica da zona costeira – Plataforma Continental'' and PROFIT – ''Interdisciplinary study of oceanographic processes underlying the phytoplankton dynamics in the Portuguese upwelling system'', PDCTE/CTA/50386/2003. C.R. Mendes grant and the HPLC analyses were supported by INTAGUS (POCTI/MAR/ 58022/2004). We thank M. Falcão for performing all nutrient analysis. We also thank to A. Morais, T. Quental and F. Quintela for all the assistance during the sampling period. We are grateful to T. Moita for the helpful comments to the manuscript.

References

Barlow, R.G., Mantoura, R.F.C., Gough, M.A., Fheman, T.W., 1993. Pigment signatures of the phytoplankton composition in the north-eastern Atlantic during the 1990 spring bloom. Deep Sea Research II 40, 459–477.

Dodge, J.D., 1982. Marine Dinoflagellates of the British Isles. Her Majesty's Stationary Office, London, 299 pp.

Dortch, Q., Whitledge, T.E., 1992. Does nitrogen or silicon limit phytoplankton production in the Mississippi River plume and nearby regions? Continental Shelf Research 12 (11), 1293–1309.

Ediger, D., Soydemir, N., Kideys, A.E., 2006. Estimation of phytoplankton biomass using HPLC pigment analysis in the southwestern Black Sea. Deep Sea Research II 53, 1911–1922.

Fatela, F., Taborda, R., 2002. Confidence limits of species proportions in microfossil assemblages. Marine Micropaleontology 45, 169–174.

Gameiro, C., Cartaxana, P., Brotas, V., 2007. Environmental drivers of phytoplankton distribution and composition in Tagus estuary, Portugal. Estuarine, Coastal and Shelf Science 75 (1/2), 21–34.

Gibb, S.W., Cummings, D.G., Irigoien, X., Barlow, R.G., Fauzi, R., Mantoura, C., 2001. Phytoplankton pigment chemotaxonomy of northeastern Atlantic. Deep Sea Research II 48, 795–823.

Gieskes, W.W.C., Kraay, G.W., 1983. Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. Marine Biology 75, 179–185.

Grasshoff, K., 1983. Methods of Seawater Analysis. Verlag Chemie, New York. Hallegraeff, G.M., 1981. Seasonal study of phytoplankton pigments and species at coastal station off Sydney: importance of diatoms and nannoplankton. Marine Biology 61, 107–118.

Hasle, G.R., 1978. Phytoplankton manual: the inverted microscope method. In: Sournia, A. (Ed.), Monographs on Oceanic Methodology. UNESCO, Paris, pp. 88–96.

Hasle, G.R., Syvertsen, E.E., 1996. Marine diatoms. In: Tomas, C.R. (Ed.), Identifying Marine Diatoms and Dinoflagellates. Academic Press, Inc., London, pp. 5–385.

Havskum, H., Schlüter, L., Scharek, R., Berdalet, E., Jacquet, S., 2004. Routine quantification of phytoplankton groups – microscopy or pigment analyses? Marine Ecology Progress Series 273, 31–42.

Irigoien, X., Meyer, B., Harris, R., 2004. Using HPLC pigment analysis to investigate phytoplankton taxonomy: the importance of knowing your species. Helgoland Marine Research 58, 77–82.

Jeffrey, S.W., Hallegraeff, G.M., 1987. Phytoplankton pigment, species and light climate in a complex warm-core eddy of the east Australian Current. Deep Sea Research I 45, 1141–1468.

Jeffrey, S.W., Vesk, M., 1997. Introduction to marine phytoplankton and their pigment signatures. In: Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (eds.), Phytoplankton Pigments in Oceanography. UNESCO, Paris, pp. 37–84.

Jeffrey, S.W., Mantoura, R.F.C., Bjørnland, T., 1997. Data for the identification of 47 key phytoplankton pigments. In: Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (eds.), Phytoplankton Pigments in Oceanography. UNESCO, Paris, pp. 449–559.

Jeffrey, S.W., Wright, S.W., Zapata, M., 1999. Recent advances in HPLC pigment analysis of phytoplankton. Marine and Freshwater Research 50, 879–896.

Kevin, J.G., Spicer, J.I., 2004. Biodiversity: an Introduction. Blackwell Publishing, Oxford, 208 pp.

Mackey, M.D., Mackey, D.J., Higgins, H.W., Wright, S.W., 1996. CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Marine Ecology Progress Series 144, 265–283.

Mackey, D.J., Higgins, H.W., Mackey, M.D., Holdsworth, D., 1998. Algal class abundances in the western equatorial Pacific: estimation from HPLC measurements of chloroplast pigments using CHEMTAX. Deep Sea Research I 45, 1141–1468.

Millie, D.F., Paerl, H.W., Hurley, J.P., 1993. Microalgal pigment assessments using high performance liquid chromatography: a synopsis of organismal and ecological applications. Canadian Journal of Fisheries and Aquatic Science 50, 2513–2527.

Peeken, I., 1997. Photosynthetic pigment fingerprints as indicators of phytoplankton biomass and development in different water masses of the Southern Ocean during austral spring. Deep Sea Research II 44, 261–282.

Schlüter, L., Havskum, H., 1997. Phytoplankton pigments in relation to carbon content in phytoplankton communities. Marine Ecology Progress Series 155, 55–65.

Stolte, W., Kraay, G.W., Noordeloos, A.M., Riegman, R., 2000. Genetic and physiological variation in pigment composition of *Emiliania huxleyi* (Prymnesiophyceae) and the potential use of its pigment ratios as a quantitative physiological marker. Journal of Phycology 36, 529–539.

Throndsen, J., 1978. Phytoplankton manual: preservation and storage. In: Sournia, A. (Ed.), Monographs on Oceanic Methodology. UNESCO, Paris, pp. 69–75.

Young, J., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I., Ostergaard, J., 2003. A guide to extant coccolithophore taxonomy. Journal of Nannoplankton Research Special Issue 1, 123 pp.

Zapata, M., Garrido, J.L., 1991. Influence of injection conditions in reversed-phase high-performance liquid chromatography of chlorophylls and carotenoids. Chromatographia 31, 589–594.

Zapata, M., Rodríguez, F., Garrido, J.L., 2000. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. Marine Ecology Progress Series 195, 29–45.

CHAPTER 6: CONCLUSIONS

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The sampling station seemed to be a good indicator of regional phytoplankton variations and associated processes. At the site it was possible to monitor the changes between upwelling and onshore advection conditions and the phytoplankton response to these variations. However, it should be taken in account the retention features of the site. Nutrients tend to be permanently available what can lead to higher cell numbers. Though and both assuming the site as representative of the influence of hydrographic processes in Lisbon bay and equal cell growth rates over the years, the higher cell counts observed can suggest higher offshore abundances attaining at the sampling site. The shallow depth sometimes influenced the higher surface temperatures measured, and should be considered in the analysis of summer values. A possible greater influence of winter conditions was sometimes noticed. But, the presence of oceanic species as coccolithophores or upwelling species as *Pseudo-nitzschia* spp. insures the influence of these conditions at the site. The fine scale sampling allowed accurate associations between hydrographic conditions and phytoplankton.

On Lisbon bay the annual phytoplankton succession was characterized by a seasonal cycle typical of a temperate area and by short succession cycles associated with the intensity and persistence of upwelling and downwelling events, changes in temperature and salinity and nutrient availability. Figure 11 is a general schematic representation of the seasonal and interannual distribution of the three major groups (diatoms, coccolithophores and dinoflagellates) in relation to weekly upwelling. During the four years, the phytoplankton community presented variability scales from interannual, seasonal to short-term. The influence of persistent upwelling conditions, observed earlier in the year, seemed to play an important role in the increase in biomass (Chl *a*), through the years, more exactly 0,76 $\mu g.l^{-1}$. Chlorophyll *a* reflected the major trends in phytoplankton development and the pigments detected under the HPLC showed a good correlation with phytoplankton identifications. In contrast with the following years, 2002, was characterized by the upwelling and downwelling seasons clearly distinguished and low precipitation. The highest phytoplankton concentrations were counted and the community was dominated by diatoms under upwelling conditions and coccolithophores when onshore advection prevailed. The following years were characterized by longer periods of mild upwelling as in 2004, when the next high concentrations were observed. However, the assemblage was dominated by diatoms and, instead of coccolithophores, by dinoflagellates with two short and

expressive peaks. The lower development of coccolithophores, during summer-early autumn coincided with the lowest concentrations of nitrites and nitrates, important for calcification processes as well as with an overall decreased in phosphates and limiting silicates, during this year. Between those two years, 2003 was particularly characterized by longer periods of intense precipitation and strong fluctuations in salinity and lower temperatures. Phytoplankton maxima were observed later in the year and attended in very low numbers.

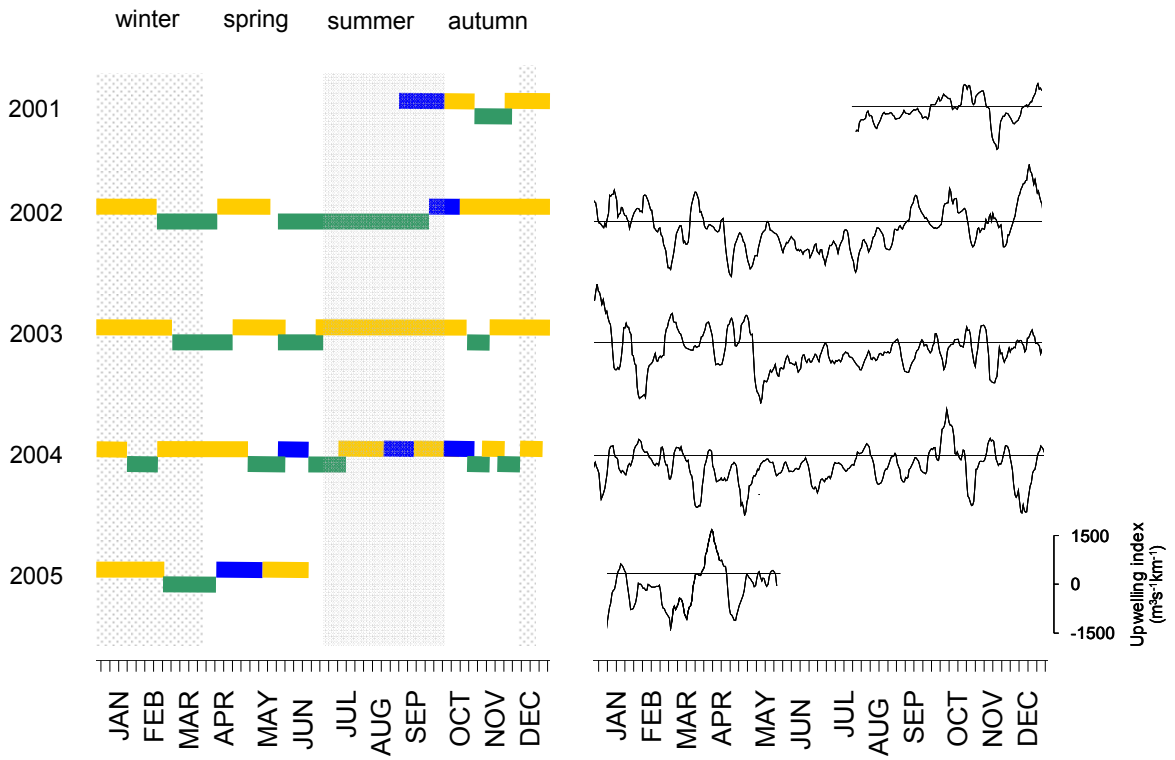


Figure 11 - Schematic representation of the seasonal and inter-annual distribution of the three major groups: **GREEN – diatoms, ORANGE – coccolithophores** and **BLUE – dinoflagellates** in relation to weekly upwelling.

The phytoplankton community was mainly composed by diatoms, dinoflagellates and coccolithophores which explained more than 90% of the assemblage. Fucoxantin, peridinin and 19'-hexanoyloxyfucoxanthin appeared as good indicators, for diatoms, dinoflagellates and coccolithophores, respectively, with synchronized seasonal variations and significant positive correlations.

From a general point view, over the years, the species succession occurred along a gradient of decaying turbulence and nutrient availability as previously described by several authors. Small chain diatoms were the first to react to turbulence and were followed by larger forms as upwelling conditions persisted, mainly during spring. When onshore advection prevailed, coastal turbulence was reduced but nutrients were still available, diatoms were replaced by coccolithophores and finally by dinoflagellates. These conditions were usual during autumn, the short transition period from upwelling to downwelling seasons.

Diatoms were the most abundant group, however, it was noticed that short upwelling pulses appeared to be unfavourable for the maintenance and development of this group. Diatoms exploited periods of higher nutrient availability with peaks interspersed with silicates maxima. Diatoms blooms consume silicates, sometimes until limitation values, influencing the subsequent low cell numbers. Silicates were highly available, especially after rainy periods and higher runoff, what possibly favoured the first major peaks recurrently observed through February months, the transition from winter to spring conditions. During spring, diatoms dominated the phytoplankton assemblage contributing almost exclusively to Chl *a*. Fucoxanthin also provided a solid proxy for monitoring diatoms seasonal variations, in this region. The group diversity and preferences remained similar over seasons and the community was composed by 104 identified taxa. The dominant species were *Chaetoceros* spp., *Thalassiosira* spp., *Pseudo-nitzschia* spp., *Skeletonema costatum*, *Asterionelopsis glaciallis*, *Leptocylindrus danicus*, *Detonula pumila*, *Guinardia delicatula*, *Guinardia* spp., *Thalassionema nitzschioides* and *Cylindrotheca closterium*. The presence of *D. pumila* seemed to have a great contribution to Chl *a* and a role in the inconsistency between lower cell counts and higher biomass levels. In contrast, higher counts and lower Chl *a* concentrations can be associated with small chain diatoms and coccolithophores.

The coccolithophores *Emiliania huxleyi* and *Gephyrocapsa* genus also developed during spring, reaching concentrations closer to diatoms. These species most contributed to coccolithophores maxima during spring and should be considered with diatoms to explain spring biomass values. In lower concentrations, *Coccolithus pelagicus* appeared throughout the upwelling season with maxima densities during spring. This coccolithophore revealed a preference for cold waters associated with moderate turbulence and in 2002 reached the highest concentrations coincident with a strong upwelling season. As a recognized proxy for the presence of an upwelling front, in Lisbon bay, *C. pelagicus* abundance can indicate the position of the upwelling plume

rooted at Cape Roca, in relation to the Cascais site. The upwelling filament observed during the summer cruise in 2005 was highlighted by the patchy distribution of this species both in front of the filament and south of cape Roca (data not shown).

Coccolithophores were the second most abundant group and appeared capable of resisting to coastal processes such as turbulence, yet decreased throughout the sampling. This decline was probably a result of shorter and less intense convergence periods and of an increase in the number of days with upwelling, especially from autumn until winter. Twenty two species were identified and the optimum conditions lay between 14º and 20ºC and salinities of 34 to 36.5. In opposition to diatoms, coccolithophore composition changed seasonally and the group thrived in a remarkable variety of oceanographic conditions. The most abundant species during summer – autumn were *Helicosphaera carteri*, *Coronosphaera mediterranea*, *Rhabdosphaera clavigera*, *Syracosphaera pulchra*, *E. huxleyi* and *Gephyrocapsa* spp. This assemblage thrived in environments resulting from the confluence of warmer and oligotrophic oceanic waters with the coastal and low turbulent nutrient enriched. *C. mediterranea* distributed in the widest range of turbulence, temperature and salinity while *H. carteri* and *R. clavigera* occurred in a narrower range and did not seem to respond so quickly to nutrient enrichment. This last specie was repeatedly absent nearshore as upwelling intensified. *S. pulchra* appeared for the first time in June 2004 dominating the coccolithophore community and coincident with an overall decrease in nutrient availability. *E. huxleyi*, *Gephyrocapsa oceanica*, *Gephyrocapsa muellerae* and *Gephyrocapsa ericsonii* were always observed in samples from Lisbon bay and increased under low turbulent conditions, during the two most productive periods (spring and summer). *E. huxleyi*, *G. muellerae* and *G. ericsonii* could indicate the presence of colder waters associated with the beginning of the upwelling season usual during spring while *G. oceanica* was particularly indicative of productive periods during summer. *E. huxleyi* and *Gephyrocapsa* spp. could be used as proxies for highly productive environments generated by upwelling and surrounding areas of upwelling centers.

Dinoflagellates, like some coccolithophores, preferred warmer stratified conditions, with maxima during summer. However, this group decreased through the four years what was related to the decrease of lasting convergence periods and an intensification of upwelling conditions. We can also hypothesize a competitive disadvantage in relation to coccolithophores. The group seemed to have a narrow tolerance to changes in turbulence and temperature. From the 83 taxa identified the

community was dominated by a permanent development of *Scripsiella* cf. *trochoidea* and in lower numbers by the species *Ceratium fusus*, *Prorocentrum micans*, *Ceratium furca* and several others included in the genus *Ceratium* spp., *Prorocentrum* spp., *Protoperidinium* spp., *Dinophysis* spp. and *Gymnodinium* spp.. It was also observed a significant development of a phytoplankton assemblage with less than 20 μm, representing 50% of the Chl *a* measured during this season and until autumn.

From spring to autumn the phytoplankton species succeeded and peaked according to particular environmental conditions. The winter season was in general characterized by a strong decreased of all phytoplankton groups: the influence of onshore advection of oceanic waters was recurrently observed as well as high nutrient availability and lower SST. Increments in precipitation and runoff were followed by a decrease in salinity. Diatoms seemed to be light dependent and influenced by low salinities and temperatures once silicates were highly available to be fully utilized by this group. Dinoflagellates, in particular, evidenced a narrow tolerance to winter conditions while the coccolithophores, *Calcidiscus quadriperforatus* and *Calcidiscus leptoporus*, dominated the phytoplankton assemblage. These two species are typical components of the transition from cold to warmer water floras and seemed more influenced by changes in turbulence and salinity once decreased both when upwelling intensified associated with high salinities or when advection conditions coincided with rainy periods (salinity below 30 as in 2003 and 2004). Both species seemed to react independently but having a co-occurring seasonal pattern nearshore, developing in different concentrations throughout the seasons. *C. quadriperforatus* dominated over *C. leptoporus* during winter, while the last remained sole in the samples until early spring, related to higher SST. In Lisbon bay, the data suggested that *Calcidiscus* species responded to nutrient availability associated with low temperatures and promptly developed nearshore when turbulence decays as a result of weakening northlies during the upwelling season (spring) or prevailing onshore advection conditions, during winter. From 2001 until 2004, the genus was always absent, during summer, over the shelf revealing the influence of colder and turbulent waters but during summer 2005 the species was unusually reported nearshore. The genus distributed south of the upwelling filament due to the presence of an intrusion of oceanic warmer waters promoted by the occurrence of two counter-rotating mesoscale eddies. This intrusion influenced the orientation of the upwelling filament, with a sole westward extension (and not the usual southward jet). The species traced the displacement nearshore of these offshore warmer waters. It was also observed an increase in the

size of *C. quadriperforatus* coccospheres, probably influenced by nutrient availability and low turbulence conditions in the retentive upwelling shadow area south of cape Roca. *C. quadriperforatus* and *C. leptoporus* were observed offshore forming two spatially separated patches. Both species developed within a coccolithophore assemblage composed by the summer-autumn species observed during the time-series (chapter 2) and by more than 20 coccolithophore species, describe in chapter 3.

Through the time series, distinct nutrient proportions coincided with the development of each group and particular species. This suggests that nutrients by themselves can not act as a triggering signal for phytoplankton development. A combination of factors as light, turbulence, salinity and nutrient ratios are needed to explain biomass and phytoplankton variability. However we are inclined to suggested in broad terms a combination of turbulent conditions, light and silicates availability as most favourable for diatoms development, lower nutrient demands and stratification for dinoflagellates and intermediate turbulence and availability of nitrates for coccolithophores.

Sampling on a weekly basis allowed exhaustive observations of phytoplankton composition and seasonality. Such an effort was required to observe how species reacted to shorter time scales as upwelling events and to other coastal processes. Hence, it was possible to determine precise associations between species and different regional oceanographic regimes. Sampling further offshore and in deeper levels, permitted the observation of an additional great number of coccolithophores, especially holococcolithophores and other species never observed in the bay as *U. sibogae* and *F. profunda*. The chemotaxonomic analysis was a helpful and faster way of analyze larger changes of the phytoplankton community by the quantification of biomarker pigments, with relatively much less effort compared to microscopic studies. This type of analysis permitted to recognize the presence of phytoplankton taxa difficult to identify and enumerate by microscopy, such as cryptophytes, prasinophytes, chlorophytes and cyanobacteria. These groups face problems mainly concerned with their small size making the HPLC approach an accurate tool to access and describe the total phytoplankton biomass. However, microscopic studies were crucial to an exact assignment of marker pigments to phytoplankton taxa and thus permit a reliable study of phytoplankton community structure and dynamics. For instance, similar Chl *a* values were found to be related with differences in floral composition and cell counts, indicating the importance of microscope identifications in the interpretation of peaks.

A bi-weekly sampling would be adequate to reduce observation effort and still ensure the results. A larger gap between samples would result in a loss of information both biological and hydrographic. Biodiversity would be reduced and it would be difficult to establish correlations between species and the environment.

Contrasting evidences in environmental parameters controlling coccolithophore blooms were observed around the world by several authors, especially concerning physical parameters and nutrient concentrations and ratios. A combination of parameters seemed to be required and further research could be focus on intra-specific differences (e.g. morphological, genotypic), carbonate saturation state, grazing, competition between species and groups or even the presence of virus.

CHAPTER 7: COCCOLITHOPHORES FROM LISBON BAY - PLATES

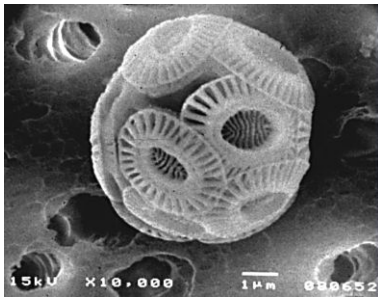
CHAPTER 7: COCCOLITHOPHORES FROM LISBON BAY - PLATES

The present chapter gathers SEM micrographs of some coccolithophores from Lisbon bay. The order of the plates followed the taxonomical sections adopted by Young et al. (2003).

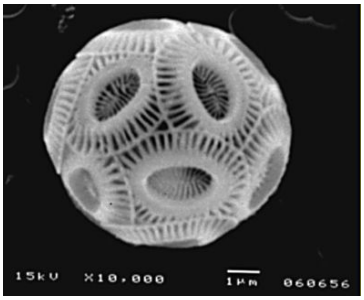
Classification of species followed “Young, J.R., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I, Ostergaard, J. (2003). A guide to extant coccolithophore taxonomy. Journal of Nannoplankton Research, Special Issue 1” and “Cros, L., Fortuno, J-M. (2002). Atlas of Northwestern Mediterranean Coccolithophores. Scientia Marina 66, Suppl.1. Institut de Ciències del Mar, CMIMA-CSIC Barcelona, Spain.”

PLATE 1 – Isochrysidales (Noelaerhabdaceae)

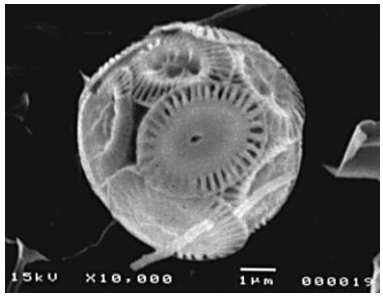
1.1 *Emiliania huxleyi*



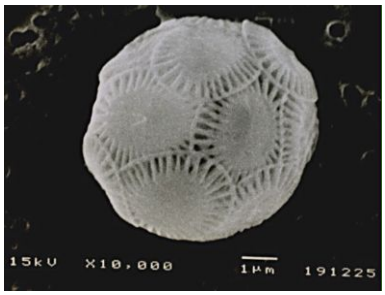
type A



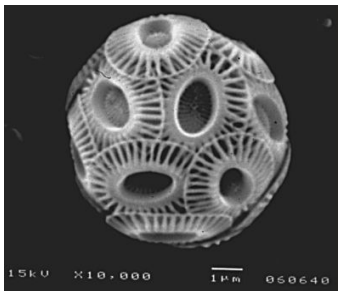
type A



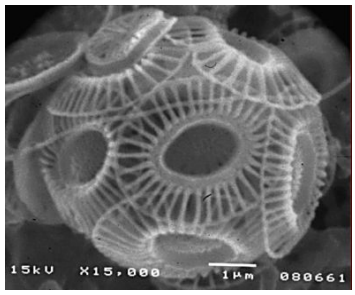
type A overcalcified



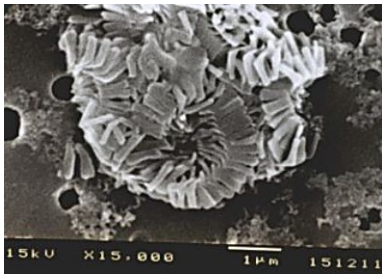
type A overcalcified



type B



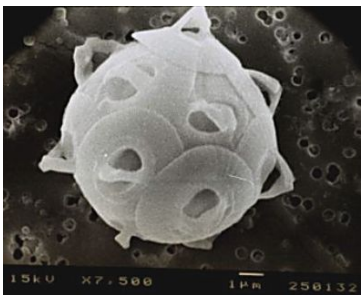
type B/C



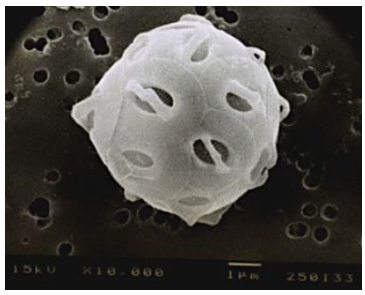
E. huxleyi - dissolved

1.2 *Gephyrocapsa*

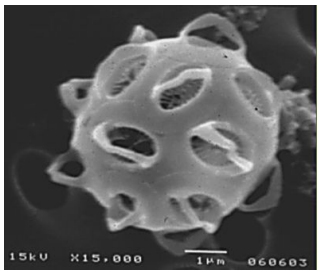
G. oceanica



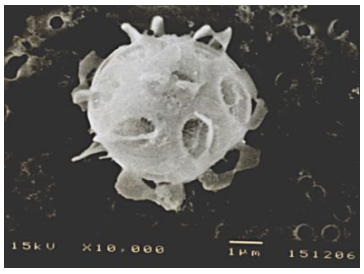
G. oceanica



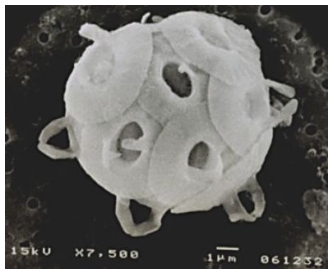
G. muellerae



G. ericsonii



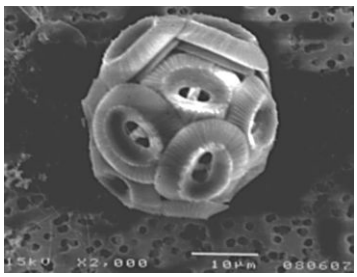
G. ornata



Gephyrocapsa with elevated bridge

PLATE 2 – Coccosphaerales (Coccolithaceae & Calcidiscaceae)

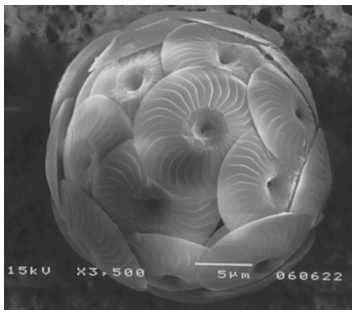
2.1 Coccolithaceae



Coccolithus pelagicus ssp. *braarudii*

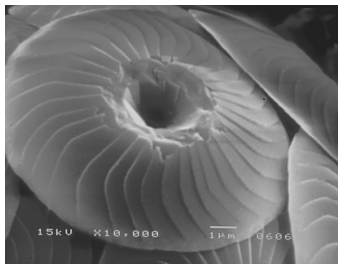
2.2 Calcidiscaceae

2.2.1 *Calcidiscus* and *Oolithotus*

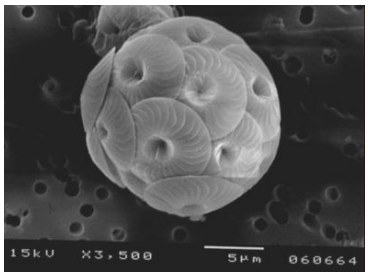


C. quadriperforatus

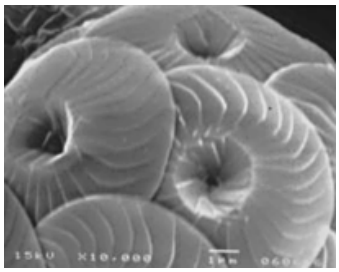
C. quadriperforatus - large



C. quadriperforatus

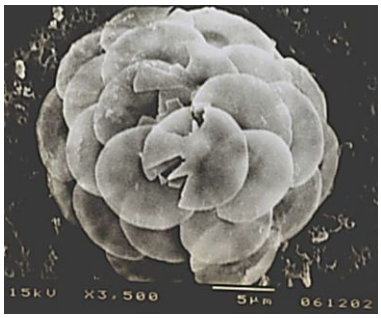


C. leptoporus



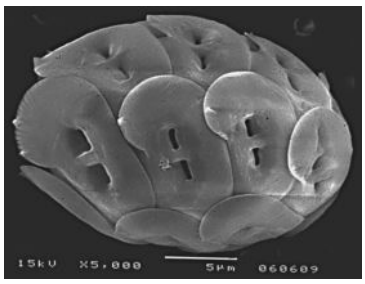
C. leptoporus

Calcidiscus spp. – dissolved

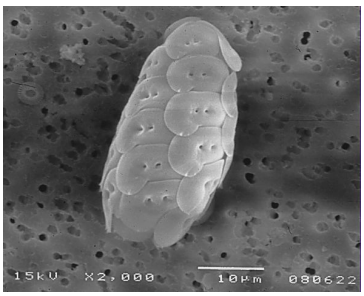


Oolithotus fragilis

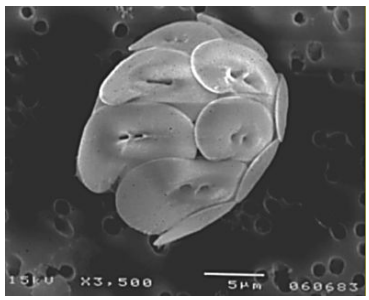
Plate 3 – Zygodiscales (Helicosphaeraceae)



H. carteri



H. carteri

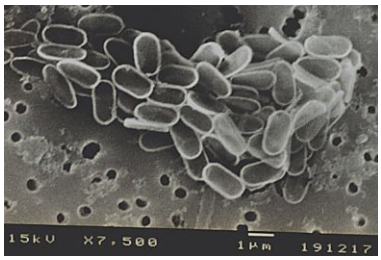


H. carteri

Plate 4 – Syracosphaerales (Syracosphaeraceae and Rhabdosphaeraceae)

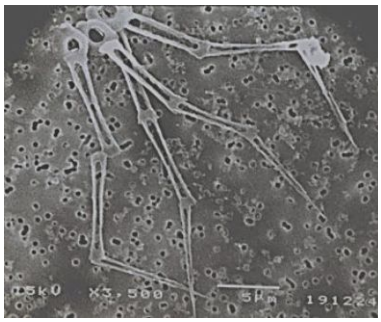
4.1 Genera with appendages

4.1.1 *Calciopappus*



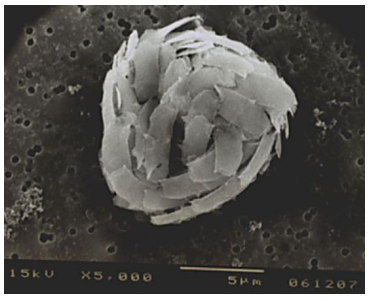
C. caudatus

4.1.2 *Michaelsarsia*



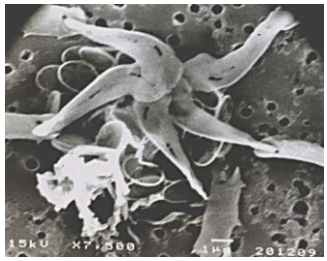
M. elegans

4.1.3 *Ophiaster*



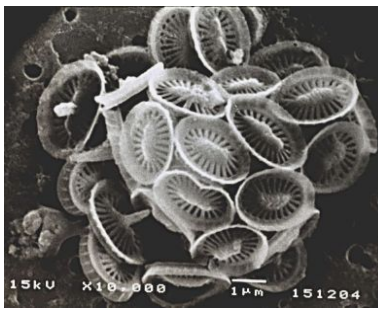
O.formosus (the legs of *Ophiaster* which appear to envelop the cell)

O. hydroideus

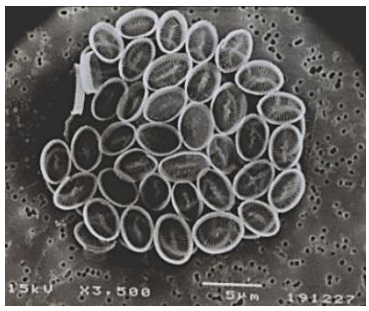


O. hydroideus

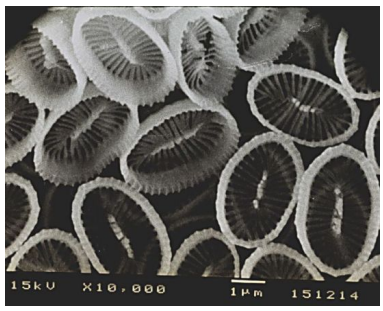
4.2 *Syracosphaera*



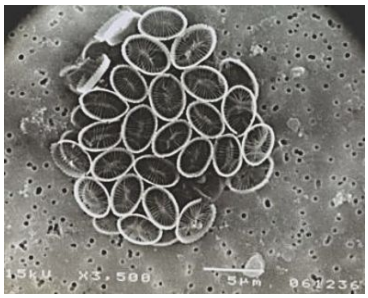
S. nodosa



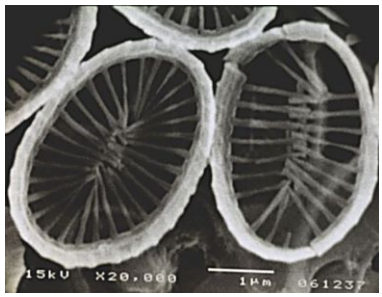
S. cf. *lamina*



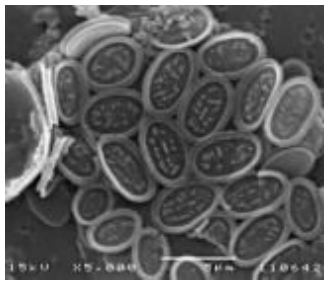
S. cf. *lamina*



S. tumularis

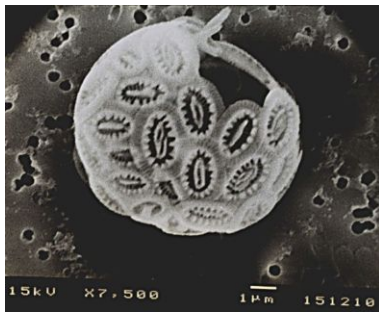


S. tumularis

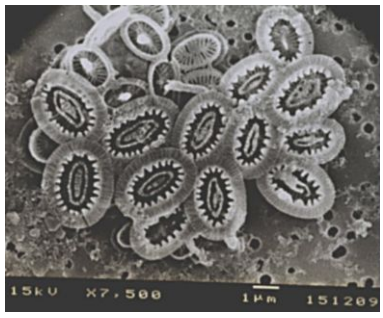


S. borialis type 2

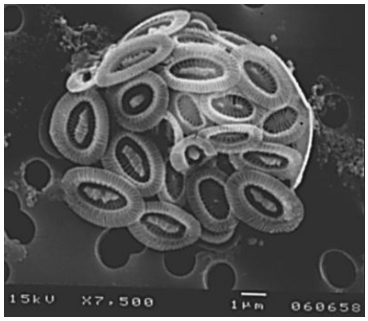
S. molischii type 1



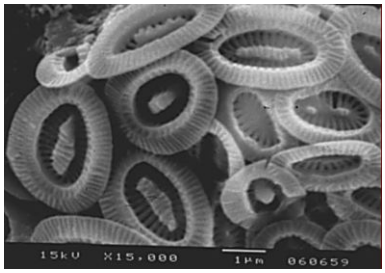
S. molischii type 2



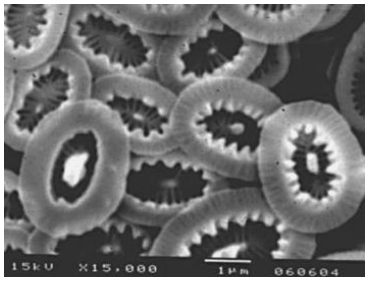
S. molischii type 2



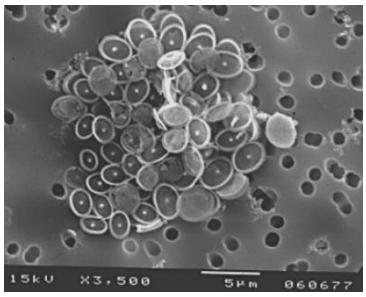
S. molischii type 3



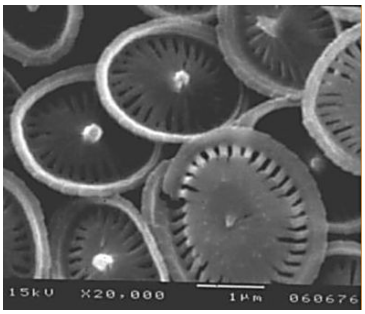
S. molischii type 3



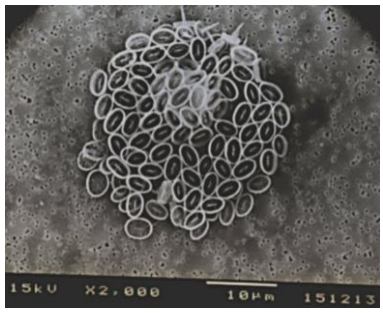
S. molischii type 4



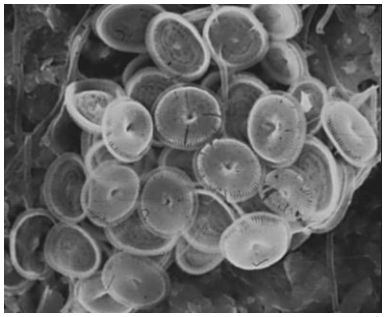
Syracosphaera sp.



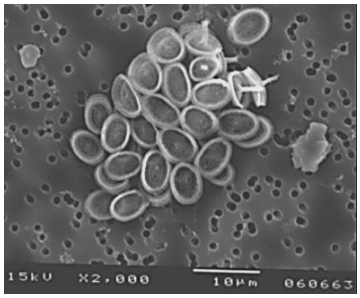
Syracosphaera sp.



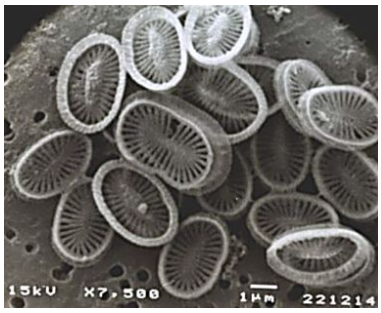
Syracosphaera sp.



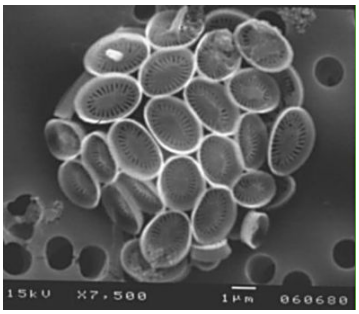
S. pulchra (x3500)



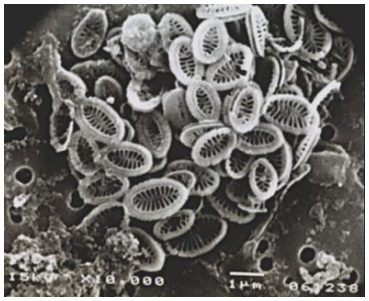
S. pulchra



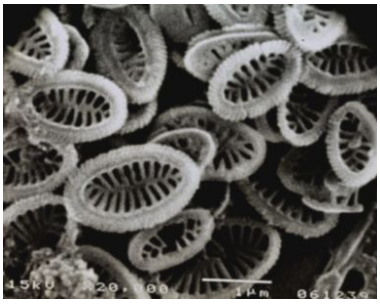
Syracosphaera sp. type D of Kleijne 1993



Syracosphaera sp. type L of Kleijne 1993



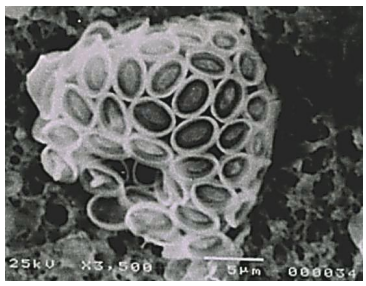
Syracosphaera sp. type I of Kleijne 1993



Syracosphaera sp. type I of Kleijne 1993

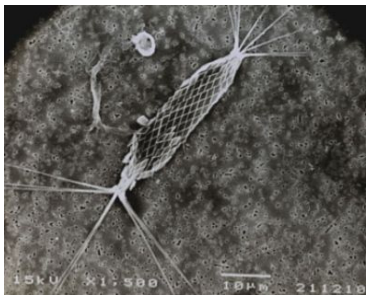
4.3 Syracosphaerales – Genus *incertae sedis Coronosphaera*

C. mediterranea

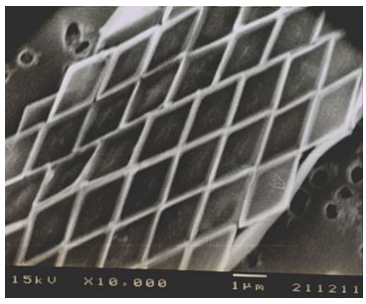


C. maxima

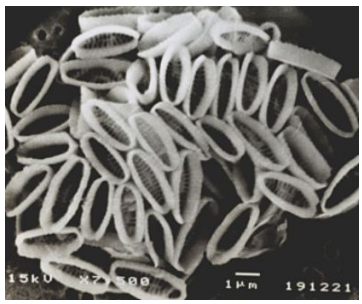
4.4 Calciosoleniaceae



Calciosolenia murrayi

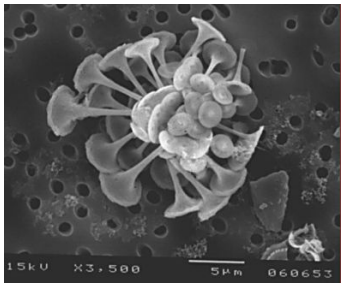


C. murrayi

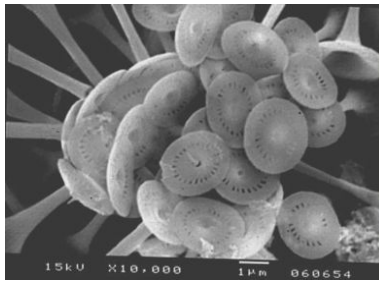


Alveosphaera bimurata

4.5.2 *Discosphaera*

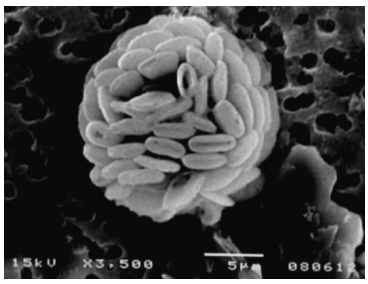


D. tubifera

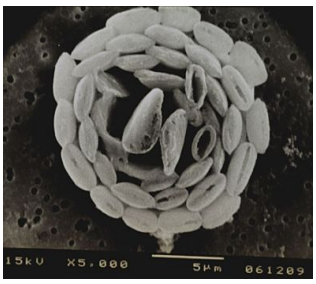


D. tubifera

4.5.3 *Algirosphaera*



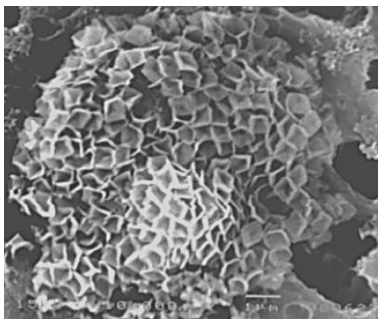
A. robusta



A. robusta

Plate 5 - Heterococcolith families and genera *incertae sedis*

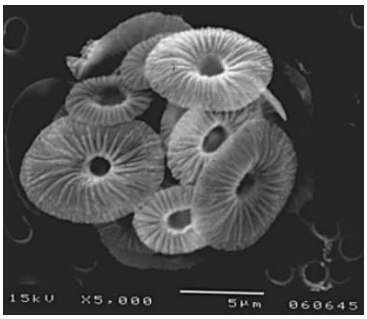
5.1 Alisphaeraceae



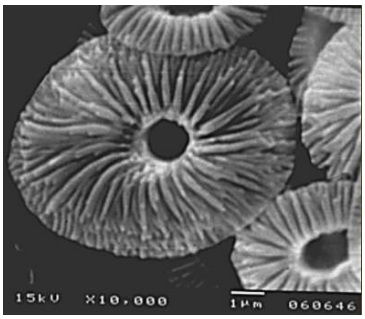
Polycrater galapagensis

5.2 Umbelosphaeraceae

Umbelosphaera tenuis
type II

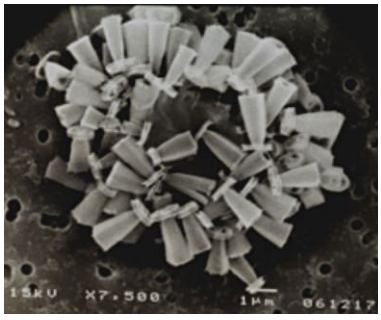


Umbelosphaera tenuis
type IV

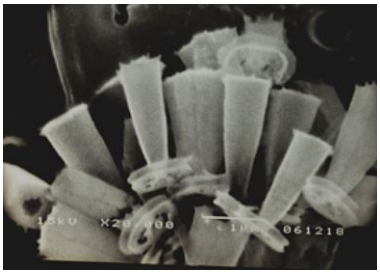


Umbelosphaera tenuis
type IV

5.3 Narrow-rimed placoliths



Turrilithus latericioides

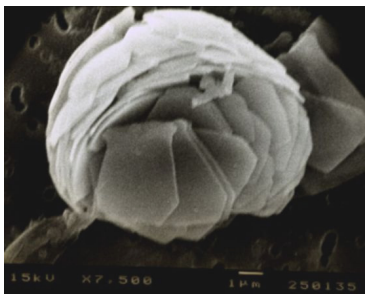


Turrilithus latericioides

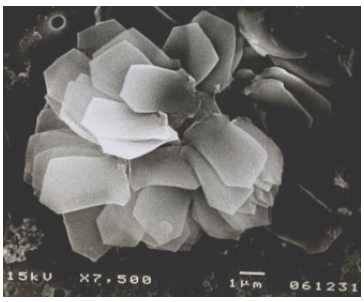
5.4 Papposphaeraceae

Papposphaera lepida

Plate 6 - Nannoliths *incertae sedis*



Florisphaera profunda

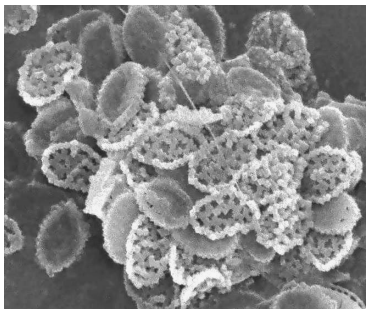


Florisphaera profunda

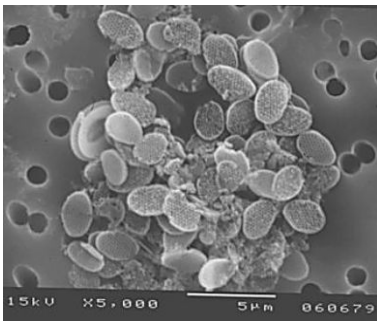
Plate 7 - Holococcoliths (Calyptrosphaeraceae)

7.1 Tubeless planar

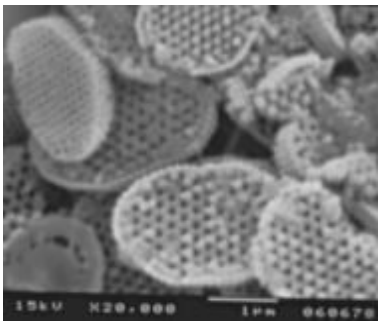
Planar, monomorphic



Coccolithus pelagicus ssp. *pelagicus* HOL ("*Crystallolithus hyalinus*")



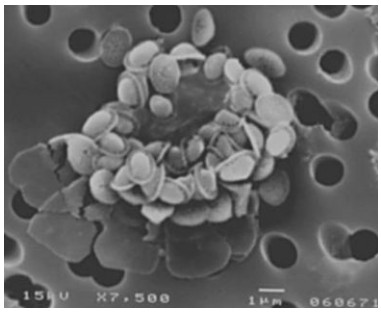
Calcidiscus leptoporus ssp. *leptoporus* HOL ("*Crystallolithus rigidus*")



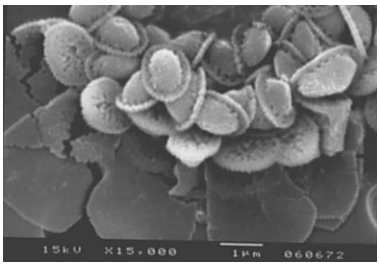
Calcidiscus leptoporus ssp. *leptoporus* HOL ("*Crystallolithus rigidus*")

7.2 Tubeless conical

***Anthosphaera* – fried egg shape, dimorphic**



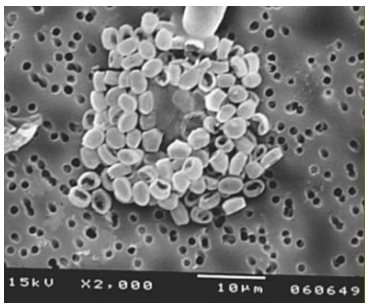
Anthosphaera fragaria



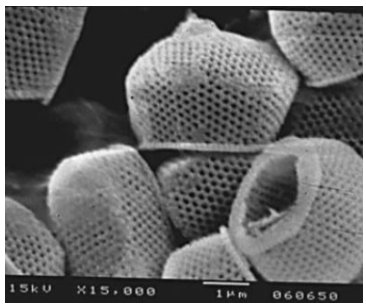
Anthosphaera fragaria

7.3 Convex-covered tube

Convex, regular form



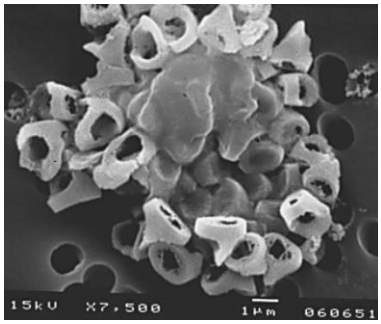
Syracosphaera pulchra HOL *oblonga* type ("*Calyptrosphaera oblonga*")



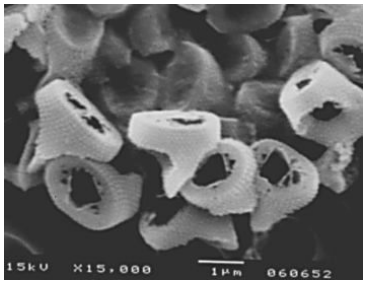
Syracosphaera pulchra HOL *oblonga* type ("*Calyptrosphaera oblonga*")

7.4 BCs Bridged tube

7.4.1 *Homozygosphaera* – monomorphic with zygoliths

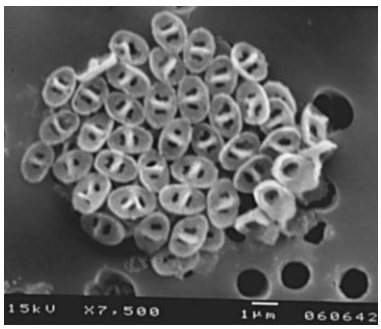


Homozygosphaera spinosa



Homozygosphaera spinosa

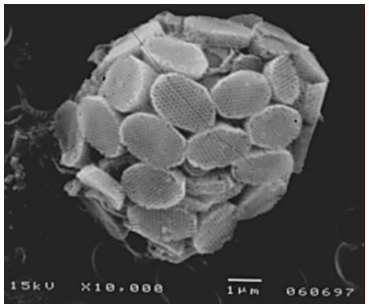
7.4.2 *Corisphaera* – weakly dimorphic



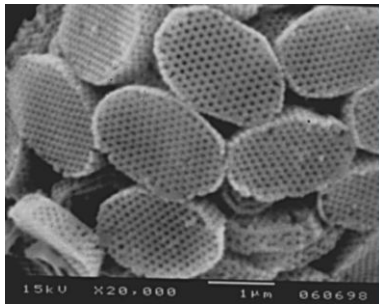
Corisphaera sp.

7.5 Flat-covered tube

7.5.1 *Coronosphaera* holococcoliths and associated species

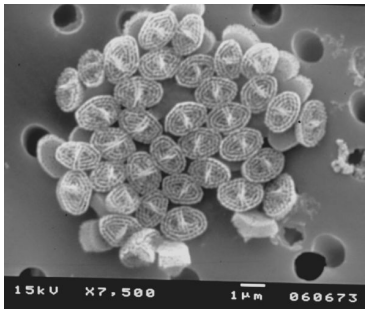


Calyptrolithophora papillifera

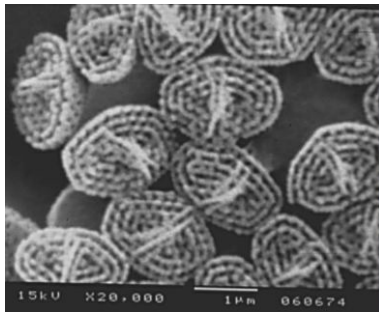


Calyptrolithophora papillifera

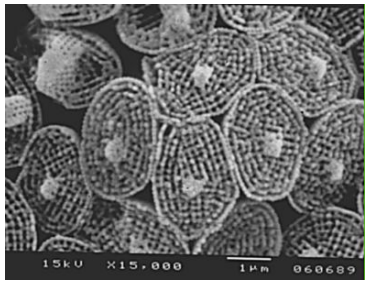
7.5.2 *Zygosphaera* – concentric wall ultrastructure



Zygosphaera marsilii



Zygosphaera marsilii



Zygosphaera hellenica