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Dynamics of phytoplankton communities around the Antarctic Peninsula and off the Portuguese coast

Carlos Rafael Borges Mendes

DOUTORAMENTO EM BIOLOGIA

(ECOLOGIA)

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“*O que sabemos é uma gota;*

o que não sabemos é um oceano.”

(*Sir Isaac Newton*)

Dedico esta tese ao Miguel, à Helena e ao Dinis.

Os “putos” da minha vida.

Dissertação apresentada à

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Carlos Rafael Borges Mendes

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Declaração

Para efeitos do disposto nº2 do Art. 8º do Dec-Lei 388/70, o autor da dissertação declara que interveio na concepção do trabalho experimental, na interpretação dos resultados e na redacção dos manuscritos publicados e submetidos para publicação.

Carlos Rafael Borges Mendes

Dezembro de 2011

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ÍNDICE

ABSTRACT

The study of phytoplankton dynamics is a fundamental tool in environmental assessment and monitoring, studies of trophic relationships and modeling ecosystems. The present study was a rare opportunity to study two different ecosystems, such as a polar zone, the Antarctic, and a temperate zone influenced by upwelling processes, the Portuguese coast. The main objective of this study was to understand the physical-chemical processes related to the phytoplankton communities' distribution in these areas. Particularly, the Nazaré submarine canyon region (coast of Portugal) and around the tip of the Antarctic Peninsula, and characterize their communities through pigment analysis (HPLC) and identifying dominant species by microscopy. A great spatial variability in chlorophyll *a* (Chl *a*) was observed in the Antarctic Peninsula: highest levels in the vicinity of the James Ross Island (exceeding 7 mg m^{-3} in 2009), intermediate values (0.5 to 2 mg m^{-3}) in the Bransfield Strait, and the lowest concentrations in the Weddell Sea and Drake Passage (below 0.5 mg m^{-3}). On the other hand, in the region of Nazaré submarine canyon, a clear onshore-offshore gradient was visible: high Chl *a* concentrations were recorded in the canyon head, near the coast, where values greater than 4 mg m^{-3} were observed; in contrast, Chl *a* was relatively low in offshore regions, with values below 0.5 mg m^{-3}. The use of taxonomic tools such as CHEMTAX software allowed efficient quantification of the contribution of different taxonomic groups present to total Chl *a* (biomass index). This study also showed that the spatial distribution of macronutrients is one of the key factors regulating the distribution of phytoplankton communities in the Nazaré Canyon region, while in the neighboring Antarctic Peninsula, other factors, such as light availability and/or iron distribution, mostly associated with the structure of the water column, determined the spatial composition of phytoplankton communities.

The results presented are important to understand the oceanographic dynamics of these regions, and provide new insights for the overall knowledge of phytoplankton dynamics. Moreover, this work contributed with invaluable *in situ* data, which can be used in ecosystem modeling.

Keywords: Antarctic Peninsula; Portugal coast; Upwelling region; Phytoplankton communities; Phytoplankton Pigments; High-Performance Liquid Chromatography.

RESUMO

O ambiente marinho contém comunidades biológicas muito diversas que estão interligadas em complexas cadeias tróficas. Na base dessas cadeias tróficas encontra-se o fitoplâncton (principal produtor primário dos oceanos), que embora representando menos de 1% da biomassa vegetal do planeta, é responsável por cerca de metade da produção primária na Terra. O estudo da dinâmica do fitoplâncton constitui uma ferramenta fundamental na monitorização e avaliação ambiental, nos estudos das relações tróficas e na modelação dos ecossistemas. A presente dissertação constituiu uma rara oportunidade de estudar dois ecossistemas tão distintos como uma zona polar, a Antártica, e uma zona temperada influenciada por processos de afloramento, a costa portuguesa. Apesar de o oceano Austral ser, usualmente, caracterizado como uma região de elevadas concentrações de nutrientes, mas com baixas concentrações de clorofila *a* (Chl *a*), existem determinadas zonas ao redor da Antártica onde se registam elevadas taxas de produtividade marinha, que servem de importantes áreas de alimentação para herbívoros e que são cruciais para captação de CO_2 atmosférico em termos globais. Por sua vez, as regiões costeiras caracterizadas pelos processos de afloramento, como o caso da costa de Portugal, estão entre os ecossistemas marinhos mais produtivos dos oceanos e são conhecidas por sustentar algumas das regiões de pesca mais importantes do mundo.

O principal objectivo deste estudo foi compreender os processos físico-químicos responsáveis pela distribuição das comunidades de fitoplâncton na região da Península Antártica e na região do canhão da Nazaré (costa de Portugal), caracterizando as respectivas comunidades através da análise

pigmentar por HPLC (*high-performance liquid chromatography,* que se poderá traduzir como cromatografia líquida de elevada precisão) e identificando as espécies dominantes por microscopia. A utilização de ferramentas químico-taxonómicas como o programa CHEMTAX permitiu uma eficiente quantificação da contribuição das diversas classes taxonómicas para o total de Chl *a* (índice de biomassa).

O estudo ecológico efectuado na região da costa de Portugal encontra-se descrito e discutido no Capítulo II. Este trabalho baseou-se em uma campanha oceanográfica efectuada na região do canhão da Nazaré, numa altura do ano em que predominaram ventos favoráveis (quadrante norte) para a ocorrência de processos de afloramento costeiro. Esta situação foi evidenciada pelo surgimento de uma banda de águas mais frias, junto à costa, observada nas imagens de satélite da temperatura da superfície da água do mar. Nesta região da costa de Portugal foram observadas maiores concentrações de Chl *a*, relacionadas com a presença de diatomáceas e/ou dinoflagelados, nas regiões costeiras em associação com a presença de águas mais frias e ricas em nutrientes. Nas regiões mais afastadas da costa, fora da região de afloramento, observou-se um aumento da estratificação, menores concentrações de nutrientes e um total domínio de primnesiófitas, com um incremento na contribuição das cianobactérias nas estações mais oceânicas. Por outro lado, a presença do canhão submarino determinou um diferencial Norte-Sul na circulação dos nutrientes, que proporcionou uma injecção de maiores quantidades de nutrientes (bem visível na distribuição espacial do fosfato) para a região costeira a Sul do canhão, favorecendo o desenvolvimento de diatomáceas nesta área. Os dinoflagelados, por sua vez, competindo com as

diatomáceas por nutrientes e, provavelmente, melhor adaptados a regimes com menores concentrações de fosfato estabeleceram-se numa região a norte da cabeceira do canhão, onde este nutriente foi observado em concentrações muito baixas. Esta região de elevada biomassa (valores de Chl *a* superiores a 4 mg m^{-3}) foi caracterizada por um forte florescimento de um dinoflagelado tóxico formador de cadeias longas – *Alexandrium affine* – em conjunto com outros dinoflagelados como *Ceratium candelabrum*, *Ceratium furca*, *Ceratium fusus*, *Dinophysis acuta* e *Dinophysis caudata*.

O Capítulo III diz respeito ao estudo acerca da distribuição espacial das comunidades de fitoplâncton no entorno da Península Antártica, baseado nos resultados obtidos em dois cruzeiros oceanográficos realizados em dois anos consecutivos (2008 e 2009) no final do verão austral. As maiores biomassas registaram-se nas estações mais costeiras e próximas à Península, que apresentaram uma coluna de água mais homogénea e, possivelmente, uma maior disponibilidade em ferro devido aos processos de mistura. Estas zonas mais costeiras, em especial a região ao redor da ilha de James Ross (onde se registaram valores de Chl *a* superiores a 7 mg m^{-3}), foram associadas com um predomínio de diatomáceas. Em zonas mais oceânicas (mar do Weddell e Passagem de Drake) verificou-se um aumento da estratificação, provavelmente restringindo os níveis de ferro na camada eufótica, o que limitou a biomassa e favoreceu o crescimento de organismos nanoplanctónicos, como as criptófitas e/ou *Phaeocystis antarctica* (primnesiófita). A utilização de índices pigmentares permitiu avaliar alguns processos fisiológicos das comunidades de fitoplâncton em resposta a determinadas condições ambientais (disponibilidade de ferro, luz e/ou herbivoria). Esta informação serviu para uma melhor compreensão dos

complexos processos responsáveis pela dinâmica do fitoplâncton ao redor da Península Antártica.

A região do Estreito de Bransfield apresentou uma grande variabilidade espacial e temporal das suas características físico-químicas devido, em parte, a uma influência repartida de águas provenientes do mar de Weddell e do mar de Bellingshausen. Este facto reflectiu-se na grande variabilidade observada na distribuição da biomassa (concentrações de Chl *a* entre 0.5 e 2 mg m^{-3}) e, consequentemente, das comunidades de fitoplâncton.

Na sequência desta grande variabilidade observada para o Estreito de Bransfield e juntando uma terceira campanha oceanográfica realizada em 2010, na mesma época do ano (final do verão austral) e nas mesmas estações de amostragem realizadas em 2008 e 2009, efectuou-se um estudo específico para a região do Estreito de Bransfield, apresentado no Capítulo IV, sobre a variabilidade interanual das comunidades de fitoplâncton (variação na dominância dos principais grupos taxonómicos) em relação às condições físico-químicas e climáticas observadas. Foi ainda possível associar o início do degelo marinho, para a região do Estreito de Bransfield, com as variações observadas nas comunidades de fitoplâncton no final do verão austral (período em que se realizaram as amostragens). Tal como já observado por diversos autores para outras regiões ao redor da Península Antártica, uma alteração no início e/ou intensidade do degelo marinho causa um desfasamento (atraso ou antecipação) na natural sucessão fitoplanctónica da região. No ano de 2010, onde se verificaram temperaturas atipicamente frias durante o verão austral, o início/intensidade do degelo (gatilho fundamental para o desenvolvimento dos primeiros florescimentos de diatomáceas) foi de tal forma atrasado que resultou

em valores de biomassa bastante reduzidos durante todo o verão austral de 2010. Além disso, este processo resultou em um domínio de criptófitas em detrimento das diatomáceas, naquele ano, com presumíveis implicações para toda a cadeia trófica marinha da região.

Palavras-chave: Península Antártica; costa de Portugal; sistema de afloramento; fitoplâncton; pigmentos fitoplanctónicos; cromatografia líquida de elevada eficiência.

CAPÍTULO I

Introdução

1.1. Introdução geral

O ambiente marinho contém comunidades biológicas muito diversas que estão interligadas em complexas cadeias tróficas. Na base dessas cadeias tróficas encontra-se o fitoplâncton (principal produtor primário dos oceanos), que embora representando menos de 1% da biomassa vegetal do planeta, é responsável por cerca de metade da produção primária na Terra [Falkowski, 2002]. O fitoplâncton desempenha um papel fundamental na regulação dos níveis de dióxido de carbono (CO_2) nas camadas superficiais do oceano, através dos processos fotossintéticos, influenciando as trocas entre oceano e atmosfera. O recente conceito de "bomba biológica" ilustra o papel do fitoplâncton no sequestro de CO_2, através da qual ocorre a sedimentação das células fitoplanctónicas e outros detritos orgânicos para o fundo oceânico; ou seja, existe um transporte de carbono para o leito oceânico e sua consequente retirada do ciclo superficial marinho por longos períodos [Falkowski, 2002]. Estima-se que diariamente o fitoplâncton seja responsável pela absorção de aproximadamente 100 milhões de toneladas de carbono da atmosfera [Behrenfeld et al., 2006].

O fitoplâncton cresce nas camadas mais superficiais do oceano (i.e., na zona eufótica, onde a intensidade da luz decresce até 1% daquela existente à superfície), necessitando de luz e nutrientes para se desenvolver. Os nutrientes, quando esgotados na superfície, são repostos principalmente por processos físicos como o afloramento ("upwelling"), turbulência e outros. Numa situação de abundância de nutrientes e de luz favorável, estes organismos podem-se multiplicar rapidamente desencadeando um florescimento ou

"bloom". Estes episódios podem ser sazonais (algumas semanas) ou pontuais (alguns dias) [Cloern, 1996]. Durante este processo existe uma sucessão de eventos transitórios, com várias magnitudes, que envolvem um número variado de espécies e grupos taxonómicos de fitoplâncton. Os mecanismos que controlam o início, a magnitude e a duração dos florescimentos podem ser bastante diversificados consoante o tipo de ecossistema em estudo [Townsend et al., 1994].

As alterações climáticas podem gerar significativas alterações nos padrões conhecidos de sucessão do fitoplâncton marinho. Estas questões têm sido objecto de muitos, e importantes, trabalhos científicos, mas nem todos com resultados concordantes. Utilizando técnicas de detecção remota, registou-se uma diminuição na produção primária líquida total durante a última década, que se relacionou com o recente aumento da temperatura da superfície dos oceanos [Behrenfeld et al., 2006]. Por outro lado, de acordo com Doney [2006], esta variação da temperatura influenciará os processos de mistura das águas, o que irá favorecer, por um lado, o crescimento do fitoplâncton nas zonas polares e, por outro, uma diminuição nos trópicos e nas zonas de latitude média. Outro aspecto que tem vindo a ser associado às mudanças climáticas é a antecipação dos florescimentos de primavera. Edwards & Richardson [2004], utilizando séries de observações microscópicas de longo termo, verificaram a antecipação no florescimento de diversas espécies fitoplanctónicas, com consequências no crescimento de espécies de zooplâncton e toda a sua implicação na restante cadeia trófica. Por outro lado, Kahru et al. [2011], utilizando imagens de satélite, verificaram uma antecipação do florescimento de primavera no Ártico, estimado em 50 dias para o período

entre 1997 e 2009. O desenvolvimento de programas de monitorização do fitoplâncton em escala global é crucial na identificação de futuras alterações nos ecossistemas marinhos [Hays et al., 2005].

Neste trabalho efectuaram-se estudos sobre o fitoplâncton e as variáveis ambientais que influenciam sua dinâmica em dois ecossistemas bastante produtivos que apresentam particularidades bastante distintas: a região Antártica e a zona costeira de Portugal.

A região Antártica, embora remota e isolada geograficamente, exerce uma profunda influência no clima do planeta e, por consequência, nos ecossistemas e na sociedade. As comunidades marinhas antárticas são reconhecidas por um alto nível de endemismo, que em contraste com as áreas emersas, podem atingir, em determinadas regiões, biomassa e biodiversidade elevadas [Brandt et al., 2007]. Como tal, esta região é um laboratório excepcional e único no planeta para o estudo de processos evolutivos e adaptativos dos diversos organismos nela existentes.

O oceano Austral, regra geral, é habitualmente caracterizado como sendo uma região com elevados teores em macronutrientes, mas com baixas concentrações de clorofila *a* (representando a biomassa fitoplanctônica) [Chisholm & Morel, 1991]. Considerando as águas ricas em macronutrientes do oceano Austral, a luz actua como o principal factor limitante durante a primavera austral [Smith & Gordon, 1997], enquanto os baixos teores de ferro como variável limitativa da produção primária durante o verão [Sedwick & DiTullio, 1997]. Além disso, é possível que ocorra uma co-limitação de ambos os factores [Tremblay & Smith, 2007], uma vez que o ferro e a limitação de luz interagem entre si [Sunda & Huntsman, 1997; Sedwick et al., 2007]. No

entanto, existem determinadas regiões do oceano Austral onde se têm observado elevadas biomassas fitoplantónicas, caracterizando florescimentos na primavera e/ou verão. Estas áreas são tipicamente associadas com: (1) a estabilidade sazonal da camada de mistura, provocada pela camada superficial de baixa salinidade devido ao descongelamento do gelo marinho nas zonas marginais [Smith & Nelson, 1986; Mitchell & Holm-Hansen, 1991; Holm-Hansen et al., 1989]; (2) os principais sistemas oceânicos frontais, gerados por intrusão de água da Corrente Circumpolar Antártica [Boyd et al., 1995; Savidge et al., 1995] e/ou ressurgência da Água Profunda Circumpolar Superior [Prézelin et al., 2000, 2004]; (3) as condições meteorológicas favoráveis, tais como baixa intensidade de vento [Lancelot et al., 1993; Smith et al., 1998]; e (4) a formação de gelo marinho [Smetacek et al., 1992]. Estes florescimentos são controlados principalmente pela luz [van Oijen et al., 2004; Smith et al., 2000], micronutrientes como o ferro [Martin et al., 1990; Coale et al., 2004; Hare et al., 2007], herbivoria por microzooplâncton [Burkill et al., 1995] e/ou tempestades [Mitchell & Holm-Hansen, 1991; Fitch & Moore, 2007]. Durante estes períodos de alta produção no oceano Austral, o fitoplâncton funciona como um importante meio de captação de CO_2 e, além da sedimentação direta, processos como excreção celular, lise e herbivoria podem retirar das camadas superiores da coluna da água grandes quantidades de material orgânico, num fluxo superior a 1-2 g C m^{-2} d^{-1} [Fischer et al., 2002].

A região Antártica estudada neste trabalho engloba a ponta da Península Antártica, que abrange o Estreito de Bransfield, parte da Passagem de Drake e o Noroeste do mar de Weddell (Fig. 1). No lado leste da Península Antártica, particularmente nas zonas ao redor do gelo marinho no mar de

Weddell, extensos florescimentos de fitoplâncton têm sido detectados durante a primavera e verão [Sullivan et al., 1993; Park et al., 1999; Kang et al., 2001], constituindo importantes áreas de alimentação para herbívoros. Além disso, as baías e zonas rasas a sudoeste do Estreito de Bransfield funcionam como extraordinários viveiros para uma série de organismos, especialmente o *krill* [Zhou et al., 1994], como resultado das elevadas biomassas sazonais fitoplanctónicas observadas nessas regiões [e.g., Karl et al., 1991; Castro et al., 2002].

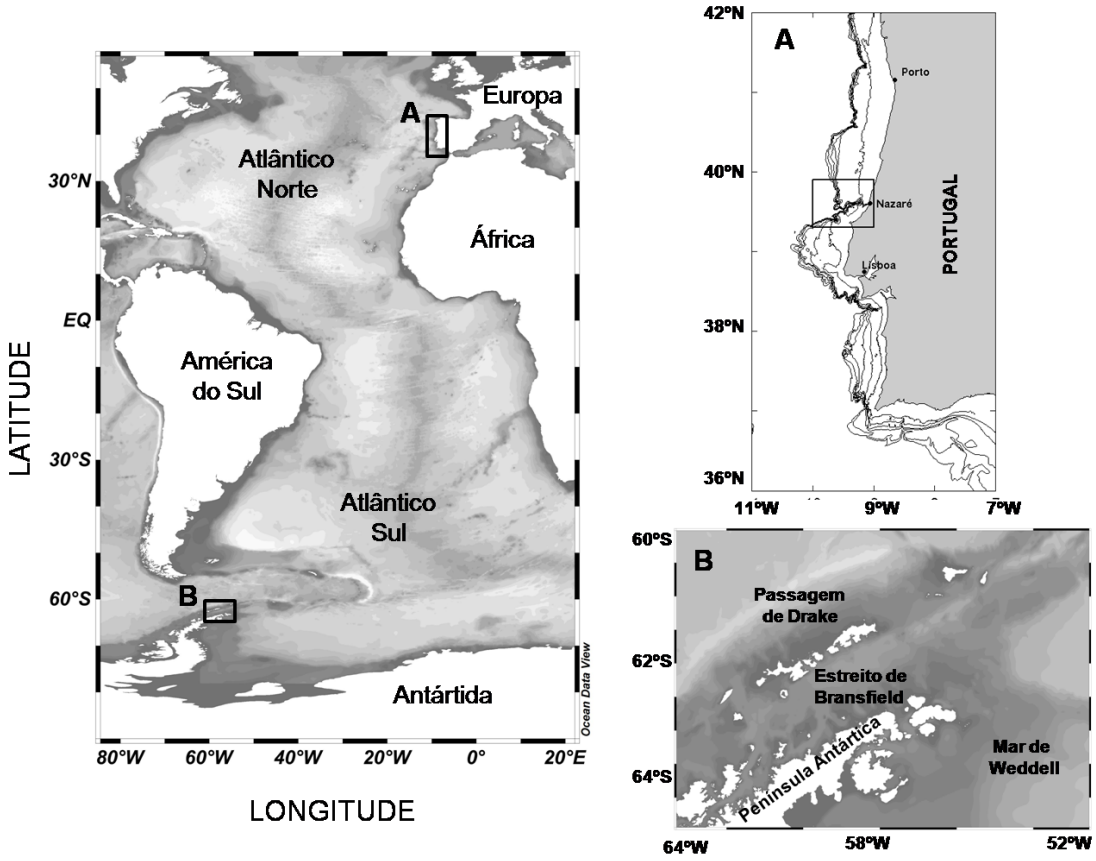

Figura 1: Localização geográfica das regiões de estudo. (A) Região do canhão da Nazaré, Portugal; (B) região da Península Antártica, Antártica.

Na região Antártica existem zonas, tais como a Península Antártica, onde as alterações climáticas têm sido bastante acentuadas, tendo-se registado um aquecimento gradual e significativo, tanto da atmosfera como das

águas superficiais e subsuperficiais, ao longo das últimas décadas [Meredith & King, 2005; Clarke et al., 2007]. Como consequência destas alterações climáticas regionais, têm sido observadas importantes mudanças na distribuição e composição das comunidades desta região (e.g., fitoplâncton, krill e pinguins) [Smith et al., 1999, 2001; Montes-Hugo et al., 2009].

Por sua vez, a zona costeira de Portugal é um ecossistema particularmente caracterizado por processos de afloramento. No fenômeno de afloramento costeiro, as águas superficiais afastam-se da costa em direção ao largo, sendo substituídas por águas mais frias e ricas em nutrientes dissolvidos (nitratos, fosfatos e silicatos). Isto acontece porque as águas subsuperficiais, que estão a ser transportadas para a superfície, possuem maior concentração desses nutrientes do que as próprias águas da superfície, que estão depletas devido ao consumo por parte do fitoplâncton. Desta forma, existe um transporte de nutrientes para a zona eufótica permitindo as condições ideais, de luz e nutrientes, para o desenvolvimento do fitoplâncton. Este aumento da produtividade primária provoca o desenvolvimento de toda a restante cadeia alimentar marinha. Em geral, estas zonas de afloramento são mosaicos múltiplos e variáveis, que apresentam uma grande heterogeneidade espacial e temporal das suas propriedades físicas e químicas [Kudela et al., 2005; Lachkar & Gruber, 2011]. A dinâmica do processo de afloramento costeiro varia com o regime dos ventos dominantes e com as diferenças batimétricas e topográficas existentes, como por exemplo, a extensão da plataforma continental, a existência de canhões submarinos e de acentuadas descontinuidades da linha de costa [Crépon et al., 1984; Kudela et al., 2005; Ryan et al., 2005]. Os sistemas de afloramento estão entre os ecossistemas

marinhos mais produtivos dos oceanos e são conhecidos por sustentar algumas das regiões de pesca mais importantes do mundo [Bakun, 1990; Pauly & Christensen, 1995; Carr & Kearns, 2003]. Apesar de representarem menos de 1% da área total dos oceanos, as regiões costeiras de afloramento são responsáveis por cerca de 11% da produção primária marinha [Chavez & Toggweiler, 1995] e cerca de 20% da captura mundial de pescado [Pauly & Christensen, 1995].

Ao longo da costa oeste portuguesa, o regime de ventos induz diferentes padrões de afloramento, que estão relacionados com as características morfológicas da costa, com a batimetria da plataforma continental/talude superior e com o regime dos ventos locais [Fiúza, 1983; Relvas et al., 2007]. Geralmente, os processos de afloramento ocorrem sazonalmente, desde Abril até Setembro, sob ventos do quadrante norte (predominantes nessa altura do ano), enquanto que processos de advecção de águas oceânicas oligotróficas são observados durante o outono e inverno, quando os ventos sul passam a dominar [Fiúza et al., 1982; Peliz et al., 2005]. No entanto, episódios de inversão no regime de ventos podem ocorrer durante ambos os períodos.

O afloramento costeiro tem sido identificado como a maior fonte de variabilidade sazonal e espacial do fitoplâncton na costa portuguesa, associado à disponibilidade de nutrientes na região eufótica e com alterações da estabilidade da coluna de água [e.g., Moita, 2001; Silva et al., 2009]. Trabalhos prévios na costa portuguesa baseados em dados de satélite [Fiúza, 1983; Haynes et al., 1993; Sousa & Bricaud, 1992; Peliz & Fiúza, 1999; Oliveira et al., 2009] têm evidenciado, durante o verão, padrões de temperatura superficial da água do mar e de clorofila *a* similares aos de outras regiões típicas de

afloramento, caracterizadas pela ocorrência periódica (na costa e em direção a regiões mais oceânicas) de filamentos de águas mais frias e ricas em clorofila *a*. Entre os vários locais onde estes filamentos aparecem recorrentemente, a área central da costa de Portugal (entre os 38°N e 40°N) tem sido assinalada como uma região propícia ao desenvolvimento de florações de algas nocivas [Moita et al., 2003; Amorim et al., 2004]. Esta é uma região com grandes alterações na orientação da linha de costa, com acentuadas irregularidades topográficas e com influência de um grande rio (o rio Tejo), proporcionando complexos padrões de correntes superficiais com centros activos de afloramento e, também, de zonas mais resguardadas e menos turbulentas [Moita et al., 2003]. Episódios de florescimentos de diatomáceas e/ou dinoflagelados têm sido associados à dinâmica dos processos de afloramento que caracterizam a circulação oceânica ao sul do cabo da Roca [Oliveira et al., 2009]. As diferentes condições hidrológicas e ecológicas resultantes desta dinâmica têm sido indicadas como os principais mecanismos de distribuição de determinadas espécies típicas desta região. Amorim et al. [2004] observaram, ao estudar a distribuição de quistos de dinoflagelados, distintos nichos ecológicos de *Gymnodinium catenatum* e de *Lingulodinium polyedrum* que foram relacionados com a presença das plumas de afloramento. Por exemplo, o desenvolvimento de *G. catenatum* (espécie não endémica, introduzida no início do século passado na região da costa portuguesa [Amorim & Dale, 2006]) é beneficiado por mecanismos físicos que favorecem a sua acumulação em regiões situadas entre as plumas de afloramento e a zona costeira, normalmente relacionadas com a presença de correntes oceânicas menos intensas [Moita et al., 2003].

A região da costa portuguesa abordada neste estudo é caracterizada pela presença do maior desfiladeiro submarino da Europa, denominado canhão da Nazaré, que possui uma extensão de cerca de 200 km e atinge os 5000 m de profundidade nas regiões mais abissais (ver Fig. 1). Trabalhos baseados em dados físico-químicos e geológicos têm revelado uma interferência do canhão submarino nos processos de afloramento costeiro desta região [Vitorino, 2005; Oliveira et al., 2007], nomeadamente na dinâmica da distribuição de nutrientes para as camadas superficiais da coluna de água. Esta é uma região ainda pouco estudada quanto à dinâmica das comunidades de fitoplâncton, apesar de alguns estudos pontuais já terem registado, por exemplo, a presença de dinoflagelados potencialmente tóxicos nesta região (e.g., *Dinophysis acuta*, *Dinophysis caudata* e *Alexandrium affine*) [Moita, 2001]. Contudo, sem associação directa à presença do canhão submarino.

Ao longo dos últimos anos, as técnicas tradicionais, através do uso da microscopia ótica, usadas no estudo das comunidades de fitoplâncton têm vindo a ser complementadas por técnicas mais expeditas e analíticas, tais como o uso de biomarcadores taxonómicos e/ou técnicas de detecção remota. Neste contexto, a análise dos pigmentos do fitoplâncton por cromatografia líquida de elevada eficiência (HPLC, *high-performance liquid chromatography*) tem-se revelado uma importante ferramenta, muito utilizada em estudos sobre dinâmica do fitoplâncton, na quantificação da biomassa e sua composição em classes taxonómicas [Mackey et al., 1996; Schlüter et al., 2000]. O programa CHEMTAX (CHEMical TAXonomy, [Mackey et al., 1996]), método químico-taxonómico usado neste trabalho, é um método que tem sido amplamente utilizado na determinação da composição do fitoplâncton em várias regiões do

globo, incluindo o oceano Austral [e.g., Mackey et al., 1998; Schlüter et al., 2000; Carreto et al., 2008; Wright et al., 2009, 2010]. Além de ser um método de fácil reprocessamento (caso seja necessário), o CHEMTAX permite estimar a composição do fitoplâncton em conjuntos numerosos de amostras, cuja determinação seria impraticável utilizando-se técnicas mais tradicionais, tal como a microscopia. Os resultados obtidos pela utilização do CHEMTAX apresentam uma forte correlação com os dados de microscopia e, em alguns casos, têm revelado a presença de grupos não detectados por esses métodos tradicionais (e.g., criptófitas, [Wright et al., 1996; Havskum et al., 2004]). As principais precauções na utilização destes métodos químico-taxonómicos estão relacionadas com a presença de pigmentos partilhados por vários grupos taxonómicos e a possíveis flutuações das razões pigmentares entre espécies de um mesmo grupo e/ou dentro da própria célula, sob a influência de vários parâmetros ambientais como a luz e/ou a disponibilidade em nutrientes [Jeffrey, 1981; Goericke & Montoya, 1998; Wright & Jeffrey, 2006; DiTullio et al., 2007]. No entanto, com as devidas precauções acerca das implicações destes factores e com um conhecimento mínimo acerca das populações das regiões de estudo [Irigoien et al., 2004], o programa CHEMTAX pode ser considerado um método bastante viável e confiável para a determinação da composição das comunidades de fitoplâncton [Mackey et al., 1998].

O desenvolvimento desta Tese permitiu à equipa científica brasileira do grupo GOAL (Grupo de Oceanografia de Altas Latitudes), que tem desenvolvido estudos detalhados na região da Patagónia [e.g., Garcia et al., 2008; Ferreira et al., 2009; Garcia et al., 2011], investigar a ecologia do fitoplâncton numa região Antártica e, simultaneamente, introduzir no grupo uma

nova metodologia a aplicar ao estudo das comunidades de fitoplâncton – a análise dos pigmentos fitoplanctónicos por HPLC. Por outro lado, o estudo na região do canhão da Nazaré (costa de Portugal), região ainda pouco estudada no que respeita a distribuição das comunidades de fitoplâncton, permitiu aprofundar o conhecimento existente sobre a dinâmica populacional do fitoplâncton marinho nas regiões sob influência de processos de afloramento ao longo da costa de Portugal.

1.2. Objectivos

O principal objectivo deste estudo foi compreender os processos físico-químicos responsáveis pela distribuição das comunidades de fitoplâncton na região da Península Antártica e na costa de Portugal.

Os objectivos específicos foram os seguintes:

1) Estudar os principais mecanismos responsáveis pela distribuição dos grupos taxonómicos do fitoplâncton nas duas regiões em estudo;
2) Caracterizar as comunidades de fitoplâncton utilizando a análise pigmentar por HPLC, complementada por observações ao microscópio;
3) Testar o uso dos pigmentos fotossintéticos no desenvolvimento de índices que reflictam o estado fisiológico das comunidades de fitoplâncton relativamente a diferentes condições ambientais.

1.3. Estrutura da tese

Os objectivos específicos desta Tese são abordados em estudos independentes que se encontram nos capítulos seguintes (**II**, **III** e **IV**) sob a forma de trabalhos publicados ou em processo de publicação. Desta forma, o **Capítulo II** intitulado "*Spatial distribution of phytoplankton assemblages in the Nazaré submarine canyon region (Portugal): HPLC-CHEMTAX approach*" procura responder aos objectivos específicos 1 e 2, no que se refere à região da costa de Portugal; o **Capítulo III**, "*Dynamics of phytoplankton communities during late summer around the tip of the Antarctic Peninsula*", engloba todos os três objectivos para a região da Península Antártica; e o **Capítulo IV**, "*Cryptophytes dominated diatoms in the Bransfield Strait (Antarctic Peninsula) in the late summer 2010*", atende aos objetivos 1 e 2, numa tentativa de compreender e associar a variabilidade interanual observada nas comunidades de fitoplâncton da região da Península Antártica à variação dos factores abióticos e/ou climáticos. A síntese dos principais aspectos metodológicos é apresentada no tópico seguinte (1.4.) enquanto o **Capítulo V** corresponde à conclusão geral desta Tese.

1.4. Metodologias usadas

1.4.1. Amostragem

A amostragem, em ambos os ecossistemas, foi efectuada através de sistemas de carrossel com garrafas de "*Niskin*" existentes nos navios utilizados durante as campanhas oceanográficas. Conjuntamente, foram determinados

perfis verticais de temperatura, salinidade e fluorescência a partir de sensores acoplados ao sistema CTD (*Conductivity*, *Temperature*, *Depth*). Amostras de água foram recolhidas a diferentes profundidades para determinação da concentração de nutrientes, pigmentos fitoplanctónicos (clorofilas e carotenóides) e para determinação da composição taxonómica, através de microscopia óptica.

Na região da Antártica foram realizados três cruzeiros oceanográficos no final do verão austral (Fevereiro/Março de 2008, 2009 e 2010) junto à plataforma continental da Península Antártica, com estações efectuadas no Estreito de Bransfield, Passagem de Drake, e noroeste do mar de Weddell (ver Fig. 1). Este trabalho está inserido dentro do projecto internacional, multi-institucional e interdisciplinar denominado SOS-CLIMATE (*Southern Ocean Studies for Understanding Global Climate Issues*) e coordenado por investigadores da Universidade Federal do Rio Grande (FURG), Brasil, tendo decorrido no âmbito do Ano Polar Internacional (Março de 2007 a Março de 2009).

Na zona costeira portuguesa foi realizada uma única campanha oceanográfica (Junho/Julho de 2006), na região do Canhão da Nazaré (ver Fig. 1), utilizando um cruzeiro oceanográfico que decorreu no âmbito do projecto HERMES (*Hotspot Ecosytem Research on the Margins of European Seas*), coordenado por investigadores do Instituto Hidrográfico (IH), Portugal.

No total, foram efectuadas amostragens em 262 estações oceanográficas, tendo sido amostradas, e posteriormente analisadas cerca de 700 amostras para determinação, por HPLC, dos diversos pigmentos fotossintéticos.

1.4.2. Análise de nutrientes

A determinação da concentração de nutrientes inorgânicos dissolvidos (nitrato, nitrito, amónia, silicato e fosfato) nas amostras de água, recolhidas em várias profundidades, foi efectuada recorrendo a métodos colorimétricos utilizando analisadores automatizados. As amostras recolhidas na região da costa portuguesa foram armazenadas (no navio) a uma temperatura de -20ºC e posteriormente analisadas na Divisão de Química e Poluição do Meio Marinho do Instituto Hidrográfico, Portugal (para maiores detalhes ver capítulo II). Por sua vez, as amostras referentes à região da Península Antártica foram recolhidas e imediatamente processadas, sob responsabilidade de investigadores do Laboratório de Biogeoquímica do Instituto de Biologia da Universidade Federal do Rio de Janeiro, em laboratório instalado no próprio navio (para maiores detalhes ver capítulo IV).

1.4.3. Determinação dos pigmentos fitoplanctónicos por HPLC

Nas estações oceanográficas realizadas recolheram-se amostras de água (com um volume aproximado entre 1 e 5L, dependendo da biomassa presente), que foram imediatamente filtradas, utilizando-se filtros Whatman GF/F (0.7 µm de poro). Os filtros foram prontamente congelados em azoto líquido e armazenados a -80ºC até serem processados em laboratório. A extracção dos pigmentos foi efectuada com 2/3 mL de metanol tamponizado (com 2% de acetato de amónio). Durante o processo de extracção, procedeu–se à maceração do filtro com a ajuda de uma vareta de vidro,

submeteu-se a um banho de ultrassons durante 1 minuto e deixou-se a extrair durante 30 minutos a -20ºC. Em seguida centrifugou-se a 2500 rpm, durante 15 minutos, a uma temperatura de 4ºC. Retirou-se o material sobrenadante e, antes de se proceder à injecção no HPLC, filtrou-se usando filtros de membrana Millipore (0.2 µm de poro). O aparelho utilizado foi um HPLC Shimadzu, que inclui um módulo distribuidor de solventes (LC-10ADVP) com um sistema de controlo (SCL-10AVP), um detector de fotodiodos (SPDM10AVP) e um detector de fluorescência (RF-10AXL). A separação cromatográfica dos pigmentos foi efectuada seguindo duas metodologias distintas que acompanharam o desenvolvimento da técnica, no laboratório do Instituto de Oceanografia da Universidade de Lisboa, ao longo deste estudo. Desta forma, o processamento das amostras recolhidas durante o cruzeiro efectuado na costa portuguesa seguiu uma metodologia diferente (método C18; Kraay et al. [1992], adaptada por Brotas & Plante-Cuny [1996]) da usada no processamento das amostras resultantes das campanhas na região da Península Antártica (método C8; Zapata et al. [2000]). As vantagens/desvantagens de ambos os métodos estão amplamente descritas e discutidas em Mendes et al. [2007]. Adicionalmente, e numa fase mais recente, foi utilizado um padrão interno (Trans-β-apo-8'-carotenal) no processamento das amostras (cruzeiro oceanográfico de 2010 realizado na Península Antártica), de forma a possibilitar a quantificação de quaisquer anomalias resultantes de todo o processo de extracção/separação dos pigmentos. As características da fase móvel (solventes) e da fase estacionária (coluna) de ambos os métodos encontram-se resumidas, respectivamente, na Tabela 1 e 2. A identificação e quantificação dos picos referentes aos pigmentos

fotossintéticos foram realizadas usando como referência padrões comerciais da DHI (*Institute for Water and Environment, Denmark*). A concentração foi calculada a partir do sinal obtido pelo detector de fotodiodos e/ou pelo detector de fluorescência, para o caso dos pigmentos clorofilianos.

Tabela 1: Gradientes e composição das fases móveis utilizadas pelas duas metodologias (método C18 e C8).

		Solventes		
	Tempo (min)	A* Metanol:água (85:15 v/v) %	B Acetonitrilo:água (90:10 v/v) %	C Acetato de etilo %
Método C18	0	60	40	0
	2	0	100	0
	7	0	80	20
	17	0	50	50
	21	0	30	70
	28.5	0	30	70
	29.5	0	100	0
	30.5	60	40	0
	35	60	40	0
	Tempo (min)	A Metanol:acetonitrilo:piridina aquosa† (50:25:25 v/v/v) %	B Metanol:acetonitrilo:acetona (20:60:20 v/v/v) %	
Método C8	0	100	0	
	20	60	40	
	26	5	95	
	38	5	95	
	40	100	0	

* Solvente A tamponizado com 0.5 M de acetato de amónia (concentração final)

† Solução aquosa de piridina (0.25 M) com pH ajustado a 5.0 pela adição de ácido acético

Tabela 2: Características das colunas cromatográficas usadas neste trabalho.

Coluna	Tipo	Dimensões (mm)	Tamanho do poro (Å)	Tamanho das partículas (µm)	Área da superficie (m^{-2} g^{-1})	Carbono (%)
Supelcosil LC-18 **(método C18)**	Monomérica C18 (Octadecilsílica)	250 × 4.6	100	5	170	11
Symmetry C8 **(método C8)**	Monomérica C8 (Octilsílica)	150 × 4.6	100	3.5	337	12.27

1.4.4. CHEMTAX

A quantificação da contribuição dos principais grupos taxonómicos para o total de clorofila *a* foi efectuada utilizando a versão 1.95 do programa CHEMTAX [Mackey et al., 1996; Wright et al., 1996, 2009]. Este programa utiliza um processo interativo de factorização matricial de forma a optimizar a associação entre os diferentes pigmentos presentes e a determinar a mais adequada composição em grupos taxonómicos [Mackey et al., 1996]. Desta forma, e com o objectivo de uma maior precisão na atribuição da clorofila *a* pelos diferentes grupos de fitoplâncton, o CHEMTAX requer uma matriz de entrada que contenha as classes esperadas (informação obtida através de uma prévia visualização ao microscópio e/ou através da presença de determinado tipo de pigmentos e suas respectivas associações) e as razões de pigmentos iniciais dessas mesmas classes. Usando o processo interativo numa dada matriz, o software optimiza as razões dos pigmentos para cada grupo e aplica uma razão final para o total de clorofila *a*, de forma a determinar a composição em cada amostra. Geralmente, a construção da matriz das razões pigmentares de entrada é baseada em trabalhos já publicados que utilizam a mesma metodologia em regiões geograficamente semelhantes. Esta informação pode, também, ser complementada através de razões pigmentares obtidas em trabalhos laboratoriais com culturas de espécies/grupos similares. No presente trabalho, as matrizes de entrada foram adaptadas de outras matrizes já disponíveis na bibliografia e desenvolvidas em regiões próximas às abordadas neste estudo. Detalhes sobre o procedimento de construção destas matrizes

encontram-se descritos/explicados no capítulo II (para a região do canhão da Nazaré) e no capítulo III (para a região da Península Antártica).

Para cada região em estudo foram aplicadas abordagens diferentes de utilização do programa CHEMTAX e baseadas em procedimentos descritos na bibliografia [Latasa, 2007; Wright et al., 2009]. Estas abordagens estão detalhadamente descritas nos respectivos capítulos (II e III).

Os dados de saída foram obtidos em termos de valores absolutos (mg m^{-3}) de clorofila *a* atribuídos a cada grupo taxonómico, ou como uma quantidade relativa (percentual) do total de clorofila *a* de uma amostra.

1.4.5. Análises de microscopia

As análises microscópicas efectuadas nas amostras seleccionadas da costa de Portugal tiveram apenas um carácter qualitativo, de forma a identificar espécies-chave (para maiores detalhes ver capítulo II). A observação e identificação destas espécies foram realizadas no Instituto de Investigação das Pescas e do Mar (IPIMAR) sob supervisão da Doutora Graça Vilarinho. Por sua vez, as análises microscópicas das amostras da região da Península Antártica foram recolhidas com o objectivo de efectuar uma comparação entre os resultados obtidos pelo CHEMTAX e pelas contagens efectuadas ao microcópio. Estas análises foram efectuadas pelo investigador MSc. Márcio Silva de Souza, no Laboratório de Fitoplâncton e Microorganismos Marinhos do Instituto de Oceanografia da FURG e os detalhes protocolares inerentes à técnica usada estão descritos detalhadamente no capítulo III.

1.4.6. Análises estatísticas

Foram aplicados diferentes procedimentos estatísticos (correlações simples, análises de agrupamento e/ou análises multivariadas) de forma a selecionar as variáveis físico-químicas, para cada região, com maior contribuição para a variância encontrada entre os pontos de amostragem. Estes procedimentos, quando usados, encontram-se devidamente comentados nos respectivos capítulos.

Referências

Amorim A, Dale B (2006). Historical cyst record as evidence for the recent introduction of the dinoflagellate Gymnodinium catenatum in the north-eastern Atlantic. African Journal of Marine Science 28(2): 193-197.

Amorim A,MoitaT, Oliveira P (2004).Dinoflagellate blooms related to coastal upwelling plumes off Portugal. In:Steidinger K, Landsberg J,Thomas C, Vargo G (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, Intergovernmental Oceano-graphic Commission of UNESCO, pp 89–91.

Bakun A (1990). Global climate change and intensification of coastal ocean upwelling. Science 247: 198-201.

Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Feldman GC, Milligan AJ, Falkowski PG, Letelier RM, Boss ES (2006). Climate-driven trends in contemporary ocean productivity. Nature 444: 752-755.

Boyd PW, Robinson C, Savidge G, leB Williams PJ (1995). Water column and sea-ice primary production during Austral spring in the Bellingshausen Sea. Deep-Sea Research II 42: 1177-1200.

Brandt A, Gooday AJ, Brandão SN, Brix S, Brökeland W, Cedhagen T, Choudhury M, Cornelius N et al. (2007). First insights into the biodiversity and biogeography of the Southern Ocean deep sea. Nature 447: 307-311.

Brotas V, Plante-Cuny MR (1996). Identification et quantification des pigments chlorophylliens et caroténoïdes des sédiments marins: un protocole d'analyse par HPLC. Oceanologica Acta 19: 623–634.

Burkill PH, Edwards ES, Sleigh MA (1995). Microzooplankton and their role in controlling phytoplankton growth in the marginal ice zone of the Bellingshausen Sea. Deep-Sea Research II 42: 1277-1290.

Carr ME, Kearns EJ (2003). Production regimes in four Eastern Boundary Current systems. Deep-Sea Research II 50: 3199-3221.

Carreto JI, Montoya N, Akselman R, Carignan MO, Silva RI, Colleoni DAC (2008). Algal pigment patterns and phytoplankton assemblages in different water masses of the Río de la Plata maritime front. Continental Shelf Research 28: 1589-1606.

Castro CG, Rios AF, Doval MD, Perez FF (2002). Nutrient utilisation and chlorophyll distribution in the Atlantic sector of the Southern Ocean during Austral summer 1995-96. Deep-Sea Research II 49: 623-641.

Clarke A, Murphy EJ, Meredith MP, King JC, Peck LS, Barnes DKA, Smith RC (2007). Climate change and the marine ecosystem of the western Antarctic Peninsula. Phylosophical Transactions of the Royal Society 362: 149-166.

Cloern JE (1996). Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. Reviews of Geophysics 34: 127-168.

Chavez FP, Toggweiler JR (1995). Physical estimates of global new production: The upwelling contribution. In: Summerhayes CP, Emeis K-C, Angel MV, Smith RL, Zeitzschel B (Eds.), Upwelling in the Ocean: Modern Processes and Ancient Records. John Wiley, New York, pp. 313-320.

Chisholm SW, Morel FMM (1991). Preface: What controls phytoplankton production in nutrient-rich areas of the open sea? Limnology and Oceanography 36.

Coale KH, Johnson KS, Chavez FP, Buesseler KO, Barber RT, Brzezinski MA, Cochlan WP, Millero FJ, Falkowski PG, Bauer JE, Wanninkhof RH, Kudela RM et al. (2004). Southern ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. Science 304: 408-414.

Crépon M, Richez C, Chartier M (1984). Effects of coastline geometry on upwellings. Journal of Physical Oceanography 14: 1365-1382.

DiTullio GR, Garcia N, Riseman SF, Sedwick PN (2007). Effects of iron concentration on pigment composition in *Phaeocystis antarctica* grown at low irradiance. Biogeochemistry 83: 71-81.

Doney SC (2006). Plankton in a Warner World. Nature 444: 695-696.

Ducklow HW, Baker K, Martinson DG, Quetin LB, Ross RM, Smith RC, Stammerjohn SE, Vernet M, Fraser W (2007). Marine pelagic ecosystems: the West Antarctic Peninsula. Philosophical Transactions of the Royal Society B 362: 67-94.

Edwards M, Richardson AJ (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430: 881-884.

Falkowski PG (2002). The ocean's invisible forest - Marine phytoplankton play a critical role in regulating the earth's climate. Could they also be used to combat global warming. Scientific American 287: 54-61.

Ferreira A, Garcia VMT, Garcia CAE (2009). Light absorption by phytoplankton, non-algal particles and dissolved organic matter at the Patagonia shelf-break in spring and summer. Deep-Sea Research I 56: 2162-2174.

Fischer G, Gersonde R, Wefer G (2002). Organic carbon, biogenic silica and diatom fluxes in the marginal winter sea-ice zone and in the polar front region: interannual variations and differences in composition. Deep-Sea Research II 49: 1721-1745.

Fitch DT, Moore JK (2007). Wind speed influence on phytoplankton bloom dynamics in the southern ocean marginal ice zone. Journal of Geophysical Research 112, C08006.

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

Fiúza AFG, Macedo ME, Guerreiro MR (1982). Climatological space and time variation of the Portuguese coastal upwelling. Oceanologica Acta 5: 31-40.

Garcia CAE, Garcia VMT, Dogliotti AI, Ferreira A, Romero SI, Mannino A, Souza MS, Mata MM, Garcia VMT (2011). Environmental conditions and bio-optical signature of a coccolithophorid bloom in the Patagonian shelf. Journal of Geophysical Research 116, C03025.

Garcia VMT, Garcia CAE, Mata MM, Pollery RC, Piola AR, Signorini SR, Mcclain CR, Rodriguez MDI (2008). Environmental factors controlling the phytoplankton blooms at the Patagonia shelf-break in spring. Deep-Sea Research I 55: 1150-1166.

Goericke R, Montoya JP (1998). Estimating the contribution of microalgal taxa to chlorophyll a in the field - variations of pigment ratios under nutrient- and light-limited growth. Marine Ecology Progress Series 169: 97-112.

Hare CE, DiTullio GR, Riseman SF, Crossley AC, Popels LC, Sedwick PN, Hutchins DA (2007). Effects of changing continuous iron input rates on a Southern Ocean algal assemblage. Deep-Sea Research I 54: 732-746.

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

Haynes R, Barton E, 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.

Hays GC, Richardson AJ, Robinson C (2005). Climate change and marine plankton. Trends in Ecology and Evolution 20: 337-344.

Holm-Hansen O, Mitchell BG, Hewes CD, Karl DM (1989). Phytoplankton blooms in the vicinity of Palmer Station, Antarctica. Polar Biology 10: 49-57.

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

Jeffrey SW (1981). Light quality and pigment adaptations in microalgae. Proceedings of the International Botanical Congress: 179.

Kahru M, Brotas V, Manzano-Sarabia M, Mitchell BG (2011). Are phytoplankton blooms occurring earlier in the Arctic? Global Change Biology 17: 1733-1739.

Kang SH, Kang JS, Lee S, Chung KH, Kim D, Park MG (2001). Antarctic phytoplankton assemblages in the marginal ice zone of the northwestern Weddell Sea. Journal of Plankton Research 23: 333-352.

Karl DM, Holm-Hansen O, Taylor GT, Tien G, Bird DF (1991). Microbial biomass and productivity in the western Bransfield Strait, Antarctica during the 1986-87 austral summer. Deep-Sea Research I 38: 1029-1055.

Kraay GW, Zapata M, Veldhuis MJW (1992). Separation of chlorophylls c1, c2, and c3 of marine phytoplankton by reversed-phase-C18-High-Performance Liquid Chromatography. Journal of Phycology 28: 708-712.

kudela R, Pitcher G, Probyn T, Figueiras F, Moita T, Trainer V (2005). Harmful Algal Blooms in Coastal Upwelling Systems. Oceanography 18: 184-197.

Lancelot C, Mathot S, Veth C, Debaar H (1993). Factors controlling phytoplankton ice edge blooms in the marginal ice zone of the northwestern Weddell Sea during sea ice retreat: field observations and mathematical modelling. Polar Biology 13: 377-387.

Lachkar Z, Gruber N (2011). What controls biological production in coastal upwelling systems? Insights from a comparative modeling study. Biogeosciences 8: 2961-2976.

Latasa M (2007). Improving estimations of phytoplankton class abundances using CHEMTAX. Marine Ecology Progress Series 329: 13-21.

Mackey MD, Higgins H, Mackey M, Holdsworth D (1998). Algal class abundances in the western equatorial Pacific: Estimation from HPLC measurements of chloroplast pigments using CHEMTAX. Deep-Sea Research II 45: 1441-1468.

Mackey MD, Higgins HW, Mackey DJ, Wright SW (1997). CHEMTAX user's manual: a program for estimating class abundances from chemical markers — application to HPLC measurements of phytoplankton pigments. 229, CSIRO Marine Laboratories, Hobart, Australia.

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996). CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Marine Ecology Progress Series 144: 265-283.

Martin JH, Gordon RM, Fitzwater SE (1990). Iron in Antarctic Waters. Nature 345: 156-158.

Mendes CR, Cartaxana P, Brotas V (2007). HPLC determination of phytoplankton and microphytobenthos pigments: comparing resolution and sensitivity of a C18 and a C8 method. Limnology and Oceanography: Methods 5: 363-370.

Meredith MP, King JC (2005). Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century. Geophysical Research Letters 32.

Mitchell BG, Holm-Hansen O (1991). Observations and modeling of the antarctic phytoplankton crop in relation to mixing depth. Deep-Sea Research Part I 38: 981-1007.

Moita MT (2001). Estrutura, variabilidade e dinâmica do fitoplâncton na costa de Portugal Continental. Tese de doutoramento em Biologia, Faculdade de Ciências da Universidade de Lisboa, 272p.

Moita MT, Oliveira PB, Mendes JC, Palma AS (2003). Distribution of chlorophyll *a* and *Gymnodinium catenatum* associated with coastal upwelling plumes off central POrtugal. Acta Oecologica 24: S125-S132.

Montes-Hugo M, Doney SC, Ducklow HW, Fraser W, Martinson D, Stammerjohn SE, Schofield O (2009). Recent changes in phytoplankton communities associated with rapid regional climate change along the western Antarctic Peninsula, Science, 323, 1470-1473.

Oliveira A, Santos AI, Rodriguez A, Vitorino J (2007). Sedimentary particle distribution and dynamics on the Nazaré canyon system and adjacent shelf (Portugal). Marine Geology 246: 105-122.

Oliveira PB, Moita T, Silva A, Monteiro IT, Palma AS (2009). Summer diatom and dinoflagellate blooms in Lisbon Bay from 2002 to 2005: Pre-conditions inferred from wind and satellite data. Progress in Oceanography 83: 270-277.

Oliveira PB, Nolasco R, Dubert J, Moita T, Peliz Á (2009). Surface temperature, chlorophyll and advection patterns during a summer upwelling event off central Portugal. Continental Shelf Research 29: 759-774.

Park MG, Yang SR, Kang SH, Chung KH, Shim JH (1999). Phytoplankton biomass and primary production in the marginal ice zone of the northwestern Weddell Sea during austral summer. Polar Biology 21: 251-261.

Pauly D, Christensen V (1995). Primary production required to sustain global fisheries. Nature 374: 255-257.

Peliz A, Dubert J, Santos AMP, Oliveira PB, 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.

Peliz A, Fiúza A (1999).Temporal and spatial variability of CZCS-derived phytoplankton pigment concentrations off western Iberian Peninsula. International Journal of Remote Sensing 20: 1363–1403.

Prézelin BB, Hofmann EE, Mengelt C, Klinck JM (2000). The linkage between Upper Circumpolar Deep Water (UCDW) and phytoplankton assemblages on the west Antarctic Peninsula continental shelf. Journal of Marine Research 58: 165-202.

Prézelin BB, Hofmann EE, Moline M, Klinck JM (2004). Physical forcing of phytoplankton community structure and primary production in continental shelf waters of the Western Antarctic Peninsula. Journal of Marine Research 62: 419-460.

Relvas P, Barton ED, Dubert J, Oliveira PB, Peliz Á, da Silva JCB, Santos AMP (2007). Physical oceanography of the western Iberia ecosystem: Latest views and challenges. Progress in Oceanography 74: 149-173.

Ryan JP, Chavez FP, Bellingham JG (2005). Physical-biological coupling in Monterey Bay, California: topographic influences on phytoplankton ecology. Marine Ecology. Progress Series 287: 23-32.

Savidge G, Harbour DS, Gilpin LC, Boyd PW (1995). Phytoplankton distributions and production in the Bellingshausen Sea, Austral spring 1992. Deep-Sea Research II 42: 1201-1224.

Schlüter L, Møhlenberg F, Havskum H, Larsen S (2000). The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. Marine Ecology Progress Series 192: 49-63.

Sedwick PN, DiTullio GR (1997). Regulation of algal blooms in Antarctic shelf waters by the release of iron from melting sea ice. Geophysical Research Letters 24: 2515-2518.

Sedwick PN, Garcia N, Riseman SF, Marsay CM, DiTullio GR (2007). Evidence for high iron requirements of colonial Phaeocystis antarctica at low irradiance, Biogeochemistry 83: 83-97.

Silva A, Palma S, Oliveira PB, Moita MT (2009). Composition and interannual variability of phytoplankton in a coastal upwelling region (Lisbon Bay, Portugal). Journal of Sea Research 62: 238-249.

Smetacek V, Scharek R, Gordon LI, Eicken H, Fahrbach E, Rohardt G, Moore S (1992). Early spring phytoplankton blooms in ice platelet layers of the southern Weddell Sea, Antarctica. Deep-Sea Research I 39: 153-168.

Smith RC, Baker KS, Byers ML, Stammerjohn SE (1998). Primary productivity of the palmer long term ecological research area and the Southern Ocean. Journal of Marine Systems 17: 245-259.

Smith RC, Domack EW, Emslie SD, Fraser WR, Ainley DG, Baker KS, Kennett J, Leventer A, Mosley-Thompson E, Stammerjohn SE, Vernet M (1999). Marine ecosystems sensitivity to historical climate change: Antarctic Peninsula. BioScience 49: 393-404.

Smith RC, Baker KS, Dierssen HM, Stammerjohn SE, Vernet M (2001). Variability of primary production in an Antarctic marine ecosystem as estimated using a multi-scale sampling strategy. American Zoologist 41: 40-56.

Smith WO, Nelson DM (1986). Importance of ice edge phytoplankton production in the Southern-Ocean. Bioscience 36: 251-257.

Smith WO, Gordon LI (1997). Hyperproductivity of the Ross Sea (Antarctica) polynya during austral spring. Geophysical Research Letters 24: 233-236.

Smith WO, Marra J, Hiscock MR, Barber RT (2000). The seasonal cycle of phytoplankton biomass and primary productivity in the Ross Sea, Antarctica. Deep-Sea Research II 47: 3119-3140.

Sousa F, Bricaud A (1992).Satellite-derived phytoplankton structures in the Portuguese upwelling area. Journal Geophysical Research 97: 11343–11356.

Sullivan CW, Arrigo KR, McClain CR, Comiso JC, Firestone J (1993). Distributions of Phytoplankton Blooms in the Southern-Ocean. Science 262: 1832-1837.

Sunda WG, Huntsman SA (1997). Interrelated influence of iron, light and cell size on marine phytoplankton growth. Nature 390: 389-392.

Townsend DW, Cammen L, Holligan PM, Campbell DE, Pettigrew NR (1994). Causes and consequences of variability in the timing of spring phytoplankton blooms. Deep-Sea Research I 41: 747-765.

Tremblay JE, Smith WO (2007). Primary production and nutrient dynamics in polynyas. In: Smith WO, Barber DG (Eds), Polynyas: Windows to the World, Elsevier, Amsterdam, pp. 239-270.

van Oijen T, van Leeuwe MA, Granum E, Weissing FJ, Bellerby RGJ, Gieskes WWC, de Baar HJW (2004). Light rather than iron controls photosynthate production and allocation in Southern Ocean phytoplankton populations during austral autumn. Journal of Plankton Research 26: 885-900.

Vaughan DG, Marshall GJ, Connolley WM, Parkinson C, Mulvaney R, Hodgson DA, King JC, Pudsey CJ, Turner J (2003). Recent rapid regional climate warming on the Antarctic Peninsula. Climatic Change 60: 243-274.

Vitorino J, Beja J, Pinto J, Bernardino M, Quaresma L (2005). Physical Oceanography of the Western Iberian Margin: an Overview of Nazaré Canyon Processes Based on EUROSTRATAFORM Data. EUROSTRATAFORM Final Report, Salamanca.

Wright SW, Thomas DP, Marchant J, Higgins HW, Mackey MD, Mackey DJ (1996). Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorisation program. Marine Ecology Progress Series 144: 285-298.

Wright SW, Ishikawa A, Marchant HJ, Davidson AT, van den Enden RL, Nash GV (2009). Composition and significance of picophytoplankton in Antarctic waters. Polar Biology 32: 797-808.

Wright SW, Jeffrey SW (2006). Pigment markers for phytoplankton production. In: Volkmann JK (Ed.), Marine Organic Matter: Biomarkers, Isotopes and DNA. Spring-Verlag, Berlin, pp. 71-104.

Wright SW, van den Enden RL, Pearce I, Davidson AT, Scott FJ, Westwood KJ (2010). Phytoplankton community structure and stocks in the Southern Ocean (30–801E) determined by CHEMTAX analysis of HPLC pigment signatures. Deep-Sea Research II 57: 758-778.

Zapata M, Rodriguez F, Garrido JL (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.

Zhou M, Nordhausen W, Huntley M (1994). ADCP measurements of the distribution and abundance of Euphausiids near the Antarctic Peninsula in Winter. Deep-Sea Research I 41: 1425-1445

CAPÍTULO II

Spatial distribution of phytoplankton assemblages in the Nazaré submarine canyon region (Portugal): HPLC-CHEMTAX approach

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Mendes CR, Sá C, Vitorino J, Borges C, Garcia VMT, Brotas V (2011). Spatial distribution of phytoplankton assemblages in the Nazaré submarine canyon region (Portugal): HPLC-CHEMTAX approach. Journal of Marine Systems 87: 90-10.

ABSTRACT

The distribution and composition of phytoplankton assemblages were studied in the Nazaré submarine canyon, during an upwelling event, using high-performance liquid chromatography (HPLC) pigment analysis, complemented by microscopic qualitative observations. High chlorophyll *a* (Chl *a*) concentrations were recorded in the canyon head, near the coast, where values greater than 4 μg L^{-1} were observed. In contrast, Chl *a* was relatively low in offshore regions, with values below 0.5 μg L^{-1}. The most abundant accessory pigments were fucoxanthin, peridinin, diadinoxanthin and 19'-hexanoyloxyfucoxanthin. Pigment data information was analysed using the CHEMTAX program to estimate the contribution of different taxonomic groups to total Chl *a*. North of the canyon head, an area with high concentration of peridinin-containing dinoflagellates was identified (with presence of chain-forming toxic dinoflagellates). The presence of these organisms was associated with mixed water columns and phosphate values lower than the ones south of the canyon head, where a dominance of diatoms was recorded. The rest of the study region showed a dominance of prymnesiophytes and a significant contribution of cyanobacteria at oceanic stations. This study demonstrates the usefulness of using pigment analysis to study spatial distribution of phytoplankton in relation to a complex physical environment.

Keywords: phytoplankton, photosynthetic pigments, HPLC, Portuguese coast, upwelling system, Nazaré submarine canyon

2.1. Introduction

Phytoplankton plays a crucial role in marine ecosystems, affecting the structure and efficiency of food webs, nutrient cycling and the flux of particles to deep waters. Thus, in order to understand the dynamics of pelagic ecosystems, knowledge of both phytoplankton composition and biomass is important. The determination of photosynthetic pigment concentrations by HPLC, besides providing an accurate quantification of Chl *a* concentration, allows the study of phytoplankton assemblage composition and structure, since some carotenoids and chlorophylls can be used as taxonomic indicators of phytoplankton groups [Gieskes & Kraay, 1983; Schlüter & Havskum, 1997; Ediger et al., 2006]. This technique has the advantage of detecting nano- and pico-planktonic organisms, which are normally difficult to identify by light microscopy. Furthermore, permits a relatively fast analysis of a large number of phytoplankton samples, important in monitoring abundance and composition of phytoplankton populations, which can become impracticable by microscopy [Wright & Jeffrey, 2006]. However, pigments data interpretation can be difficult as some pigments are present in several algal groups. For instance, fucoxanthin, which is a major pigment in diatoms, is also present in chrysophytes and prymnesiophytes [Jeffrey & Vesk, 1997; Wright & Jeffrey, 2006]. The development of statistical tools such as CHEMTAX [Mackey et al., 1996; Wright et al., 1996] has overcome the problem of non-specificity of some pigments. This software applies matrix factorization to pigment data in order to estimate the contribution of phytoplankton groups to total Chl *a*. Pigment analysis by HPLC followed by data analysis with CHEMTAX has proven to be an effective method for determining the

abundance of phytoplankton, even for groups without specific biomarker pigments [Mackey et al., 1996; Mackey et al., 1998; Schlüter et al., 2000; Latasa, 2007; Carreto et al., 2008]. Nevertheless, it is recognized that light microscopy can provide a better taxonomic resolution than pigment analysis (except for some very small, nano and pico fraction as mentioned previously or fragile cells not resistant to fixatives) and it is crucial for identification of key species (e.g. toxic species) with potential ecological implications. Thus, a combination of both approaches is desirable and has been recommended [Silva et al., 2008a and references therein).

In the present work, the spatial pattern of phytoplankton was studied around the Nazaré canyon region. This canyon, located on the Portuguese west coast, is not connected to a major river system and, therefore, the dynamics in the area are mainly determined by the oceanographic conditions. Generally, the west coast of Portugal is characterized by seasonal upwelling, determined by the coastal morphology, the continental shelf/upper slope bathymetry and local winds [Fiúza, 1983]. Sustained upwelling conditions are generally observed from April to September, when persistent northerlies occur [Fiúza et al., 1982], while advection of warmer oligotrophic oceanic waters is observed during autumn and winter, when southerly winds begin to dominate, leading to downwelling conditions and an intensification of waters flowing poleward [Fiúza et al., 1982; Peliz et al., 2005]. However, episodes of reverse winds can occur during both periods. In general, wind forcing circulation interacts with topography and coastline orientation, modifying the along-shore and cross-shelf flows at different levels, resulting in amplification and/or reduction of upwelling–downwelling [Kudela et al., 2005; Ryan et al., 2005a]. Upwelling has been

identified as the major source of seasonal and spatial variability of phytoplankton for the Portuguese coast, associated with nutrient availability to the euphotic zone and alterations of the water column stability [Moita, 2001; Silva et al., 2009].

The objective of the present work was to study the phytoplankton distribution around the Nazaré canyon by means of HPLC pigment analysis and CHEMTAX software, relating the observed physical and chemical conditions of this peculiar topographic region to the spatial distribution of taxonomic groups.

2.2. Methods

2.2.1. Study site

The Nazaré canyon is one of Europe's largest submarine valleys, located on the central part of the Portuguese coast (Eastern Atlantic Ocean), oriented roughly perpendicular to the coast in an E–W direction (Fig. 1). This geomorphologic feature extends from the deep ocean shoreward, from 5000 m to about 150 m deep, where the canyon head is located 500 m from the shore. Along the upper canyon section (where it cuts the continental shelf region), the canyon width changes from less than 2 km, near the canyon head, to about 8-9 km near the canyon mouth. This width is small when compared with the natural spatial scales of adjustment of the flow to topography in the presence of Earth rotation and stratification, indicating that, regarding dynamics, the Nazaré Canyon is very narrow [Vitorino et al., 2005].

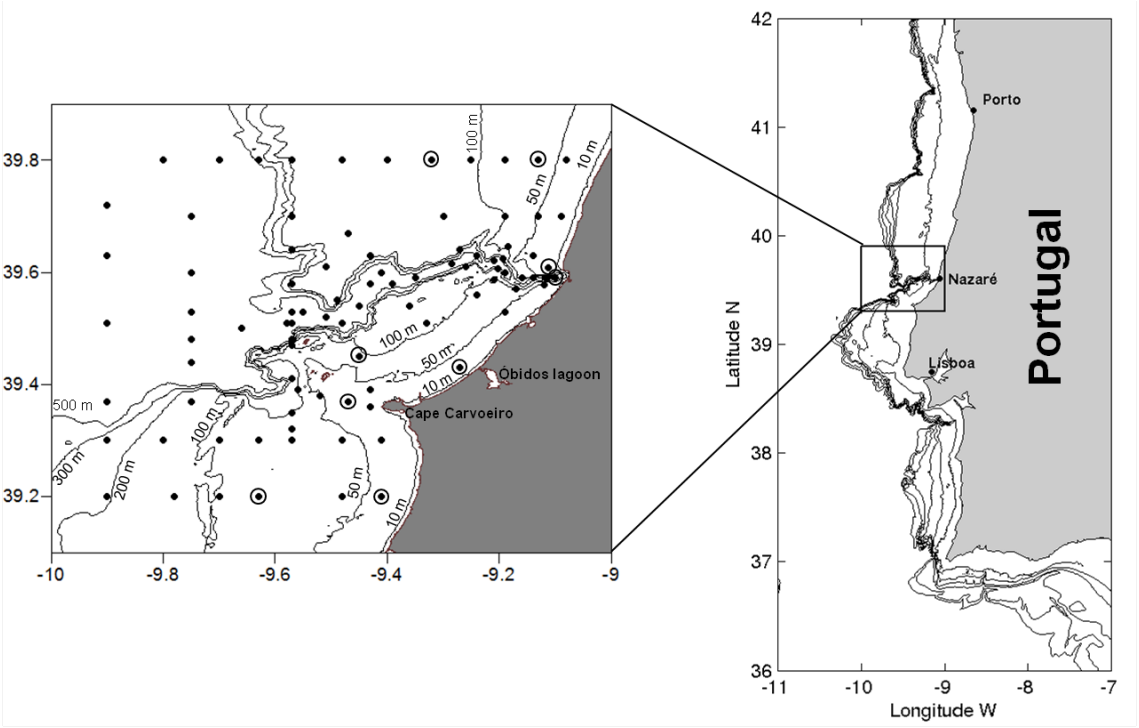

Figure 1: Location of stations sampled during HERMES06 cruise (23 June 2006 to 5 July 2006) in the Nazaré canyon region. Open circles indicate location of stations with microscopic observations**.**

2.2.2. Sample collection

Sampling was conducted between 23 June and 05 July 2006, on board the N.R.P. “D Carlos I”, during HERMES (Hotspot Ecosystem Research on the Margins of European Seas) 2006 cruise, specifically designed for physical and geological studies. Surface water samples (5 m in depth) were collected from 92 stations around the Nazaré submarine canyon for phytoplanktonic pigments and nutrients and, at selected stations, for microscopic analysis (Fig. 1). Physical data (temperature and salinity) and water samples were collected using a combined Idronaut CTD profiler and a rosette sampler. Only surface samples were collected for the present study, due to a limited number of Niskin bottles.

2.2.3. Remote-sensing data of SST

Sea Surface Temperature (SST) data acquired by the Moderate-resolution imaging spectroradiometer (MODIS) on NASAs Aqua satellite and processed by The Ocean Biology Processing Group (OBPG) were downloaded from the Ocean Color Website (http://oceancolor.gsfc.nasa.gov/). For the cruise period, swaths covering the region of interest were selected and downloaded via-ftp. After standard quality checking and masking, valid data were interpolated into an equal latitude–longitude grid and averaged for each phase of the cruise using MATLAB software. The images have a nominal resolution of 1 km.

2.2.4. Wind data

Meteorological measurements were collected with an Aanderaa AWS 2700 coastal weather station which is maintained by Instituto Hidrográfico in Ferrel (39.39ºN, 09.29ºW). The wind speed and direction data were converted to time series of northward and eastward wind components.

2.2.5. Nutrient analysis

Determination of nutrient (nitrate, nitrite, ammonium, phosphate and silicate) concentration was performed using a Skalar SANplus Segmented Flow AutoAnalyzer specially engineered for the analysis of saline waters. $N–NO_x$ and $N–NO_2$ were determined according to Strickland & Parsons [1972], with $N–NO_3$

being estimated by the difference of the previous two; $N–NH_4$ and $Si-SiO_2$ were determined according to Koroleff [1976]; $P–PO_4$ was determined according to Murphy & Riley [1962]. All methods were adapted to the methodology of segmented flow analysis and uncertainties were determined, respectively, for low and high nutrient concentrations: 11.7% and 6.0% (NO_x<2.64 µM; NO_x≥2.64 µM), 11.7% and 6.1% (NO_2<0.45 µM; NO_2≥0.45 µM), 16.6% and 8.6% (NO_3<2.64 µM; NO_3≥2.64 µM), 13.5% and 9.7% (NH_3<3.51 µM; NH_3≥3.51 µM), 11.7% and 6.1% (PO_4<0.70 µM; PO_4≥0.70 µM); 11.9% and 7% (SiO_2<1.56 µM; SiO_2 ≥1.56 µM).

2.2.6. HPLC pigment analysis

Water samples (5 L) were filtered onto Whatman GF/F filters (nominal pore size of 0.7 µm and 47 mm in diameter). The filters were deep-frozen immediately and stored at –80°C. Phytoplanktonic pigments were extracted with 5 mL of 95% cold-buffered methanol (2% ammonium acetate) for 30 min at 20°C, in the dark. Samples were sonicated (Bransonic, model 1210, w: 80, Hz: 47) for 1 min at the beginning of the extraction period. The samples were then centrifuged at 1100 g for 15 min, at 4°C. Extracts were filtered (Fluoropore PTFE filter membranes, 0.2 µm in pore size) and immediately injected in the HPLC. Pigment extracts were analyzed using a Shimadzu HPLC comprised of a solvent delivery module (LC-10ADVP) with system controller (SCL-10AVP), a photodiode array (SPD-M10ADVP), and a fluorescence detector (RF-10AXL). Chromatographic separation was carried out using a C18 column for reverse phase chromatography (Supelcosil; 25 cm long; 4.6 mm in diameter; 5 mm

particles) and a 35 min elution program. The solvent gradient followed Kraay et al. [1992] adapted by Brotas & Plante-Cuny [1996] with a flow rate of 0.6 mL min^{-1} and an injection volume of 100 µL. The limit of detection (LOD) and limit of quantification (LOQ) of this method were calculated and discussed in Mendes et al. [2007]. Pigments were identified from both absorbance spectra and retention times and concentrations calculated from the signals in the photodiode array detector or fluorescence detector (Ex. 430 nm; Em. 670 nm). The HPLC system was previously calibrated with pigment standards from Sigma (chlorophyll *a*, *b* and β-carotene) and DHI (for other pigments).

2.2.7. CHEMTAX analysis of pigment data

The relative abundance of microalgal groups contributing to total Chl *a* biomass was calculated by pigment concentration data using version 1.95 of CHEMTAX chemical taxonomy software [Mackey et al., 1996; Wright et al., 1996; Wright et al., 2009]. CHEMTAX uses a factor analysis and steepest-descent algorithm to find the best fit of the data on to an initial pigment ratio matrix. The basis for calculations and procedures are fully described in Mackey et al. [1996].

Initial pigment ratios for major algal classes were obtained from the literature [Schlüter et al., 2000; Gibb et al., 2001] (Table 1a). Based on the diagnostic pigments detected, 7 algal groups were loaded in CHEMTAX: diatoms, dinoflagellates, prymnesiophytes, chryptophytes, prasinophytes, chrysophytes and cyanobacteria (see Table 1). The pigments loaded were alloxanthin (Allo), fucoxanthin (Fuco), peridinin (Perid), prasinoxanthin (Pras),

zeaxanthin (Zea), lutein (Lut), 19'-butanoyloxyfucoxanthin (But-fuco), 19'-hexanoyloxyfucoxanthin (Hex-fuco), chlorophyll c_3 (Chl c_3), chlorophyll *b* (Chl *b*) and chlorophyll *a* (Chl *a*). Chlorophytes were excluded from this study as significantly positive correlations were found between prasinoxanthin (exclusive of prasinophytes) and concentrations of both chlorophyll *b* and lutein (pigments present in chlorophytes and prasinophytes) (R^2=0.75 and 0.50, respectively, $p<0.05$). In addition, zeaxanthin concentration (present in cyanobacteria and chlorophytes) did not correlate significantly ($p>0.05$) with chlorophyll *b* (R^2=0.25). These correlation analyses support the use of chlorophyll *b*, prasinoxanthin and lutein as indicators of prasinophytes and zeaxantin as a biomarker for cyanobacteria in this study.

Table 1: Marker pigments to Chl a ratios. Input ratios were obtained from Schlüter et al. [2000] (Prasinophytes) and Gibb et al. [2001] (all other groups). Output ratios (after 15 runs) were estimated with the CHEMTAX program – mean values of six final matrices obtained. See text for further details.

	Allo	Fuco	Perid	Pras	Zea	Lut	But-fuco	Hex-fuco	Chl *b*	Chl c_3	Chl *a*
(**a**) Input matrix											
Diatoms	0	0.760	0	0	0	0	0	0	0	0	1
Dinoflagellates	0	0	1.060	0	0	0	0	0	0	0	1
Prymnesiophytes	0	1.210	0	0	0	0	0.020	1.360	0	0.170	1
Cryptophytes	0.230	0	0	0	0	0	0	0	0	0	1
Prasinophytes	0	0	0	0.458	0.079	0.018	0	0	0.679	0	1
Chrysophytes	0	0.970	0	0	0	0	1.560	0	0	0.250	1
Cyanobacteria	0	0	0	0	0.590	0	0	0	0	0	1
(**b**) output matrix											
Diatoms	0	0.628	0	0	0	0	0	0	0	0	1
Dinoflagellates	0	0	1.001	0	0	0	0	0	0	0	1
Prymnesiophytes	0	0.143	0	0	0	0	0.001	0.637	0	0.436	1
Cryptophytes	0.486	0	0	0	0	0	0	0	0	0	1
Prasinophytes	0	0	0	0.203	0.176	0.043	0	0	0.998	0	1
Chrysophytes	0	0.019	0	0	0	0	1.720	0	0	0.001	1
Cyanobacteria	0	0	0	0	1.774	0	0	0	0	0	1

For optimization of the input matrix, a series of 60 pigment ratio tables were generated by multiplying each ratio of the initial table by a randomly function as described in Wright et al. [2009]. The best six output results (with

the smallest residual) were then selected to apply further fifteen successive CHEMTAX runs in order to check final ratio convergence, according Latasa [2007]. Using the output pigment:Chl *a* ratio matrix of each run as input for the following run, ratios should stabilize towards their most probable values [Latasa, 2007]. Fig. 2 shows the changes of main ratios for the representative taxonomic groups along the 15 successive runs, with final mean and standard deviation. The final results (ratios and abundances) were then calculated as the average of the final six outputs obtained after the processing described above. The optimized pigment ratio matrix derived by CHEMTAX is presented in Table 1b.

2.2.8. Microscopic analysis

At some coastal stations (see Fig. 1), surface samples were collected with a 20 µm mesh net and immediately preserved with Lugol's iodine solution for micro-phytoplankton qualitative analysis. Species identification was performed using an inverted light microscope Olympus IX70, at 400× magnification. Phytoplankton identification was mainly based on Hasle & Syvertsen [1996] and Dodge [1982].

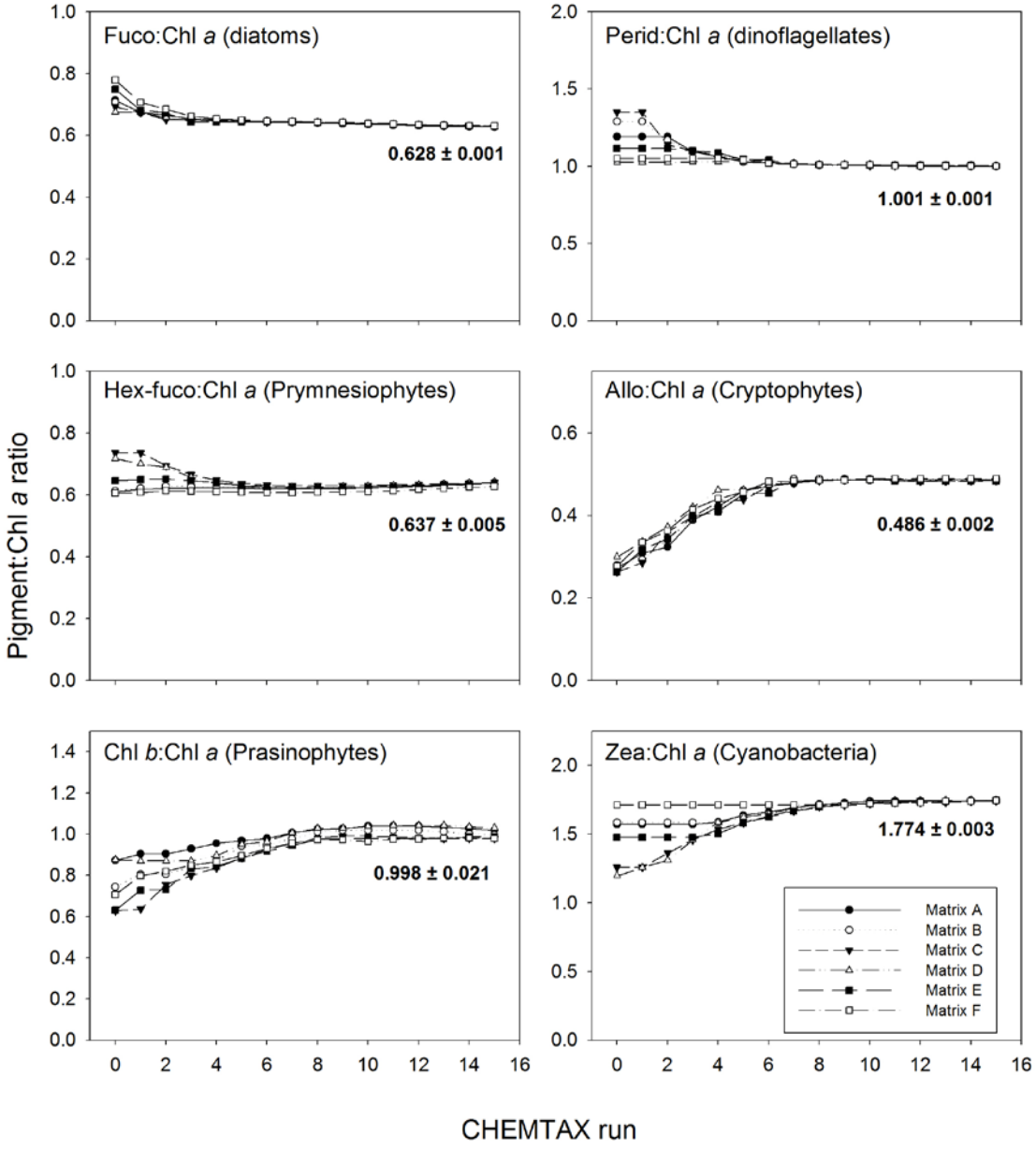

Figure 2: Evolution of pigment ratios of the six input matrices (A–F), after successive runs of CHEMTAX for main phytoplankton pigment groups, with mean and standard deviation shown for the final run. See text for initial matrix (A–F) calculation method. For pigment abbreviations, see Table 2.

2.2.9. Statistical analysis

The phytoplankton community structure at the stations was examined by a cluster analysis, using the software PRIMER6 [Clarke & Gorley, 2001]. A

dendrogram was produced using the group average linkage method based on the Bray–Curtis similarity. The mean relative contribution of each phytoplankton group to community structure was examined using the Similarity Percentage procedure (SIMPER).

In order to explore the relationships between the phytoplankton groups' biomass and environmental conditions, a Canonical Correspondence Analysis (CCA) was performed. The CCA is an ordination method effective to directly reveal correlations between spatial/temporal structure of communities and environmental factors that might be responsible for that. Water temperature, salinity, water depth, nutrient concentrations (silicate, phosphate and DIN) and N:P ratio were the environmental variables included in the CCA. The CCA was performed using CANOCO 4.5 [Ter Braak & Smilauer, 2002].

2.3. Results

2.3.1. Oceanographic conditions

During the cruise, two distinct oceanographic conditions driven by wind regime were verified (Fig. 3). The first period of the cruise (23 to 27 June 2006) was characterized by northerly winds (negative values of the northward component in Fig. 3). These winds promote an offshore transport in the surface Ekman layer, the upwelling of colder sub-surface waters by continuity, and the establishment of a southward jet over the shelf. Typically, in the NW Portuguese shelf, this response to upwelling wind events can be established in the region close to the coast in about 35 h [Mork & Jorge da Silva, 1993].

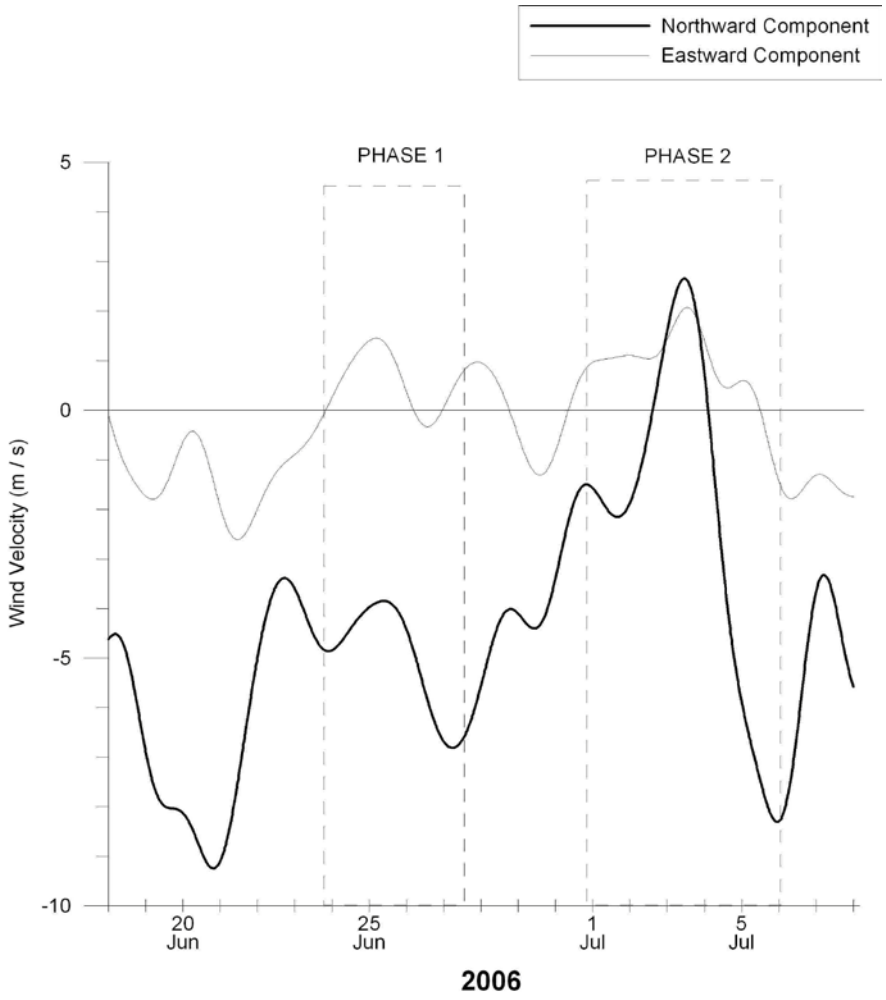

Figure 3: Times series of eastward and northward components of the wind measured at the coastal station of Ferrel during the cruise. Negative values of the northward component indicate a favorable upwelling wind. The boxes indicate the two periods of CTD measurements and water sampling.

This upwelling situation is expressed by the 30 Km width band of cold (upwelled) water, which extends along the coast (Fig. 4a), being notorious at the head of the canyon. The SST images also reveal the presence of a cold water filament off Cape Carvoeiro, which is a recurrent feature of the summer upwelling season off Western Portugal, showing frequently a westward orientation [Haynes et al., 1993].

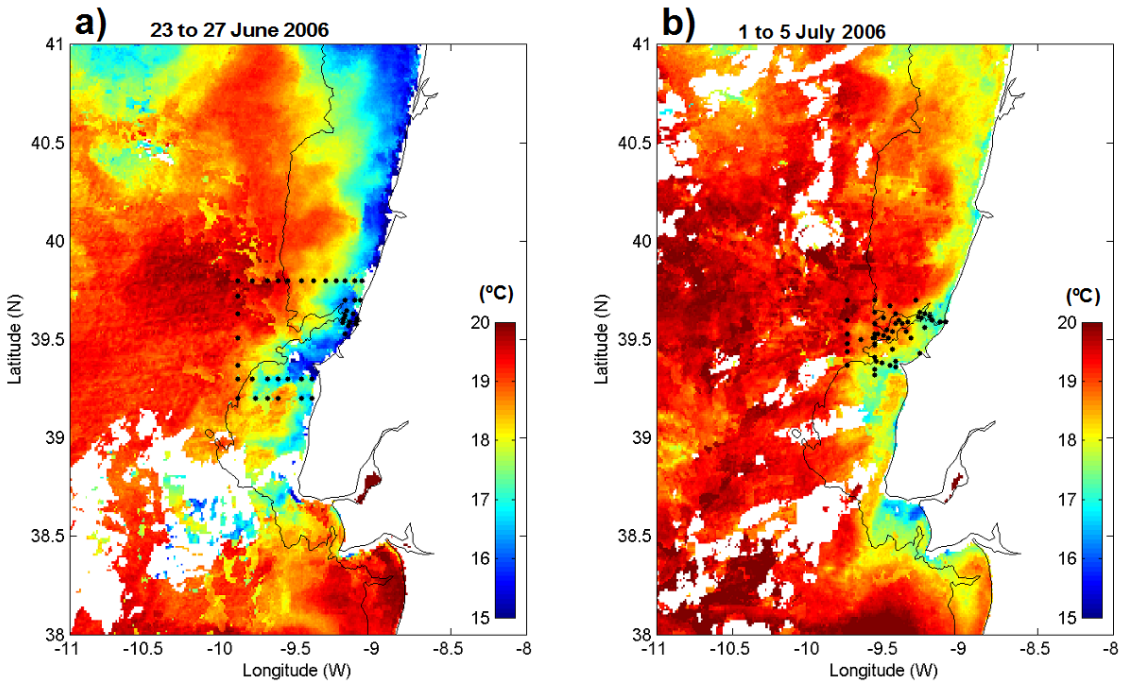

Figure 4: Average sea surface temperature (SST) from the Moderate-resolution imaging spectroradiometer (MODIS) for the period (a) 23 to 27 June 2006, and (b) 1 to 5 July 2006. Black dots represent the sampling sites during the respective periods.

In the second period (1 to 5 July 2006) the northerly winds relaxed and, for a short period between 2 and 3 July 2006, even became southerly (positive values of the northward component of the wind in Fig. 3) and downwelling favorable. These changes in the wind forcing led to a strong reduction of the cold-water band width (Fig. 4b), which expresses the response of surface waters to the upwelling relaxation. A CTD section along the canyon axis covered prior and after the wind relaxation, however, suggests that these changes affected mainly the upper 10-20 m of the water column (Fig. 5). The conditions measured at 20m depth during the second phase of the cruise were still largely representative of the upwelling conditions that were dominant before the wind relaxation.

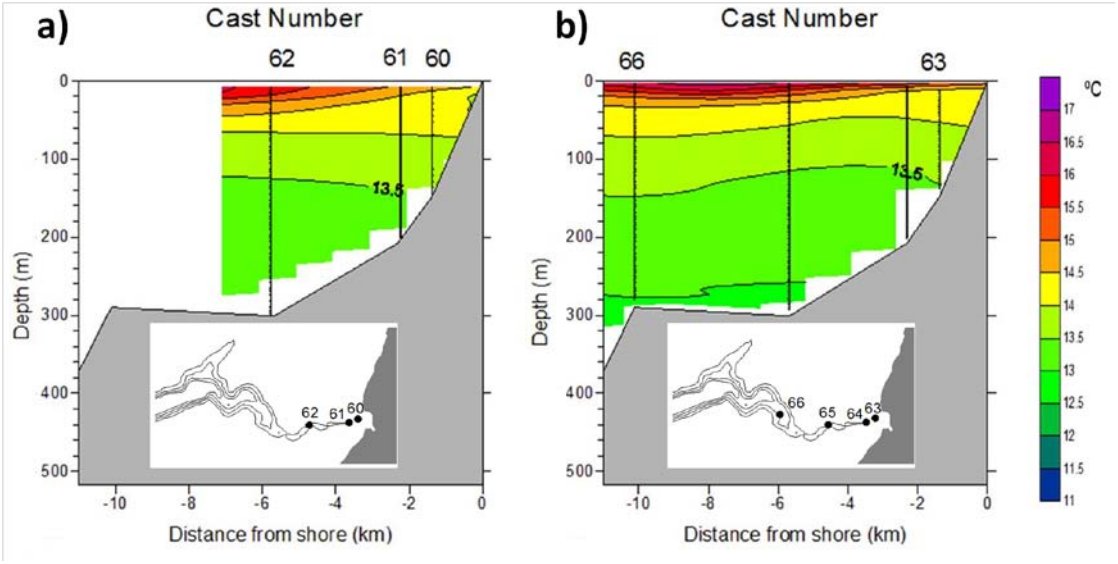

Figure 5: Temperature profiles of the repeated transect (in both cruise phases) at the canyon head. (a) Transect at the end of the first period and (b) transect at the beginning of the second period. Inset: positions of stations at each transect.

2.3.2. Nutrients

The spatial distribution of nutrients was highly variable (Fig. 6). Dissolved inorganic nitrogen (DIN) ranged from 0.21 to 7.77 µM, with higher concentrations at the canyon head (Fig. 6a). Silicates varied from 0.31 to 8.11 µM with an evident maximum north of the canyon head (Fig. 6b) and phosphates presented a range between 0.08 and 0.8 µM, with higher values south of the canyon and near the coast (Fig. 6c). N/P ratios (Fig. 6d) showed maximum values north of the canyon head. Values ranged between 1.4 and 80.

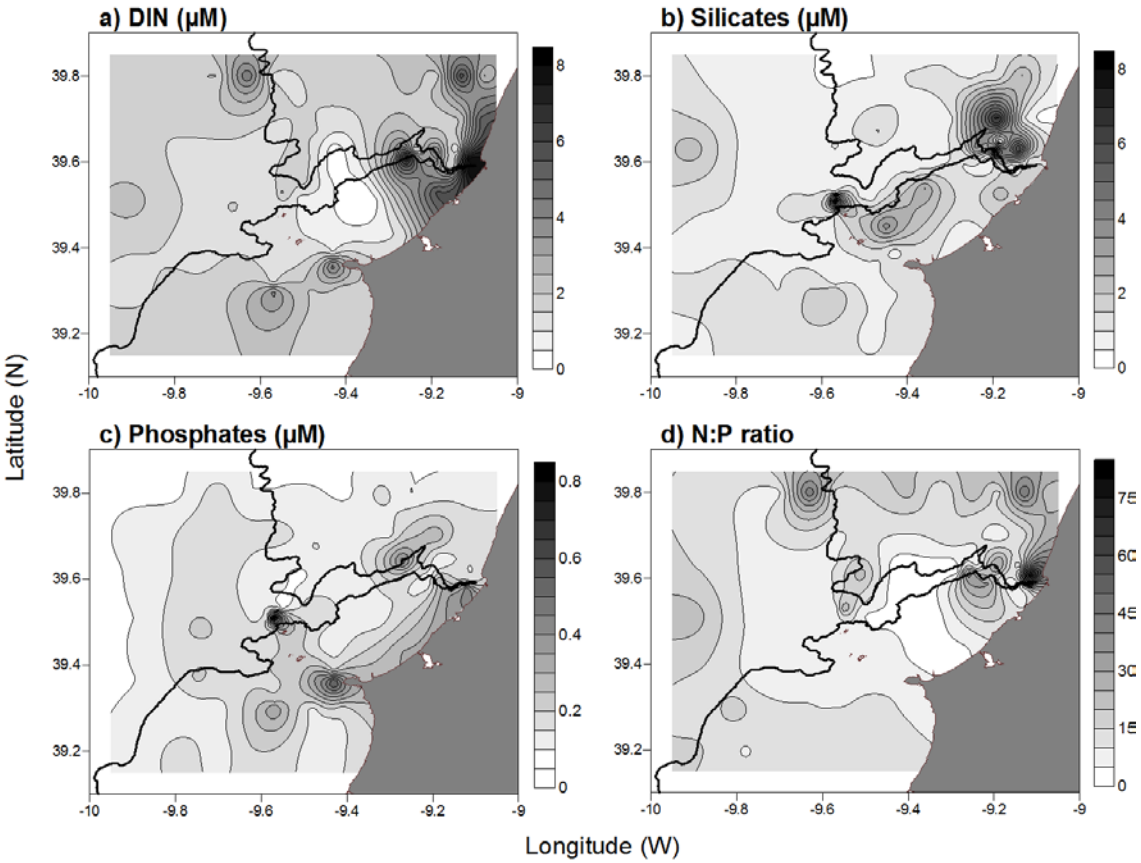

Figure 6: Surface nutrients distribution (µM) measured during this study. (a) Dissolved inorganic nitrogen (DIN), (b) silicates, (c) phosphates and (d) N:P ratios. Note the different concentration scales. Black line scheme the canyon.

2.3.3. Pigment concentrations and CHEMTAX analysis

Through the analysis of chromatograms it was possible to identify a total of 20 pigments (Table 2). Chl *a* (used as biomass index), fucoxanthin, Chl c_1+c_2, peridinin and 19'-hexanoyloxyfucoxathin were the most abundant pigments in this study, present in almost all samples, with average concentrations exceeding 0.1 µg L^{-1}. Chl *a* concentration ranged between 0.1 and 4.3 µg L^{-1}, with the highest values found near the coast and the lowest in more offshore regions (Fig. 7). Maximum biomass (3.9 and 4.3 µg L^{-1} of Chl *a*) was observed at two stations near the coast, around the canyon head, where

dinoflagellate-exclusive pigments, peridinin, dinoxanthin and p-457 were detected. At both stations, peridinin concentrations were extremely elevated, surpassing 3 µg L^{-1}. There was also a nucleus of high Chl *a* south of the canyon and southwest of Cape Carvoeiro, with values greater than 1 µg L^{-1} (Fig. 7).

Table 2: Abbreviations, names and concentrations of photosynthetic pigments detected in this study.

Abbreviation	*Pigment*	*Average concentration/minimum and maximum (µg L^{-1})*
Chl *a*	Chlorophyll *a*	0.68 (0.10 - 4.33)
Chl *b*	Chlorophyll *b*	0.06 (0.01 - 0.46)
Chl c_3	Chlorophyll c_3	0.09 (0.00 - 0.40)
Chl c_1+c_2	Chlorophyll c_1 plus c_2	0.13 (0.00 - 1.47)
Chlide *a*	Chlorophyllide *a*	trace amounts
Fuco	Fucoxanthin	0.17 (0.01 - 1.28)
Perid	Peridinin	0.16 (0.01 - 3.29)
Diadino	Diadinoxanthin	0.07 (0.01 - 0.83)
Diato	Diatoxanthin	0.01 (0.00 - 0.06)
Dino	Dinoxanthin	no standard
Hex-fuco	19'-Hexanoyloxyfucoxanthin	0.10 (0.02 - 0.38)
But-fuco	19'-Butanoyloxyfucoxanthin	0.02 (0.00 - 0.09)
Allo	Alloxanthin	0.02 (0.00 - 0.35)
Zea	Zeaxanthin	0.06 (0.02 - 0.21)
Pras	Prasinoxanthin	0.01 (0.00 - 0.10)
Lut	Lutein	0.01 (0.00 - 0.03)
Viola	Violaxanthin	trace amounts
ββ-Car	β,β-Carotene	trace amounts
βε-Car	β,ε-Carotene	trace amounts
P-457	P-457	trace amounts

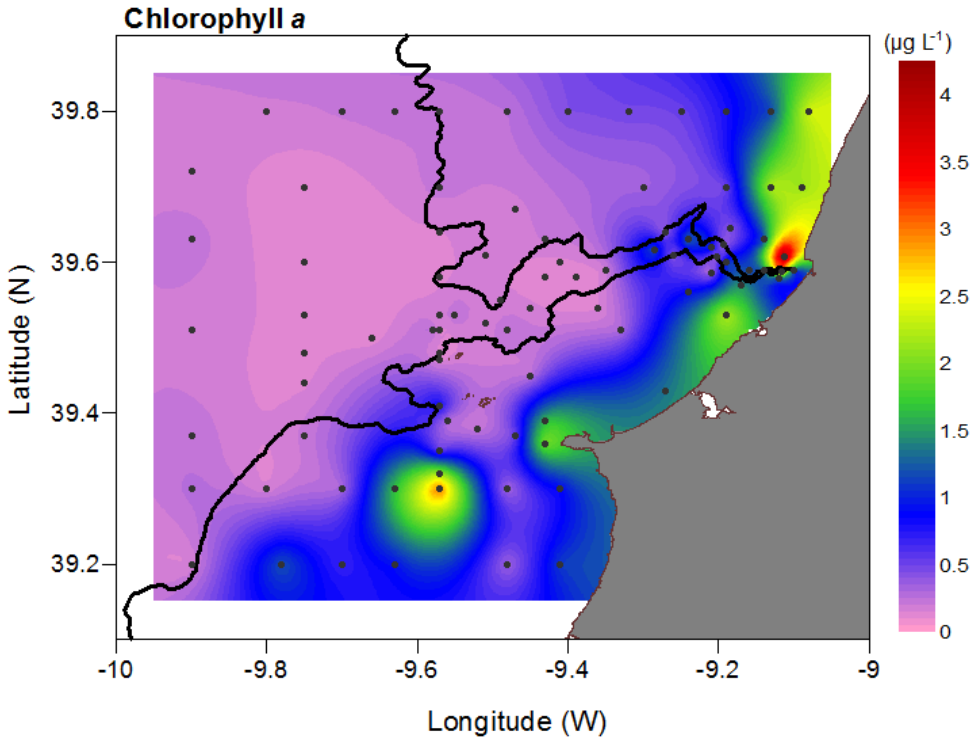

Figure 7: Surface distribution of total chlorophyll *a* (µg L^{-1}). Black points represent stations' location.

The relative contribution of the main phytoplankton groups to Chl *a*, calculated by CHEMTAX is shown in Fig. 8. Diatoms were distributed mainly in the region south of the canyon, along the coast and surrounding Cape Carvoeiro (Fig. 8a) with a maximum biomass contribution of 80%. Dinoflagellates appeared to dominate only at two stations immediately north of the canyon head, with values higher than 65% (Fig 8b). In all other stations the contribution of dinoflagellates was always below 30% of the total Chl *a*. Prymnesiophytes were the dominant group off the coast with a maximum dominance (>50% of total Chl *a*) at stations north of the canyon (Fig. 8c). Cyanobacteria appeared with a major contribution (>25%) in offshore stations (Fig. 8d) and a maximum of cryptophytes (>20%) was observed at two stations south of the canyon region, at approximately 39.3°N (Fig. 8e). Prasinophytes,

more abundant north of the canyon, were always below 20% (Fig. 8f), and crysophytes never represented more than 8% of biomass (data not shown).

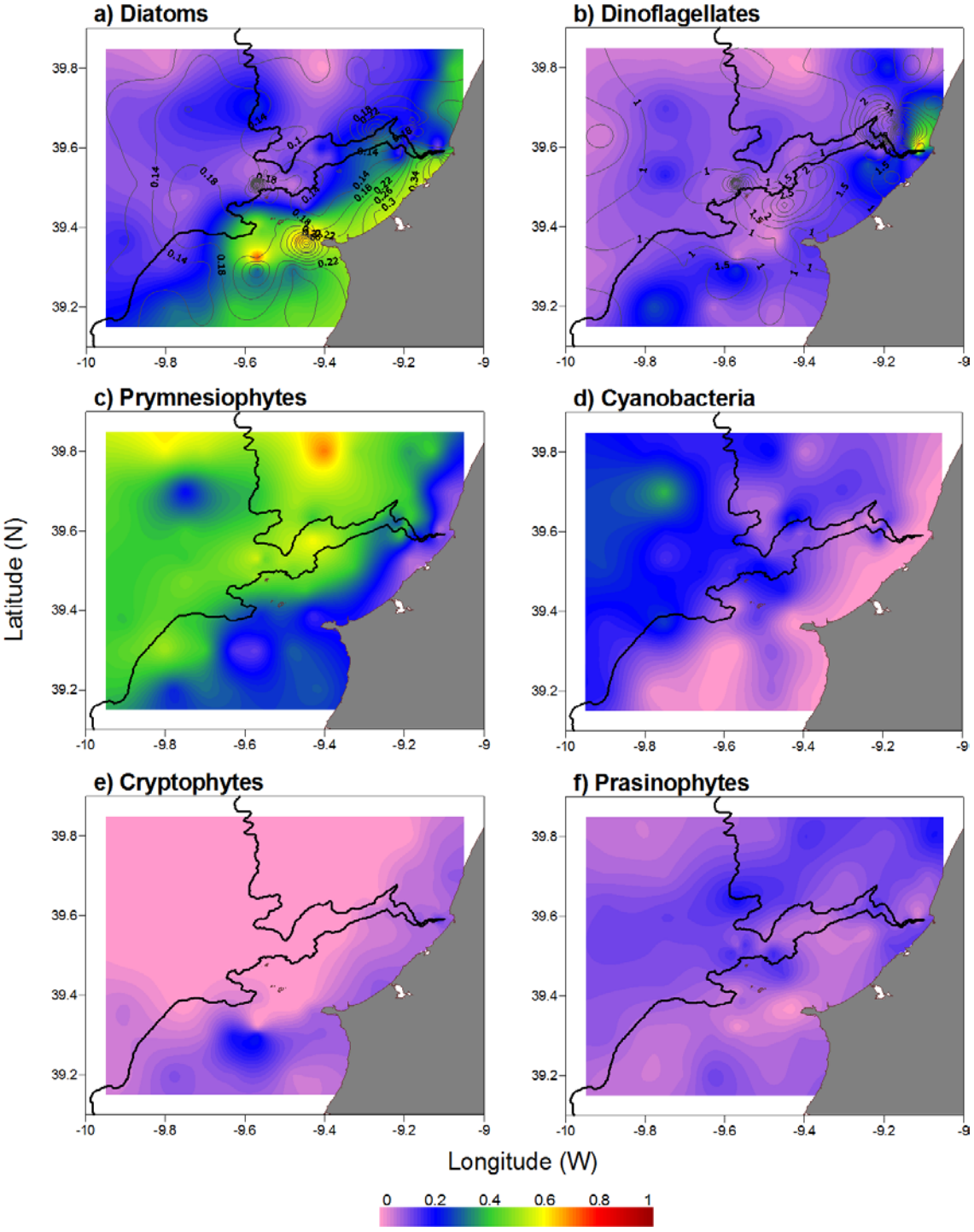

Figure 8: Surface distribution of relative percentage contribution of main phytoplankton groups to total chlorophyll *a*, estimated by interpretation of pigment HPLC data using the CHEMTAX program. (a) Diatoms (color scale) and phosphate concentrations (lines), (b) dinoflagellates (color scale) and silicate concentrations (lines), (c) prymnesiophytes, (d) cyanobacteria, (e) cryptophytes and (f) prasinophytes.

2.3.4. Spatial distribution of taxonomical groups

The Bray–Curtis Similarity Index among stations was applied to estimate the spatial variability of phytoplankton community. Cluster analysis identified three main groups of stations (60% similarity), represented in Fig. 9a. The SIMPER procedure revealed one group (closed circles in Fig. 9a) composed by two stations dominated by dinoflagellates, with 74% contribution to within-group similarity. A second group (open circles in Fig. 9a) consisted of stations dominated by diatoms which contributed 47% to the within-group similarity; and prymnesiophytes contribution of 24%. Finally, a third larger group (closed triangles in Fig. 9a) defined by stations with dominance of prymnesiophytes, with 51% contribution to station similarity. Within this last group, two stations appear separated from the rest and from one another (cluster analysis – 65% similarity). These stations (open triangle and open square in Fig. 9a) presented the highest prymnesiophyte (72%) and cyanobacteria (40%) relative contributions to biomass, respectively.

The level of water column stratification at the selected stations representing each sub-region was analyzed through temperature profiles (Fig. 9b). For the prymnesiophyte dominated group an offshore (St. 24) and a coastal (St. 32) station were selected in order to represent the spatial span of the group. The offshore station is well stratified with surface temperatures around 20ºC and 14ºC at 100 m depth. Lower surface temperatures are observed in the coastal station (around 17ºC) and water column was moderately stratified. Diatom (St. 152) and dinoflagellate-dominated (St. 56)

stations were characterized by a poorly stratified water column with a narrow temperature range with depth.

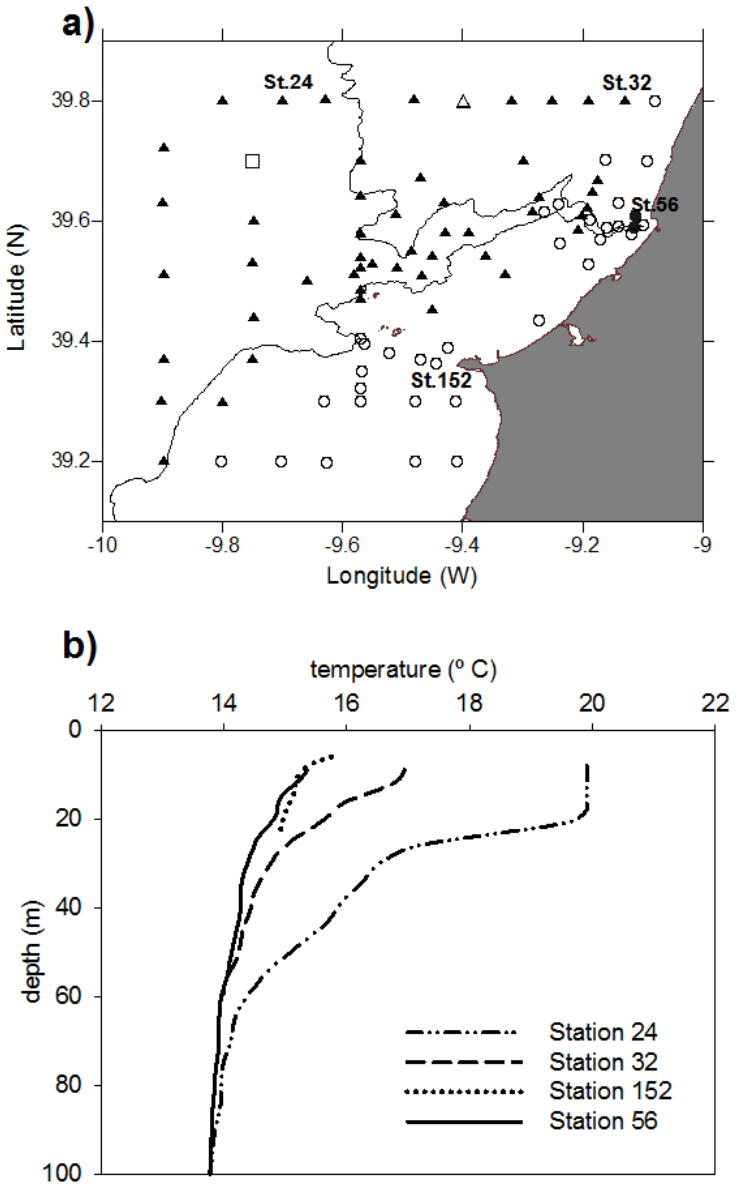

Figure 9: (a) Schematic representation of Cluster analysis results based on the relative contribution of phytoplankton groups to biomass. (○) Group with the highest diatom contributions; (●) group with dinoflagellate dominance; (▲) group with prymnesiophyte dominance; (△) station with the highest values of prymnesiophyte contribution and (□) station with the highest contribution from cyanobacteria. (b) Temperature profiles (ºC) of four selected representative stations: diatom dominated station (station 152), dinoflagellate station (station 56), coastal (station 32) and offshore (station 24) prymnesiophyte dominated stations. For station location see Fig. 9a.

A Monte Carlo test of F-ratio showed that the seven environmental variables contributed significantly to explain the spatial distribution of phytoplankton groups ($p < 0.01$) (water temperature, salinity, water depth, N:P ratio, phosphate, DIN and silicate). A Canonical Correspondence Analysis (CCA) was used to investigate the response of each group to the environmental variables analyzed. The first two ordination axes from the CCA explained 95% of the total spatial distribution of phytoplankton groups with 62% referring to the first canonical axis and 33% to the second one (Fig.10).

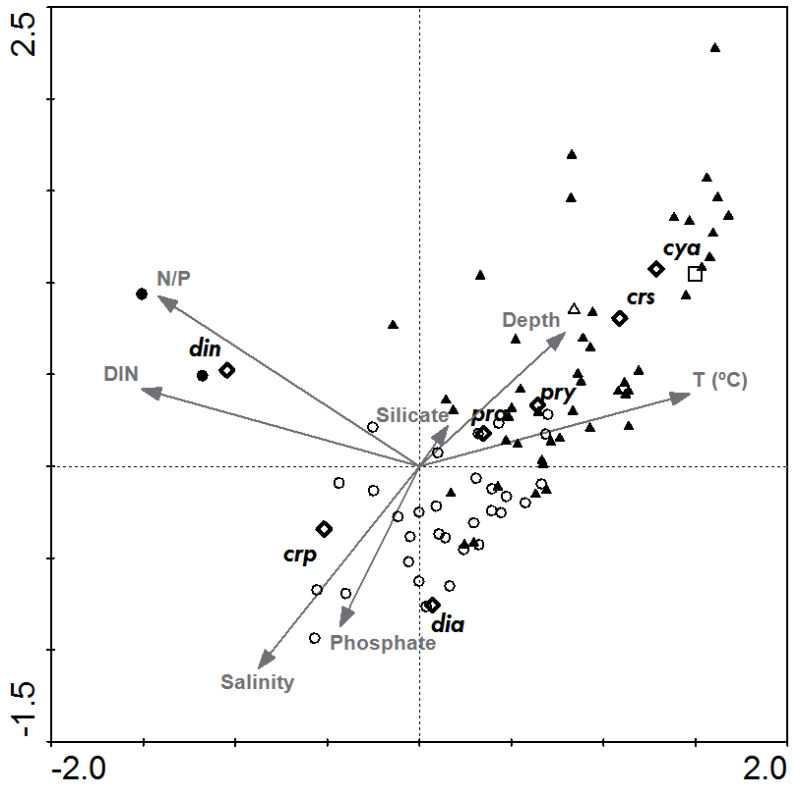

Figure 10: Canonical Correspondence Analysis ordination diagram relative to data on absolute contributions of phytoplankton groups. The first two ordination axes represent 62% of the total phytoplankton group's variance and 95% of phytoplankton groups–environment relations. Arrows refer to environmental variables (water temperature (TºC), water depth (Depth), dissolved inorganic nitrogen (DIN), N:P ratio (N/P), salinity, phosphate and silicate). Diamonds (◆) refer to absolute contribution of phytoplankton groups (***din***, dinoflagellates; ***dia***, diatoms; ***crp***, cryptophytes; ***pra***, prasinophytes; ***pry***, prymnesiophytes; ***cya***, cyanobacteria; ***crs***, crysophytes). Samples are represented with the same symbols used in Fig. 9a for better comparison with cluster analysis results.

The first axis reveals a clear distribution of phytoplankton groups (❖ in Fig.10) associated with temperature and water depth, being prasinophytes (pra), prymnesiophytes (pry), crysophytes (crs) and cyanobacteria (cya) distribution characterized by higher temperature, offshore waters. Conversely, diatoms (dia), cryptophytes (crp) and dinoflagellates' (din) contributions are related to cold and more saline waters, rich in nutrients (coastal upwelled waters). Diatoms and dinoflagellates appear related to higher phosphate and nitrate concentrations, respectively.

2.3.5. Microscopic analysis

Microscope identification of the micro-phytoplankton component (>20 μm) from coastal stations revealed the presence of the following species: the dinoflagellates *Alexandrium affine*, *Ceratium azoricum*, *Ceratium candelabrum*, *Ceratium extensum*, *Ceratium macroceros*, *Ceratium massiliense*, *Ceratium pentagonum*, *Ceratium tripos*, *Ceratium furca*, *Ceratium fusus*, *Dinophysis acuta*, *Dinophysis caudata*, *Gymnodinium catenatum*, *Prorocentrum micans*, *Protoperidinium diabolus*, *Protoperidinium divergens*, *Protoperidinium oceanicum*, *Protoperidinium steinii*, and the diatoms *Pseudo-nitzschia* sp., *Rhizosolenia* sp., *Thalassiosira* sp., *Lioloma sp.*, *Proboscia alata*, *Leptocylindrus danicus*, *Leptocylindrus mediterraneus* and *Detonula pumila*. The high-peridinin concentration stations revealed the presence of large chains of dinoflagellates (e.g. A. *affine* and C. *pentagonum*) together with some non-chain forming dinoflagellates (e.g. D. *acuta*, D. *caudata* and C. *furca*).

2.4. Discussion

Diatoms, dinoflagellates and prymnesiophytes dominated the phytoplankton assemblage in terms of contribution to total biomass (chlorophyll *a*), around the Nazaré canyon region. However, cyanobacteria were an important group in most offshore stations. Those sites, with higher values of zeaxanthin concentration, were also analyzed by C8 HPLC method [Zapata et al., 2000], adequate for picophytoplankton, but no divinyl chl *a* (prochlorophytes biomarker) was detected. Hence we can assume that the picophytoplankton assemblage did not include prochlorophytes. The C18 HPLC method mostly used in this work was selected for being less costly, faster and for showing a higher sensitivity than the C8 method [Mendes et al., 2007].

Prymnesiophytes appeared outside the upwelling areas, in stratified and nutrient-poor oceanic waters (see CCA in Fig. 10). The maximum concentrations of Hex-fuco (major pigment of prymnesiophytes) were recorded in a region north of the canyon. A previous micropalaeontological study of surface sediments from the Nazaré canyon region revealed high abundance and diversity of nannoliths (calcareous structures of nannoplankton) in exactly the same area [Guerreiro et al., 2009]. The authors suggest that locally favorable conditions for productivity of coccolithophores could be promoted in this part of the shelf by non-linear internal waves (solitons). Those, according to Quaresma et al. [2007], are generated along the northern flank of the canyon by the interaction of the dominant semi-diurnal tide with the canyon topography, and propagate northward over the shelf, leading to enhanced injection of nutrients from the bottom to surface waters. This mechanism has been proven

to be an important contributor to the enhancement of biological productivity in some shelf-break regions [Sangrà et al., 2001 and references therein]. A study of marine coccolithophores in Portuguese coastal waters emphasized the importance of these organisms with a dominance of Gephyrocapsa species and *Emiliania huxleyi* [Silva et al., 2008a; Silva et al., 2008b].

Microphytoplankton (diatoms and dinoflagellates) was abundant along the coast line, responding to the upwelling signal and corresponding to the highest values of Chl *a*. The dynamical conditions also favored high Chl *a* concentrations south of Cape Carvoeiro in an eddie-like structure (filament) where strong diatom dominance was registered. This pattern, associated with the presence of the Cape, is typical of an alternation between upwelling and downwelling processes, and frequently reported for the Portuguese coast [Relvas et al., 2007]. In the study period, upwelling was observed in the first period and relaxation in the second; however, the spatial distribution of the phytoplankton communities remained essentially unaltered, as the same species composition was observed at a station sampled at both periods on the canyon head. This condition can be seen in the similarity of the vertical temperature profiles in the repeated transect (see Fig. 5). This section shows that in response to the relaxation of the upwelling favorable winds, warm oceanic water extends onshore, increasing stratification in the upper 20-30 m. The water column below however, remains mostly unchanged during this period. This suggests there was a relaxation process during the cruise, visible at the SST images and winds' regimes, but not sufficiently strong to cause impact on the surface phytoplankton community's composition, as verified by microscopic analysis.

Pigment analysis revealed micro-phytoplankton as dominant in the thin band of upwelled water, but a distinct spatial distribution was evident between diatoms and dinoflagellates (see CCA in Fig. 10). Diatoms dominated south of the canyon, around Cape Carvoeiro, whereas dinoflagellates were more abundant at the two northern stations located at the head of the canyon (Fig. 8).

According to Kudela et al. [2005], spatial patterns of phytoplankton biomass distribution in upwelling systems are related to water-column stratification, nutrient availability, and the intensity and persistence of upwelling conditions. Stratification is a primary condition associated with blooming of red tide dinoflagellates [Margalef et al., 1979; Smayda, 1997; Ryan et al., 2005b], as it separates resources that sustain phytoplankton: light that increases towards the surface, and nutrients that increase in concentration with depth. By enabling access to separated light and nutrient resources, motility of dinoflagellates can provide competitive advantage over non-motile species [Ryan et al., 2005b]. In this study, diatoms and dinoflagellates appear in distinct regions, separated by the submarine canyon, but with similar hydrographic conditions, visible in the temperature profiles of both regions (Fig. 9b). Both groups appear in the band of upwelled waters, with relatively turbulent cool waters under a well developed thermocline. The predominance of chain-forming dinoflagellate species found in our study is in accordance with their selective advantage to survive in turbulent waters around upwelling areas [Margalef et al., 1979; Fraga et al., 1989; Moita et al., 2003].

Concerning nutrient availability, nitrogen and phosphorus strongly stimulate phytoplankton blooms, including harmful bloom species (HAB's), in coastal areas [Paerl, 1997; Baek et al., 2008]. Along the coast south of the

canyon, dominated by diatoms, concentrations of phosphates were always greater than 0.2 µM (see Fig. 7a). In contrast, north of the canyon head, at the two stations with the highest biomass and dominance of dinoflagellates, phosphate levels were less than 0.2 µM, with an extremely high N:P ratio (60 and 80, respectively) thus indicating a potential limitation by phosphate in this region, possibly restricting diatoms growth. This condition may favor dinoflagellates, since it is known that some species can grow on organic phosohorus sources by using alkaline phosphatase enzymes [Jauzein et al., 2010]. The nutrient distribution around the canyon head can be explained by the persistent input of nutrients in the south-side of the canyon, where the phosphate availability (>0.2 µM) remains apparently favorable for diatom growth. The influence of the coastal Óbidos lagoon can be discarded, as the profiles of nearby stations do not show any intrusion of a different water mass (data not shown).

Recent laboratory research revealed that the growth rates of two Ceratium species increased in high N:P (32-200) nutrient conditions (P limitation), suggesting an advantage over other algal species in phosphorus-limited conditions [Baek et al., 2008]. These authors calculated the half-saturation constants (K_s) for nitrate and phosphate of C. *furca* (0.49 and 0.05 µM, respectively) and C. *fusus* (0.32 and 0.03 µM, respectively), which are low comparing to diatom K_s values. In fact, a great variability is reported for diatom phosphorous K_s, e.g. 0.29-0.39 for *Eucampia zodiacus* [Nishikawa et al., 2007], 5.8-7 for *Thalassiosira pseudonana* [Perry, 1976], or 0.68 for *Skeletonema costatum* [Yamamoto & Tarutani, 1999], among others. In our study, some of the dinoflagellate species (e.g. C. *furca* and C. *fusus*), show the

ability to obtain nutrients through alternative nutrition sources, such as phagotrophy, which might contribute to bloom formation and population persistence [Baek et al., 2008].

Among the dinoflagellate community, some toxic species were very abundant, such as two *Dinophysis* species and A. *affine*. The occurrence of these organisms has been previously reported in this region by Moita [2001], *Dinophysis* spp in stratified summer conditions and A. *affine* in the autumn period. Our results revealed a massive bloom of dinoflagellates, both of unicellular and chain-forming species (e.g. A. *affine*, in some cases with more than 30 cells per chain) located in a well-defined region of the canyon head.
For other regions in the Northwest Portuguese coast several episodes of toxic dinoflagellate blooms have been also described [Moita et al., 2003; Moita et al., 2006 and references therein]. Many of these species (e.g. *Alexandrium* spp and *Gymnodinium catenatum)* produce benthic resting stages which accumulate in bottom sediments and may function as seed-beds for planktonic blooms [Amorim & Dale, 1998].

The contrasting nutrient conditions at north and south of the canyon are associated with particular interactions between topography and upwelling processes. The spring/summer oceanographic conditions along the west Portuguese coast are generally dominated by a coastal upwelling, characterized by a southward (left bounded) flowing shelf circulation and an important supply of nutrient-rich waters to the surface layers. Left bounded flows interacting with a narrow canyon, such as the Nazaré Canyon, are known to promote an important circulation inside canyon structures [Klinck, 1996; She & Klinck, 2000]. The pressure gradient that supports the flow in the shelf, inside canyons,

forces onshore (up-canyon) flow and intensified upwelling at the canyon's head. This circulation pattern can be advected by the incident flow, leading to intensified upwelling in the southward (downstream) rim of the canyon and the nearby shelf [Allen, 1996; Klinck, 1996]. Previous observations suggest that this kind of response to incident flow is also observed in Nazaré Canyon, under upwelling conditions. The data collected in May 2004, in particular, revealed the presence of upward motion and high surface nutrient concentrations on the southward flank of the canyon and over the shelf area just south of the canyon [Vitorino et al., 2005]. The occurrence of intensified upwelling in the southern rim of the canyon, which extends its influence to the shelf south of the canyon, can eventually persist even during the period of relaxation of upwelling winds, providing a probable explanation for the persistence of high concentrations of diatoms observed in this area, in both phases of the cruise. The significant association found between higher salinities (of typical cold upwelled waters), phosphates and diatoms' abundance (see CCA results in Fig.10) seems to reinforce the occurrence of this process.

2.5. Conclusion

In this work, the use of biomarker taxonomic pigments, combined with microscopic observations, have been used to study phytoplankton distribution in a peculiar region of the Portuguese coast. The presence of topographic features such as the submarine canyon and important capes such as Cape Carvoeiro contribute to define the regional characteristics of the coastal ocean response to upwelling winds and, consequently affect the distribution of the main

phytoplankton groups. Microscopic observations confirmed the high abundance of toxic dinoflagellate species (e.g. A. *affine* and *Dinophysis* species) in a specific region of the canyon. This important occurence with potential ecological implications was associated with oceanographic and chemical conditions influenced by peculiar characteristics of the region. However, a more comprehensive study is desirable to enlighten the real impact of the canyon on phytoplankton dynamics and HAB's occurrence in the area.

Acknowledgements

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References

Allen S (1996). Topographically generated subinertial flows within a finite length canyon. J. Phys. Oceanog. 26, 1608-1632.

Amorim A, Dale B (1998). Distribution of cysts from toxic or potentially toxic dinoflagellates along the portuguese coast. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia e Intergovernmental Oceanographic Commission of UNESCO, Santiago de Compostela, pp. 64-65.

Baek SH, Shimode S, Han MS, Kikuchi T (2008). Growth of dinoflagellates, *Ceratium furca* and *Ceratium fusus* in Sagami Bay, Japan: The role of nutrients. Harmful Algae 7, 729-739.

Brotas V, Plante-Cuny MR (1996). Identification et quantification des pigments chlorophylliens et caroténoïdes des sédiments marins: un protocole d'analyse par HPLC. Oceanol. Acta 19, 623-634.

Carreto JI, Montoya N, Akselman R, Carignan MO, Silva RI, Colleoni DAC (2008). Algal pigment patterns and phytoplankton assemblages in different water masses of the Río de la Plata maritime front. Cont. Shelf Res. 28, 1589-1606.

Clarke KR, Gorley RN (2001). Primer - Plymouth Routines In Multivariate Ecological Research - v5: User Manual/Tutorial. PRIMER-E Ltd., Plymouth, pp. 91.

Dodge JD (1982). Marine Dinoflagellates of the British Isles. Her Majesty's Stationary Office, London.

Ediger D, Soydemir N, Kideys AE (2006). Estimation of phytoplankton biomass using HPLC pigment analysis in the southwestern Black Sea. Deep-Sea Res. II 53, 1911-1922.

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

Fiúza AFG, Macedo ME, Guerreiro MR (1982). Climatological space and time variation of the Portuguese coastal upwelling. Oceanol. Acta 5, 31-40.

Fraga S, Gallagher SM, Anderson DM (1989). Chain-forming dinoflagellates: an adaptation to red tides. In: Okaichi, T., Anderson, D.M., Nemoto, T. (Eds.), Red Tides: Biology, Environmental Science and Toxicology. Elsevier, New York, pp. 281-284.

Gibb SW, Cummings DG, Irigoien X, Barlow RG, Fauzi R, Mantoura C (2001). Phytoplankton pigment chemotaxonomy of northeastern Atlantic. Deep-Sea Res. II 48, 795-823.

Gieskes WWC, Kraay GW (1983). Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. Mar. Biol. 75, 179-185.

Guerreiro C, Rosa F, Oliveira A, Cachão M, Fatela F, Rodrigues A (2009). Calcareous nannoplankton and benthic foraminiferal assemblages from the Nazaré Canyon (Portuguese continental margin): preliminary results. IOP Conf. Ser., Earth. Environ. Sci. 5, 012004 (11pp) doi: 10.1088/1755-1307/5/1/012004.

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

Haynes R, Barton ED, Pilling I (1993). Development, persistence and variability of upwelling filaments off the Atlantic coast of the Iberian Peninsula. J. Geophys. Res. 98, 22681-22692.

Jauzein C, Labry C, Youenou A, Quéré J, Delmas D, Collos Y (2010). Growth and phosphorus uptake by the toxic dinoflagellate *Alexandrium catenella* (dinophyceae) in response to phosphate limitation. J. Phycol. 46, 922-936.

Jeffrey SW, 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: guidelines to modern methods, UNESCO Monogr. Oceanogr. Methodol., Vol 10, UNESCO Publishing, Paris, pp. 37-84.

Klinck JM (1996). Circulation near submarine canyons: a modeling study. J. Geophys. Res.101, 1211-1223.

Koroleff F (1976). Determination of ammonia. In: Grasshoff. K. (Ed.), Methods of Seawater Analysis. Verlag Chemie, NewYork, pp. 126-158.

Kraay GW, Zapata M, Veldhuis MJW (1992). Separation of chlorophylls c1, c2, and c3 of marine phytoplankton by reversed-phase-C18-High-Performance Liquid Chromatography. J. Phycol. 28, 708-712.

Kudela R, Pitcher G, Probyn T, Figueiras F, Moita T, Trainer V (2005). Harmful Algal Blooms in Coastal Upwelling Systems. Oceanography 18, 184-197.

Latasa M (2007). Improving estimations of phytoplankton class abundances using CHEMTAX. Mar. Ecol. Prog. Ser. 329, 13-21.

Mackey DJ, Higgins HW, Mackey MD, Holdsworth D (1998). Algal class abundances in the western equatorial Pacific: estimation from HPLC measurements of chloroplast pigments using CHEMTAX. Deep-Sea Res. I 45, 1441-1468.

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996). CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 144, 265-283.

Margalef R, Estrada M, Blasco D (1979). Functional morphology of organisms involved in red tides, as adapted to decaying turbulence. In: Taylor, D.L., Seliger, H.H., (Eds.), Toxic Dinoflagellate Blooms, Proceedings of the Second International Conference on toxic dinoflagellate blooms. Elsevier, New York, pp. 89-94.

Mendes CR, Cartaxana P, Brotas V (2007). HPLC determination of phytoplankton and microphytobenthos pigments: comparing resolution and sensitivity of a C18 and a C8 method. Limnol. Oceanogr. Methods 5, 363-370.

Moita MT (2001). Structure, variability and dynamics of phytoplankton from the Portuguese continental coast. PhD dissertation, University of Lisbon, Lisbon.

Moita MT, Oliveira PB, Mendes JC, Palma AS (2003). Distribution of chlorophyll *a* and *Gymnodinium catenatum* associated with coastal upwelling plumes off central Portugal. Acta. Oecol. 24, 125-132.

Moita MT, Sobrinho-Gonçalves L, Oliveira PB, Palma S, Falcão M (2006). A Bloom of Dinophysis acuta in a thin layer off NW Portugal. S. Afr. J. Mar. Sci. 28, 265-269.

Mork M, Jorge da Silva A (1993). Transient upwelling off West Ibéria. ICES C.M. 1993/C43, 11pp.

Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27, 31-36.

Nishikawa T, Hori Y, Tanida K, Imai I (2007). Population dynamics of the harmful diatom Eucampia zodiacus Ehrenberg causing bleachings of Porphyra thalli in aquaculture in Harima-Nada, the Seto Inland Sea, Japan. Harmful Algae 6, 763-773.

Paerl HW (1997). Coastal eutrophication and harmful algal bloom: importance of atmospheric deposition and groundwater as 'new' nitrogen and other nutrient sources. Limnol. Oceanogr. 42, 1154-1165.

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

Perry MJ (1976). Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in oligotrophic waters at the central North Pacific, Limnol. Oceanogr. 21, 88-107.

Quaresma LS, Vitorino J, Oliveira A, Silva J (2007). Evidence of sediment resuspension by nonlinear internal waves on the western Portuguese mid shelf. Mar. Geol. 246, 123-143.

Relvas P, Barton ED, Dubert J, Oliveira PB, Peliz Á, da Silva JCB, Santos AMP (2007). Physical oceanography of the western Iberia ecosystem: Latest views and challenges. Prog. Oceanogr. 74, 149-173.

Ryan JP, Chavez FP, Bellingham JG (2005a). Physical-biological coupling in Monterey Bay, California: topographic influences on phytoplankton ecology. Mar. Ecol. Prog. Ser. 287, 23-32.

Ryan JP, Dierssen HM, Kudela RM, Scholin CA, Johnson KS, Sullivan JM, Fischer AM, Rienecker EV, Mcenaney PR, Chavez FP (2005b). Coastal Ocean Physics and Red Tides – an example from Monterey Bay, California. Oceanography 18, 246-255.

Sangrà P, Basterretxea G, Pelegri JL, Aristegui J (2001). Chlorophyll increase due to internal waves on the shelf break of Gran Canaria (Canary Islands). Sci. Mar. 65, 89–97.

Schlüter L, Havskum H (1997). Phytoplankton pigments in relation to carbon content in phytoplankton communities. Mar. Ecol. Prog. Ser. 155, 55-65.

Schlüter L, Møhlenberg F, Havskum H, Larsen S (2000). The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. Mar. Ecol. Prog. Ser. 192, 49-63.

She J, Klinck JM (2000). Flow near submarine canyons driven by constant winds. J. Geophys. Res. 105, 28671-28694.

Silva A, Mendes CR, Palma S, Brotas V (2008a). Short-time scale variation of phytoplankton succession in Lisbon bay (Portugal) as revealed by microscopy cell counts and HPLC pigment analysis. Est. Coast. Shelf. Sci. 79, 230-238.

Silva A, Palma S, Moita MT (2008b). Coccolithophores in the upwelling waters of Portugal: Four years of weekly distribution in Lisbon bay. Cont. Shelf Res. 28, 2601-2613.

Silva A, Palma S, Oliveira PB, Moita MT (2009). Composition and interannual variability of phytoplankton in a coastal upwelling region (Lisbon Bay, Portugal). J. Sea Res. 62, 238-249.

Smayda TJ (1997). Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42, 1137-1153.

Strickland JDH, Parsons TR (1972). A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottawa.

Ter Braak CJF, Smilauer P (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for Canonical Community Ordination (version 4.5). Microcomputer Power, New York, USA, pp. 500.

Vitorino J, Beja J, Pinto J, Bernardino M, Quaresma L (2005). Physical oceanography of the western Iberian margin: an overview of Nazare Canyon processes based on EUROSTRATAFORM data. EUROSTRATAFORM Final Report, Salamanca.

Wright SW, Jeffrey SW (2006). Pigment markers for phytoplankton production. In: Volkmann J.K. (Ed.), Marine Organic Matter: Biomarkers, Isotopes and DNA, Springer-Verlag, Berlin, pp. 71-104.

Wright SW, Thomas DP, Marchant J, Higgins HW, Mackey MD, Mackey DJ (1996). Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorisation program. Mar. Ecol. Prog. Ser. 144, 285-298.

Wright SW, Ishikawa A, Marchant HJ, Davidson AT, van den Enden RL, Nash GV (2009). Composition and significance of picophytoplankton in Antarctic waters. Polar Biol. 32, 797-808.

Yamamoto T, Tarutani K (1999). Growth and phosphate uptake kinetics of the toxic dinoflagellate *Alexandrium tamarense* from Hiroshima Bay in the Seto Inland Sea, Japan. Phycol. Res. 47, 27-32.

Zapata M, Rodriguez F, Garrido JL (2000). Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. Mar. Ecol. Prog. Ser. 195, 29-45.

CAPÍTULO III

Dynamics of phytoplankton communities during late summer around the tip of the Antarctic Peninsula

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Mendes CRB, de Souza MS, Garcia VMT, Leal MC, Brotas V, Garcia CAE (*accepted with revisions*). Dynamics of phytoplankton communities during late summer around the tip of the Antarctic Peninsula. Deep Sea Research I.

ABSTRACT

The composition and distribution of phytoplankton assemblages were studied around the tip of the Antarctic Peninsula during two summer cruises carried out in February/March 2008 and February/March 2009. Water samples were collected for HPLC/CHEMTAX pigment analysis and microscopic observations. A great spatial variability in chlorophyll *a* (Chl *a*) was observed in the study area: highest levels in the vicinity of the James Ross Island (exceeding 7 mg m^{-3} in 2009), intermediate values (0.5 to 2 mg m^{-3}) in the Bransfield Strait, and the lowest concentrations in the Weddell Sea and Drake Passage (below 0.5 mg m^{-3}). Phytoplankton assemblages were generally dominated by diatoms, especially at coastal stations with high Chl *a* concentration, where diatom contribution was above 90% of total Chl *a*. In open-ocean areas (e.g., Weddell Sea) nanoflagellates, such as cryptophytes and/or *Phaeocystis antarctica,* replaced diatoms. Many species of peridinin-lacking autotrophic dinoflagellates (e.g., *Gymnodinium* spp.) were also important to total Chl *a* biomass at well-stratified stations of Bransfield Strait. Iron limitation, inferred from a Fe-nutritional state index (19'-hexanoyloxyfucoxanthin:chlorophyll c_3 ratio), and water column structure were the most important environmental factors determining the biomass and distribution of the phytoplankton communities. The HPLC pigment data also allowed an assessment of different physiological responses of phytoplankton to ambient light variation. The present study provides new insights about the dynamics of phytoplankton in an undersampled region of the Southern Ocean.

Keywords: Antarctic Peninsula, Phytoplankton, Pigments, HPLC, CHEMTAX

3.1. Introduction

The Antarctic Peninsula (AP) is experiencing one of the fastest rates of regional climate change on Earth, as ocean surface temperatures at the continental margin of the western AP have undergone a pronounced warming (3-4ºC) over the past century [Turner et al., 2005; Steig et al., 2009]. Such changes promote the collapse of ice shelves, retreat of glaciers and exposure of new terrestrial habitats [Clarke et al., 2007]. Environmental features, as regional circulation system, seasonal changes in the light regime and sea ice cover, have been shown to determine a latitudinal variation in phytoplankton productivity along the western AP [Garibotti et al., 2003]. Moreover, recent studies have shown that changes in phytoplankton biomass and composition along the western shelf of the AP are associated with regional long-term climate alterations [Montes-Hugo et al., 2009].

The Southern Ocean is generally a high-nutrient and low-chlorophyll (HNLC) area, mainly due to the limitation of micronutrients, such as iron. However, high phytoplankton biomass has been observed in particular regions, especially at oceanic fronts, marginal ice zones and nearshore straits, bays, and lees of islands [Prézelin et al., 2000 and references therein]. These high biomass regions are considered critical feeding sites for higher trophic levels and play a crucial role on biogeochemical cycling of nutrients. Phytoplankton blooms in those regions (usually dominated by diatoms or haptophytes, such as *Phaeocystis antarctica*) are generally associated with the development of a shallow mixed layer (with increased light levels that enhance phytoplankton growth) and/or iron availability [Prézelin et al., 2000]. On the other hand, recent

studies have shown the increasing dominance of cryptophytes in the AP region, mostly in zones with glacial melt water [Moline & Prézelin, 1996; Moline et al., 2004]. The dominance of cryptophytes instead of diatoms may influence the trophic web, as cryptophytes are more efficiently grazed by salps than by antarctic krill [Moline et al., 2004]. Therefore, studies on phytoplankton and the influence of environmental constraints in species/groups composition are relevant for evaluation of ecosystem changes, both at short and long-term scales.

The study of phytoplankton community composition has been classically performed with light microscope examination. An alternative way to study phytoplankton community structure is through chemotaxonomic methods based on High Performance Liquid Chromatography (HPLC) analysis, which rely on the presence and relative concentration of pigments that are characteristic of distinct algal taxonomic groups [Wright & Jeffrey, 2006]. Nevertheless, the use of phytoplankton pigments in chemotaxonomic methods has drawbacks, such as non-unique pigment markers and/or potential fluctuations in pigment ratios with physiological stressors, both at species and at cellular level (e.g., irradiance and nutrients) [Wright & Jeffrey, 2006]. On the other hand, variations in the relative concentration of those pigments may be used as indicators of the physiological state of phytoplankton communities [Moline, 1998; DiTullio et al., 2007]. One of the chemotaxonomic tools that has been developed and continuously improved to minimize errors inherent to fluctuations of pigment ratios is CHEMTAX [Mackey et al., 1996]. This approach involves an iterative process of matrix factorization to optimize pigment ratios in order to estimate the contribution of phytoplankton groups to total chlorophyll *a* (Chl *a*).

CHEMTAX has been extensively used in phytoplankton communities in the Australian sector of the Southern Ocean [Wright et al., 1996; Wright & van den Enden, 2000; Wright et al., 2009; 2010] but only few studies have applied this approach to the AP region [Rodriguez et al., 2002; Kozlowski et al., 2011].

In the present work, the spatial pattern of phytoplankton communities was studied around the tip of the AP, encompassing the Bransfield Strait, part of the Drake Passage and a northwestern section in the Weddell Sea. On the eastern side of the AP, particularly in the ice edge zone of the Weddell Sea, extensive phytoplankton blooms have been detected during spring and summer [Sullivan et al., 1993; Park et al., 1999; Kang et al., 2001], which form an important feeding ground for grazers. In addition, the shallows and bays of southwestern Bransfield Strait are breeding grounds for a host of biota, especially krill [Zhou et al., 1994], as result of high surface phytoplankton biomass associated with seasonal blooms [Karl et al., 1991; Castro et al., 2002]. The main objective of the present study was to understand phytoplankton biomass variation and assemblage distribution during two late summers around the tip of AP by chemotaxonomic analysis, complemented with microscopic observations. The specific questions addressed in this study were: (1) what is the relationship between the thermohaline structure, water-column physico-chemical properties and the phytoplankton communities in different areas of the study region?; and (2) can photosynthetic pigments be used as indices (proxies) that reflect physiological state and adaptations of phytoplankton communities to environmental constraints (e.g., iron-limitation and light availability)?

3.2. Material and Methods

3.2.1. Study area and sampling collection

Oceanographic cruises were conducted in the adjacent waters of the tip of AP, from 60.6ºS to 64.3ºS and from 48.3ºW to 62.2ºW (Fig. 1), during late summer of 2008 (February/March 2008) and 2009 (February/March 2009) and as part of the SOS-CLIMATE (Southern Ocean Studies for Understanding Global-CLIMATE Issues) project. Surface water samples were taken in all CTD (conductivity-temperature-depth) stations for phytoplankton pigments and microscopic analyses (phytoplanktonic cell abundance and carbon biomass). No microscopic analyses were made for the Weddell Sea samples due to very low phytoplankton concentrations. Both physical data (conductivity, temperature and salinity) and water samples were collected using a combined Sea-Bird CTD/Carrousel 911+system® equipped with 24 five-liter Niskin bottles. Density (kg m^{-3}) was calculated for evaluation of the water column physical structure based on temperature, salinity and pressure data. The upper mixed layer (UML) depth was determined as the depth where a change of 0.05 kg m^{-3} occurred over a 5 m depth interval [Mitchell & Holm-Hansen, 1991]. At some stations, chosen according with the fluorescence profiles (WetLabs® profiling fluorometer), water samples were taken from several depths for phytoplankton pigment analysis. Phytoplankton cell abundance and carbon biomass data were calculated for surface samples, as they are representative of the UML [Garibotti et al., 2003].

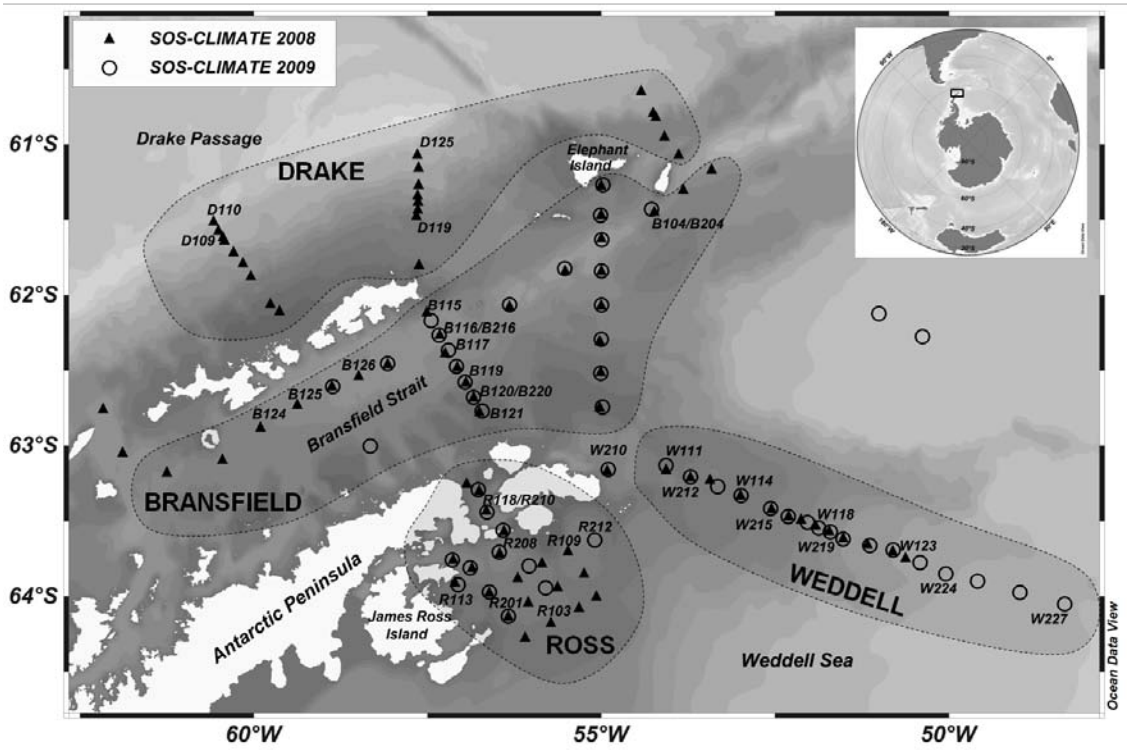

Figure 1: Study area and stations' location during SOS-CLIMATE 2008 and 2009 summer cruises. Bounded stations (dashed line) represent the geographical zonation used in this study (non-bounded stations were not used in the discussion of the results). The first letter of stations' is related to the surveyed region (D = DRAKE, B = BRANSFIELD, W = WEDDELL, R = ROSS). The number following that letter refers to the sampling period (1 = 2008 cruise, 2 = 2009 cruise). Inset map includes the South Polar orthographic projection and the box indicates the magnified region.

3.2.2. HPLC pigment analysis

During the cruises, seawater samples (1-2 L) were filtered onto Whatman GF/F filters (nominal pore size 0.7 µm and 25 mm in diameter), under vacuum pressure (< 500 mbar) and filters were immediately stored in liquid nitrogen. Phytoplankton pigments were extracted with 2 mL of 95% cold-buffered methanol (2% ammonium acetate) for 30 min at -20°C, in the dark. Samples were sonicated (Bransonic, model 1210, w: 80, Hz: 47) for 1 min at the beginning of the extraction period. Samples were then centrifuged at 1100 g for

15 min at 4°C. Extracts were filtered (Fluoropore PTFE membrane filters, 0.2 µm pore size) and immediately injected in the HPLC instrument. Pigment extracts were analyzed using a Shimadzu HPLC comprised of a solvent delivery module (LC-10ADVP) with system controller (SCL-10AVP), a photodiode array (SPD-M10ADVP), and a fluorescence detector (RF-10AXL). The chromatographic separation of pigments was achieved using a monomeric OS C8 column (Symmetry C8, 15 cm long, 4.6 mm in diameter, and 3.5 µm particle size). Mobile phases were: (A) methanol:acetonitrile:aqueous pyridine solution (0.25 M, pH adjusted to 5.0 with acetic acid) (50:25:25, v/v/v), and (B) methanol:acetonitrile:acetone (20:60:20, v/v/v). The solvent gradient followed Zapata et al. (2000) with a flow rate of 1 mL min^{-1}, with an injection volume of 100 µL, and 40 minute runs. The limit of detection and limit of quantification of this method were calculated and discussed in Mendes et al. [2007]. Pigments were identified from both absorbance spectra and retention times and concentrations calculated from the signals in the photodiode array detector or fluorescence detector (Ex. 430 nm; Em. 670 nm). The HPLC system was previously calibrated with pigment standards from Sigma (chlorophyll *a*, *b* and β-carotene) and DHI (for other pigments). Table 1 lists all pigments detected above the limit of quantification and that were considered in this study.

3.2.3. CHEMTAX analysis of pigment data

The relative abundance of microalgal groups contributing to total Chl *a* biomass was calculated by pigment concentration data using CHEMTAX v1.95 chemical taxonomy software [Mackey et al., 1996; Wright et al., 1996; Wright et

al., 2009]. CHEMTAX uses a factor analysis and steepest-descent algorithm to best fit the data on to an initial pigment ratio matrix. The basis for calculations and procedures are fully described in Mackey et al. [1996]. The initial pigment ratios of major algal classes were based on pigment matrices used in studies from the western AP region [Rodríguez et al., 2002; Kozlowski et al., 2011] (Table 2a). Based on the identified diagnostic pigments and confirmation of the higher taxonomic groups by microscopic analysis, 6 algal groups were loaded on CHEMTAX: diatoms, dinoflagellates-1 (peridinin-containing dinoflagellates), "*Phaeocystis antarctica*", cryptophytes, green flagellates (with Chl *b*) and "chemotaxonomic group". The loaded pigments were chlorophyll c_3 (Chl c_3), chlorophyll c_2 (Chl c_2), peridinin (Perid), 19'-butanoyloxyfucoxanthin (But-fuco), fucoxanthin (Fuco), 19'-hexanoyloxyfucoxanthin (Hex-fuco), alloxanthin (Allo), chlorophyll *b* (Chl *b*) and chlorophyll *a* (Chl *a*) (see Table 2a). The "chemotaxonomic group" was defined as having a pigment signature including Chl c_3, Chl c_2, But-fuco, Fuco and Hex-fuco, relative to a group including peridinin-lacking autotrophic dinoflagellates and diatoms with Chl c_3 [Wright & Jeffrey, 2006], and other algal groups whose pigment composition has not yet been exhaustively analyzed (e.g., parmales and chrysophytes).

The same initial ratio was used in data from both study years, but data from each cruise were run separately in order to detect potential variations in optimization of CHEMTAX procedures. Additionally, in order to account for variation in pigment ratios with irradiance and/or nutrient availability, data from each cruise were split into three bins according to sample depth (0-50 m, 50-100 m and >100 m).

Table 1: Concentrations (mg m^{-3}) of pigments (average and minimum-maximum concentrations by geographic region). Chl *a* = chlorophyll *a*; Chlide *a* = chlorophyllide *a*; Phytin *a* = pheophythin *a*; Phide *a* = pheophorbide *a*; Chl *b* = chlorophyll *b*; Chl c_2 = chlorophyll c_2; Chl c_3 = chlorophyll c_3; Allo = alloxanthin; Fuco = fucoxanthin; Hex-fuco = 19'-hexanoyloxyfucoxanthin; But-fuco = 19'-butanoyloxyfucoxanthin; Diadino = diadinoxanthin; Diato = diatoxanthin; Perid = peridinin.

	2008				2009		
Pigment	**Drake**	**Bransfield**	**Ross**	**Weddell**	**Bransfield**	**Ross**	**Weddell**
Chl *a*	0.39 (0.04-0.89)	0.55 (0.12-1.08)	1.76 (0.25-4.50)	0.15 (0.04-0.15)	0.92 (0.35-1.98)	3.73 (0.36-7.61)	0.27 (0.02-0.27)
Chlide *a*	0.01 (0.00-0.03)	0.02 (0.00-0.05)	0.24 (0.00-0.87)	0.01 (0.00-0.01)	0.03 (0.00-0.14)	0.33 (0.00-0.90)	0.01 (0.00-0.03)
Phytin *a*	0.02 (0.00-0.05)	0.02 (0.01-0.05)	0.07 (0.01-0.26)	0.01 (0.00-0.01)	0.04 (0.01-0.07)	0.08 (0.01-0.18)	0.01 (0.00-0.03)
Phide *a*	0.04 (0.00-0.10)	0.08 (0.01-0.22)	0.19 (0.02-0.61)	0.01 (0.00-0.03)	0.09 (0.01-0.29)	0.26 (0.03-0.55)	0.01 (0.00-0.04)
Chl *b*	0.01 (0.00-0.02)	0.01 (0.00-0.04)	0.03 (0.02-0.09)	0.01 (0.00-0.03)	0.01 (0.00-0.03)	0.02 (0.00-0.03)	0.01 (0.00-0.03)
Chl c_2	0.07 (0.01-0.20)	0.11 (0.03-0.24)	0.50 (0.03-1.27)	0.02 (0.01-0.05)	0.13 (0.03-0.28)	0.73 (0.03-1.71)	0.04 (0.00-0.13)
Chl c_3	0.10 (0.00-0.28)	0.15 (0.00-0.37)	0.06 (0.00-0.21)	0.01 (0.00-0.03)	0.09 (0.01-0.28)	0.11 (0.00-0.30)	0.02 (0.00-0.05)
Allo	0.01 (0.00-0.07)	0.02 (0.00-0.23)	0.00 (0.00-0.00)	0.02 (0.00-0.06)	0.01 (0.00-0.03)	0.00 (0.00-0.01)	0.03 (0.00-0.13)
Fuco	0.31 (0.03-0.72)	0.44 (0.10-0.96)	1.60 (0.17-3.47)	0.05 (0.02-0.14)	0.59 (0.10-1.42)	3.00 (0.2-6.95)	0.11 (0.02-0.60)
Hex-fuco	0.07 (0.02-0.12)	0.08 (0.02-0.14)	0.02 (0.00-0.07)	0.05 (0.02-0.08)	0.02 (0.01-0.06)	0.01 (0.00-0.03)	0.06 (0.01-0.14)
But-fuco	0.06 (0.01-0.19)	0.07 (0.01-0.18)	0.01 (0.00-0.02)	0.01 (0.00-0.02)	0.02 (0.01-0.06)	0.00 (0.00-0.01)	0.01 (0.00-0.02)
Diadino	0.10 (0.01-0.23)	0.13 (0.02-0.29)	0.13 (0.02-0.28)	0.02 (0.01-0.03)	0.09 (0.01-0.22)	0.29 (0.02-0.60)	0.03 (0.00-0.09)
Diato	0.01 (0.00-0.04)	0.02 (0.00-0.06)	0.02 (0.00-0.05)	0.00 (0.00-0.00)	0.02 (0.00-0.06)	0.06 (0.00-0.16)	0.00 (0.00-0.01)
Perid	0.02 (0.00-0.05)	0.05 (0.00-0.13)	0.06 (0.00-0.17)	0.00 (0.00-0.00)	0.03 (0.00-0.09)	0.03 (0.00-0.06)	0.01 (0.00-0.02)

Table 2: Pigment to chlorophyll *a* ratios used for CHEMTAX analysis of pigment data. Initial ratios before analysis (a), 2008 optimized ratios (for 0–50m bin) after analysis (b), and 2009 optimized ratios (for 0–50m bin) after analysis (c).

	Chl c_3	**Chl c_2**	**Perid**	**But-fuco**	**Fuco**	**Hex-fuco**	**Allo**	**Chl *b***	**Chl *a***
(a) Input matrix									
Diatoms	0	0.110	0	0	0.754	0	0	0	1
Dinoflagellates-1	0	0.320	0.720	0	0	0	0	0	1
Chemotaxonomic group	0.067	0.126	0	0.122	0.290	0.248	0	0	1
Phaeocystis antarctica	0.141	0.144	0	0.080	0.011	0.916	0	0	1
Cryptophytes	0	0.174	0	0	0	0	0.228	0	1
Green flagellates	0	0	0	0	0	0	0	0.945	1
(b) Output matrix: 0 - 50 m (2008 data)									
Diatoms	0	0.225	0	0	0.940	0	0	0	1
Dinoflagellates-1	0	0.274	0.926	0	0	0	0	0	1
Chemotaxonomic group	0.501	0.184	0	0.337	0.821	0.353	0	0	1
Phaeocystis antarctica	0.209	0.128	0	0.135	0.023	0.982	0	0	1
Cryptophytes	0	0.191	0	0	0	0	0.428	0	1
Green flagellates	0	0	0	0	0	0	0	0.932	1
(c) Output matrix: 0 - 50 m (2009 data)									
Diatoms	0	0.149	0	0	0.821	0	0	0	1
Dinoflagellates-1	0	0.381	0.898	0	0	0	0	0	1
Chemotaxonomic group	0.249	0.118	0	0.093	0.401	0.037	0	0	1
Phaeocystis antarctica	0.208	0.128	0	0.080	0.011	1.237	0	0	1
Cryptophytes	0	0.192	0	0	0	0	0.362	0	1
Green flagellates	0	0	0	0	0	0	0	0.879	1

For optimization of the input matrix, a series of 60 pigment ratio matrices were generated by multiplying each ratio from the initial matrix by a random function, as described by Kozlowski et al. [2011]. The average of the best six output matrices (with the lowest residual or root mean square error) were taken as the optimized results. The optimized pigment ratio matrix derived by CHEMTAX for the 0-50 m bin is presented in Tables 2b and 2c (data from 2008 and 2009, respectively). The output data are presented as absolute amounts (mg m^{-3}) of Chl *a* attributed to each phytoplankton group, and as a relative amount (percentage) of the total Chl *a* in a sample.

3.2.4. Microscopic analysis

Water samples were preserved in amber glass flasks (~250 mL) with 2% alkaline Lugol's iodine solution for phytoplankton identification and counting. Settling chambers (from 50 to 100 mL settling volume) were inspected on an Axiovert 135 ZEISS inverted microscope [Utermöhl, 1958; Sournia, 1978] in order to determine the species composition at 200×, 400× and 1000× magnification, according to specific literature [mainly, Hasle & Syvertsen, 1996; Scott & Marchant, 2005]. Staining cells with Lugol's solution allows recognition of chloroplasts and pyrenoids and provides a clear picture of the cell outline, which favors recognition of shape and size under the microscope [Sournia, 1978]. Distinction between autotrophic and heterotrophic dinoflagellates was made on either the known taxonomic trophic mode or the presence/absence of chloroplasts. Species-specific cell biovolumes were estimated by measuring cell dimensions (from microscope images - Spot Insight QE camera) and by

applying volume calculations based on the most similar geometric shapes as in Hillebrand et al. [1999]. At least 30 specimens of each species or major taxa were randomly chosen for measurements. Cell carbon content (carbon biomass) was then calculated using different carbon-to-volume ratios for diatoms and dinoflagellates according to Montagnes et al. [1994] and for all other algae groups according to Menden-Deuer & Lessard [2000].

3.3. Results

3.3.1. Spatial distribution of phytoplankton pigments

Figure 2 shows Chl *a* distribution along with Hex-fuco:chl c_3 ratio (higher Hex-fuco:chl c_3 ratio associates with iron limitation). Three spatial features are observed in Chl *a* distribution around tip of the AP: (i) a high Chl *a* region (exceeding 7 mg m^{-3} in 2009) in the vicinities of James Ross Island; (ii) a region with intermediate Chl *a* levels (0.5 to 2 mg m^{-3}) in the Bransfield Strait, and (iii) two areas with very low Chl *a* concentrations (below 0.5 mg m^{-3}), comprising the Weddell Sea section and stations located mainly offshore in the Drake Passage (only sampled in 2008). Both highest values of Hex-fuco:Chl c_3 ratios (>3) and lowest phytoplankton biomass were observed in the Weddell Sea (in both years) and offshore in the Drake Passage (Fig. 2).

Besides Chl *a*, the most abundant pigments (with maximum concentrations > 0.5 mg m^{-3}) were Fuco, Chl c_2, diadinoxanthin (Diadino) and some degradation products of Chl *a* (see Table 1). The highest concentrations of those pigments were observed near James Ross Island. Bransfield Strait

(particularly in 2008) and Drake Passage also presented relatively high values (> 0.05 mg m^{-3}) of Chl c_3, Hex-fuco and But-fuco. In the Weddell Sea region, where the lowest pigment concentrations were observed, Fuco was the main accessory pigment at coastal stations, while Allo and Hex-fuco appeared as the major carotenoids at some offshore stations.

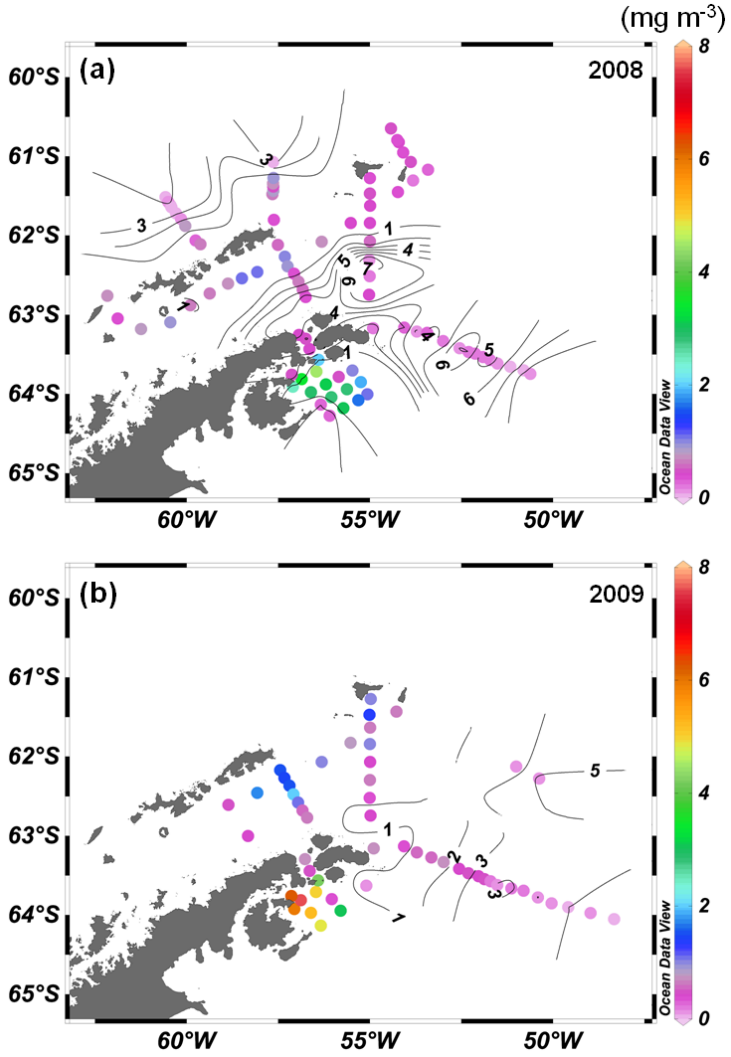

Figure 2: Surface distribution of total chlorophyll *a* (mg m^{-3}) (color scale) and Hex-fuco:Chl c_3 ratio (isolines) for SOS-CLIMATE 2008 (a) and 2009 (b).

Relationships between particular accessory pigments can be used to reveal the dominance of specific taxonomic groups. As observed in Figure 3,

the highest values of Chl c_3:Fuco (slope = 0.38) were registered in 2008 for the Bransfield Strait and Drake Passage, intermediate values (slope = 0.16) were recorded in 2009 for the Bransfield Strait and the lowest values (slope = 0.037), for both years, were observed next to James Ross Island. The different slopes of this ratio were associated with relative diatom contribution to phytoplankton community, as observed in Ross stations where higher diatom contributions were associated with a lower slope (further information on next section).

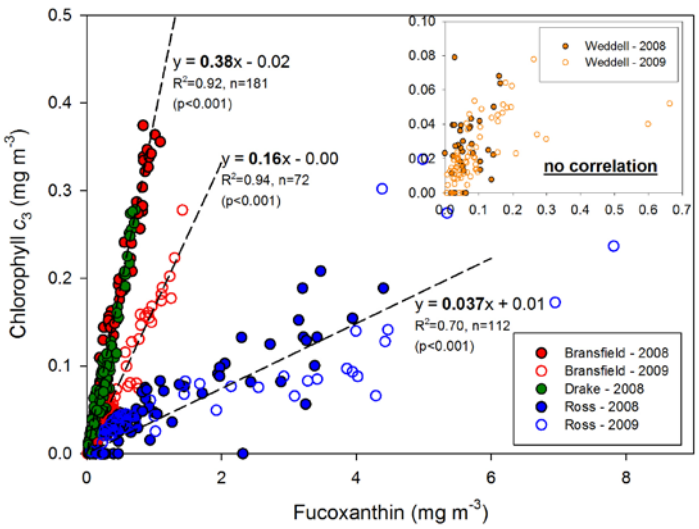

Figure 3: Relationship between chlorophyll c_3 and fucoxanthin for the different regions and sampling periods.

3.3.2. Distribution of taxonomic groups in relation to oceanography

Spatial distribution

The relative contribution of the main phytoplankton groups to surface Chl *a,* calculated by CHEMTAX, is shown in Figure 4.

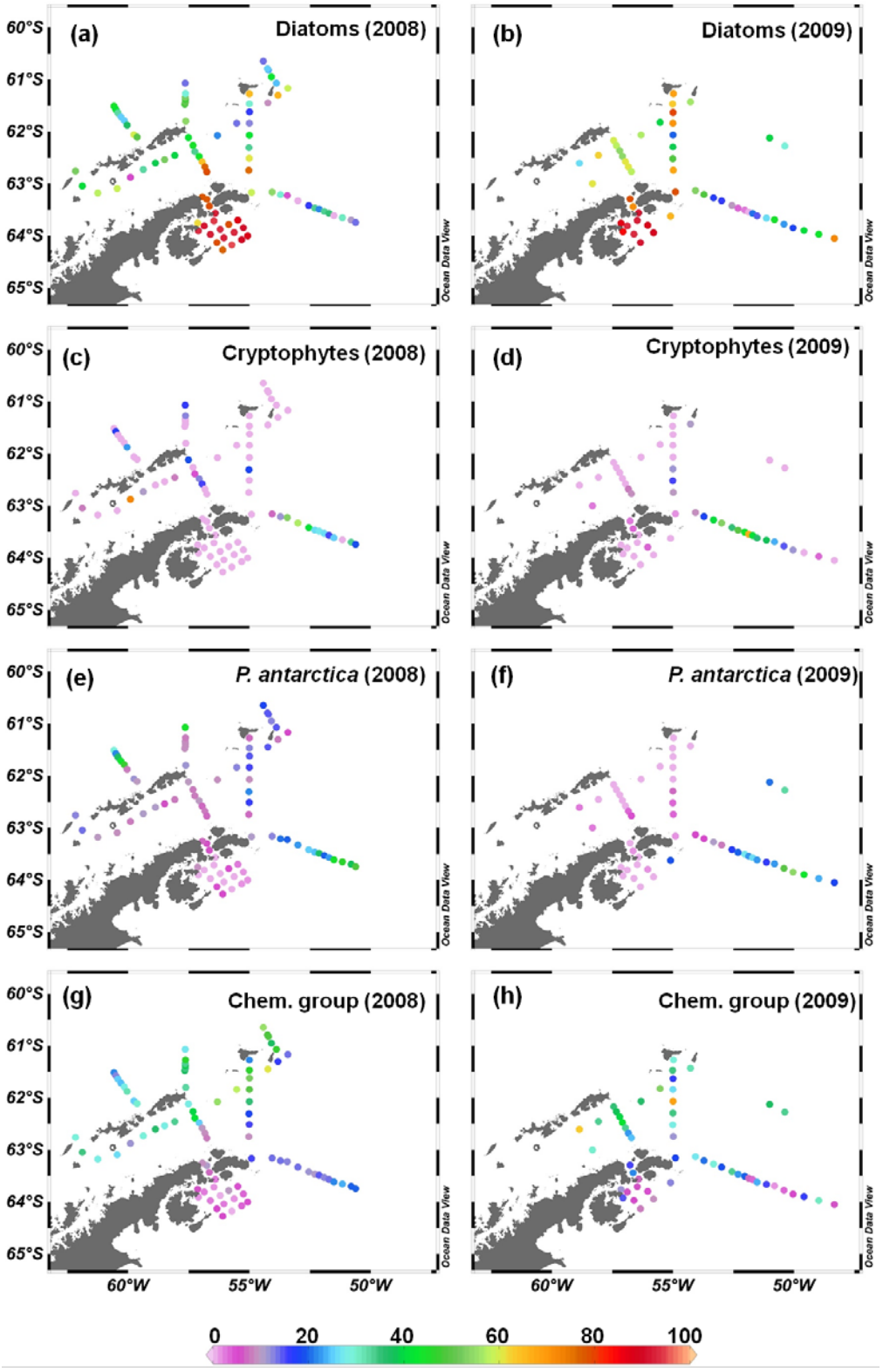

Figure 4: Surface distribution of the relative contribution (%) of main phytoplankton groups to total Chlorophyll *a* estimated by CHEMTAX program using HPLC pigment data: diatoms in 2008 (a) and 2009 (b); cryptophytes in 2008 (c) and 2009 (d); *Phaeocystis antarctica* in 2008 (e) and 2009 (f); "Chemotaxonomic group" in 2008 (g) and 2009 (h).

Phytoplankton assemblages were generally dominated by diatoms in both years (Fig. 4a and b), especially at stations with high Chl *a* concentration (mainly near James Ross Island), where diatom contribution was above 90% of total Chl *a*. Nevertheless, other important groups were also abundant at distinct areas around the tip of the AP. Cryptophytes dominated the Weddell Sea region, particularly stations with low diatom contribution, and at one station in the Bransfield Strait, in 2008 (Fig. 4c and d). The haptophyte *P*. *antarctica* showed the greatest contributions to total biomass in the Drake Passage region (only sampled in 2008) and at some Weddell Sea stations (Fig. 4e and f). The "chemotaxonomic group" was more dominant in the Bransfield Strait comparing to other regions (Fig. 4g and h). Dinoflagellates-1, more abundant in the Bransfield Strait, were always below 10% of total Chl *a*, and green flagellates never represented more than 8% of biomass (data not shown).

Microscopy vs. CHEMTAX

Direct comparisons of the estimated biomass using microscopy data and CHEMTAX showed a significant relationship for total phytoplankton (Fig. 5a) and diatom biomass (Fig. 5b). The significant correlation between microscope-derived carbon biomass and diatom-allocated Chl *a* calculated through CHEMTAX (Fig. 5b) mirrored the correlation for the total autotrophic community (Fig. 5a), denoting a clear dominance of diatoms. There was a conspicuous dominance of diatom carbon biomass in both years, with higher values (> 100 µg C l^{-1}, in average) found near James Ross Island and in agreement with CHEMTAX results (see Fig. 4a and b). Regarding the haptophyte *P.*

antarctica, differences varied with the study period. In 2009 this organism was rarely recorded in microscope observations, partly due to the lack of microscope data from Weddell Sea, where the contribution of *P. antarctica* to Chl *a* was 20-40% (determined by CHEMTAX). In 2008, with the additional data of Drake Passage, a significant correlation was observed between the two methods (Fig. 5c). Other groups (not shown in figure 5), such as cryptophytes, were barely separated from other small flagellates by microscopic analysis, except at one station in the Bransfield Strait (in 2008), where CHEMTAX data also showed a higher contribution of cryptophytes to biomass. The "chemotaxonomic group" was correlated with small flagellate biomass in the Drake Passage (R^2=0.52; p<0.05), while in the Bransfield Strait the "chemotaxonomic group" was significantly related to dinoflagellates in both years (R^2=0.62; p<0.05). This correlation found in the Bransfield Strait might indicate the presence of other types of dinoflagellates that contain other combinations of pigments instead of peridinin.

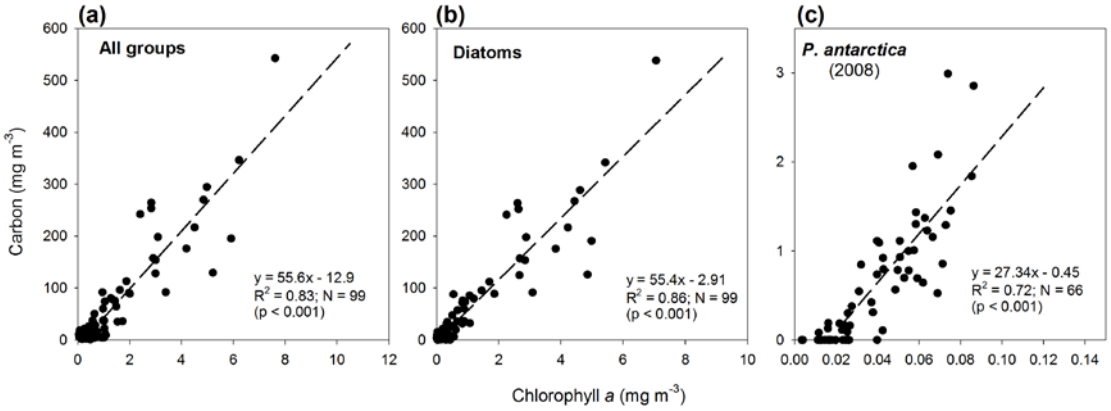

Figure 5: Relationship between chlorophyll *a* biomass estimated from CHEMTAX/HPLC pigment data and carbon biomass obtained through microscopic analysis. (a) All groups (2008 and 2009), (b) diatoms (2008 and 2009) and (c) *Phaeocystis antarctica* (only 2008).

Drake Passage (DRAKE)

Figure 6 shows the vertical profiles of Chl *a* biomass of taxonomic groups determined by CHEMTAX at a typical coastal and offshore station in the Drake Passage region.

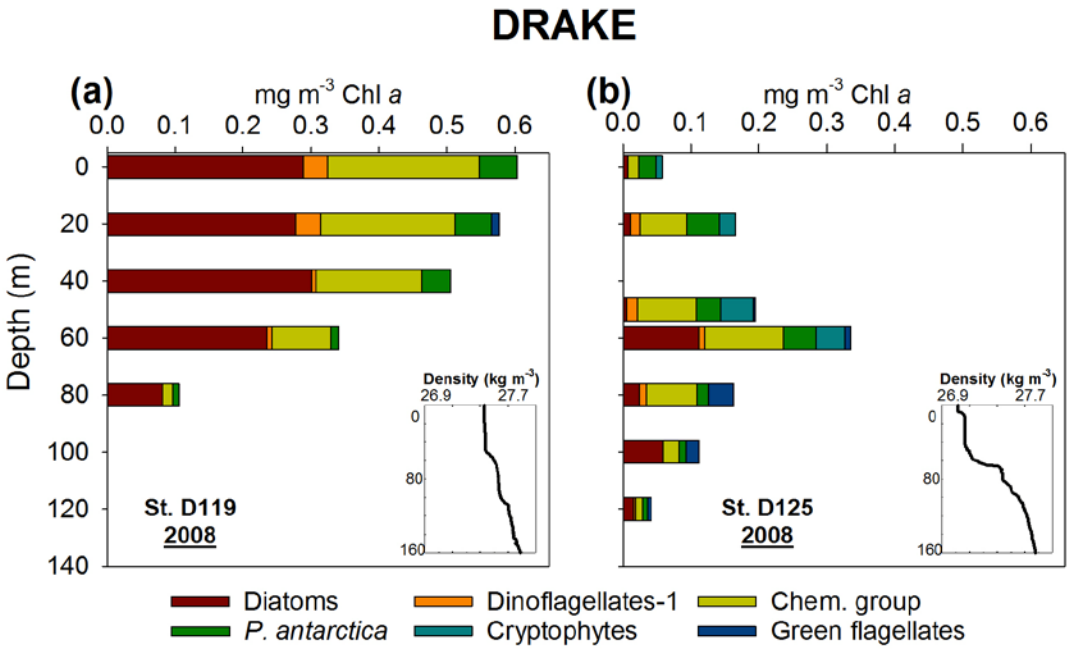

Figure 6: Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX at: (a) a coastal station (D119) and (b) offshore station (D125) in the Drake Passage region. Insets: density profiles of the respective stations (see Fig. 1 for stations' locations).

An increase in water column stratification was generally observed from coastal to offshore stations in this region, as observed in Figure 6. A coastal-offshore gradient was also observed for biomass and relative distribution of taxonomic groups, with higher Chl *a* concentration at the coastal stations and a decrease towards offshore. At the coastal station (Fig. 6a), a dominance of diatoms was observed but no deep chlorophyll maximum (DCM), which was present at the offshore station (Fig. 6b). Relatively low diatom contributions were found at the surface layers of offshore stations (below 60 m diatoms

became dominant). In those surface water masses diatoms were replaced by nanoplankton (<20 µm in greatest axial linear dimension), such as *P. antarctica*, cryptophytes and green flagellates (Fig. 6b). Although many flagellates could not be identified by microscope observations, the most representative phytoplankton species in DRAKE were the large centric diatom *Corethron pennatum*, the haptophyte *P. antarctica* and nanoflagellates, comprising dinoflagellates (e.g., *Gymnodinium* spp.), among other taxonomic groups.

Bransfield Strait (BRANSFIELD)

The Bransfield Strait region showed the highest spatial variability for both biomass and distribution of taxonomic groups, and also a significant variation between the two surveyed years (Fig. 7). Higher biomass was observed in 2009 (see also Fig. 2) compared with 2008, coupled with an increase in the relative contribution of diatoms (mainly the centric *Thalassiosira* spp., *Corethron pennatum*, the nano-sized *Chaetoceros neglectus* and the pennate *Pseudonitzschia* spp.). Generally, the highest biomass levels within the UML were registered at the deep stations in the central basin and were characterized by a major contribution of the "chemotaxonomic group" (associated with high densities of *Gymnodinium* spp.) and diatoms (Fig. 7b and e). Biomass levels decreased towards the coastal stations, where diatoms and/or cryptophytes were the major contributors to the phytoplankton community (Fig. 7a and d). In 2008, a negative relationship was found between surface Chl *a* and the UML depth ($R^2 = 0.50$, $p < 0.01$), with the deepest UML reaching 155 m near the Elephant Island (station B104). Lower biomass and an increase of small

flagellates over diatoms were seen at stations with deeper UML (see Fig. 7c). In 2009, this physical feature was not observed (UML was always less than 100 m) and biomass levels were similar to those observed at other BRANSFIELD stations, which were characterized by diatoms dominance (see Fig. 7f).

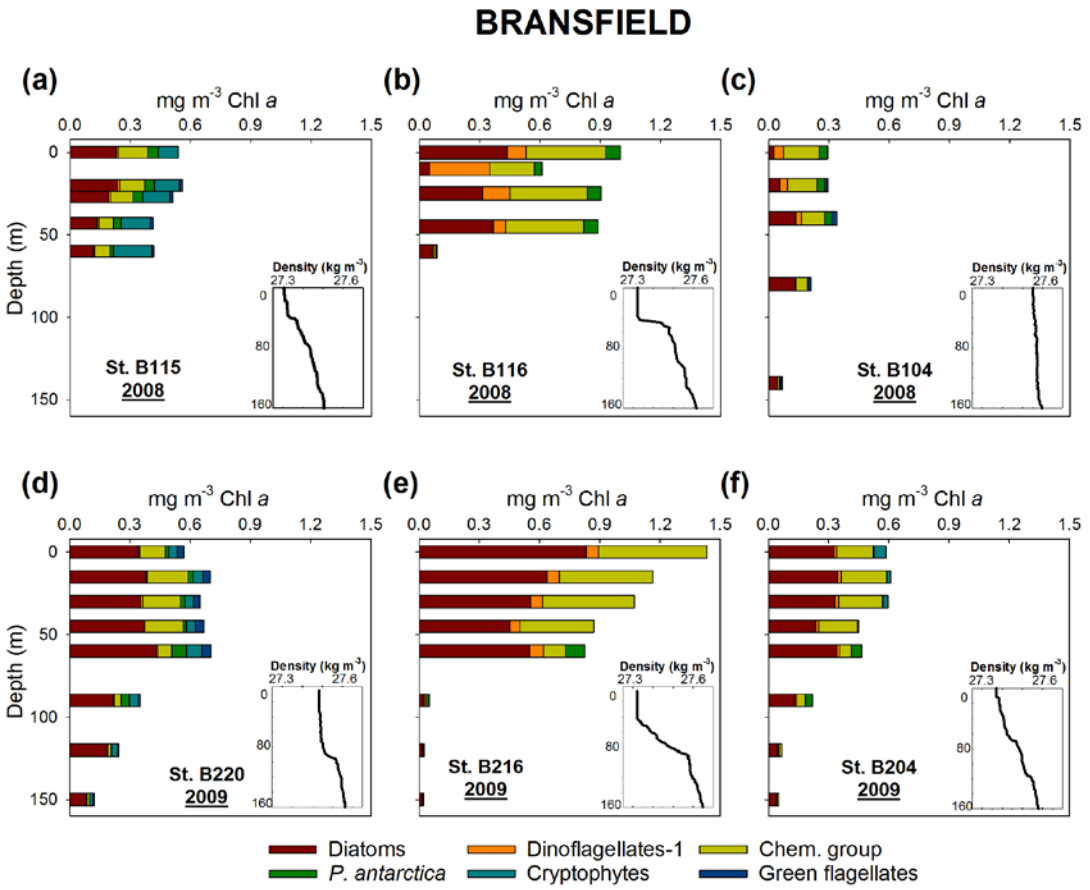

Figure 7: Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX at stations (a) B115, (b) B116 and (c) B104 (occupied in 2008); and at stations (d) B220, (e) B216 and (f) B204 (occupied in 2009) in the Bransfield Strait. Insets: density profiles of the respective stations (see Fig. 1 for stations' locations).

Generally, water column stratification and biomass levels of the main taxonomic groups were related, particularly in 2008 (Fig. 8). At stations with deep UML (stations B119, B120 and B121), mainly along the coast, diatoms

reached up to 0.4 mg m^{-3} of total Chl *a* (inset in Fig. 8), which represented over 75% of total biomass. On the other hand, other taxonomic groups (mainly dinoflagellates) reached a similar or higher relative contribution at shallow UML stations (e.g., stations B117, B125 and B126). At intermediate stratification conditions (station B124) cryptophytes became the dominant group (more than 80% of total Chl *a*). A slight stratification and low biomass, with a co-dominance of diatoms and cryptophytes was observed at the nearshore station B115 (Figs. 7a and 8).

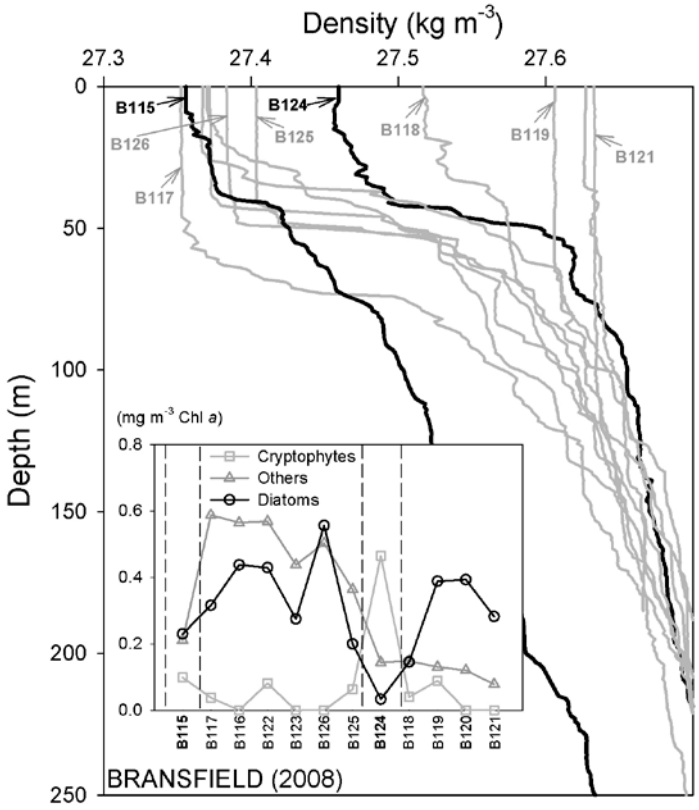

Figure 8: Vertical profiles of density at Bransfield Strait stations with surface chlorophyll *a* values above 0.5 mg m^{-3}, during the 2008 cruise. Inset: absolute contribution (mg m^{-3} of chlorophyll *a*) of major taxonomic groups at same stations shown on the main graph. Labels of some stations are displayed in order to associate with the graph inset. Density profiles of stations B115 and B124 are highlighted in black lines (see Fig. 1 for stations' locations).

Weddell Sea (WEDDELL)

In the Weddell Sea section the phytoplankton community was mainly composed by diatoms, cryptophytes and *P. antarctica* (see Fig. 4), and in both years was associated with low biomass values (Chl *a* always below 0.5 mg m^{-3}; see Fig. 2). In that section it was observed a particularly strong coastal-offshore gradient in water column stratification (Fig. 9).

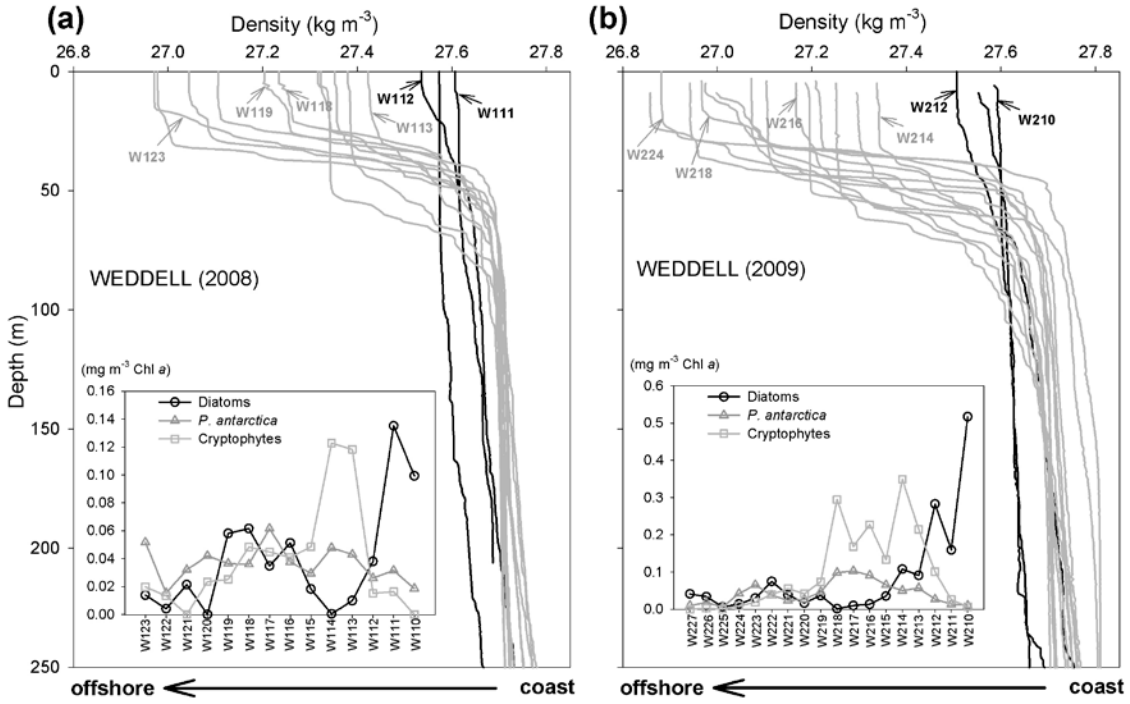

Figure 9: Vertical profiles of water column density for the Weddell Sea transect during (a) 2008 and (b) 2009. Insets: absolute contribution (mg m^{-3} of chlorophyll *a*) of major taxonomic groups along the longitude W (coastal-offshore gradient displayed by arrows) for the same stations on the main graphs (see Fig. 1 for stations' location). Labels of some stations are displayed in order to assist the geographical localization. Density profiles of more coastal stations are highlighted in black lines.

The phytoplankton community composition displayed a neat succession along this gradient (insets in Fig. 9). Diatoms were dominant in the well-mixed water column at coastal stations, associated with highest biomass

(> 0.2 mg m^{-3}), and they were gradually replaced by cryptophytes at stations with intermediate stratification. In both studied years, offshore stations were strongly stratified, which was associated with very low biomass (< 0.1 mg m^{-3}).

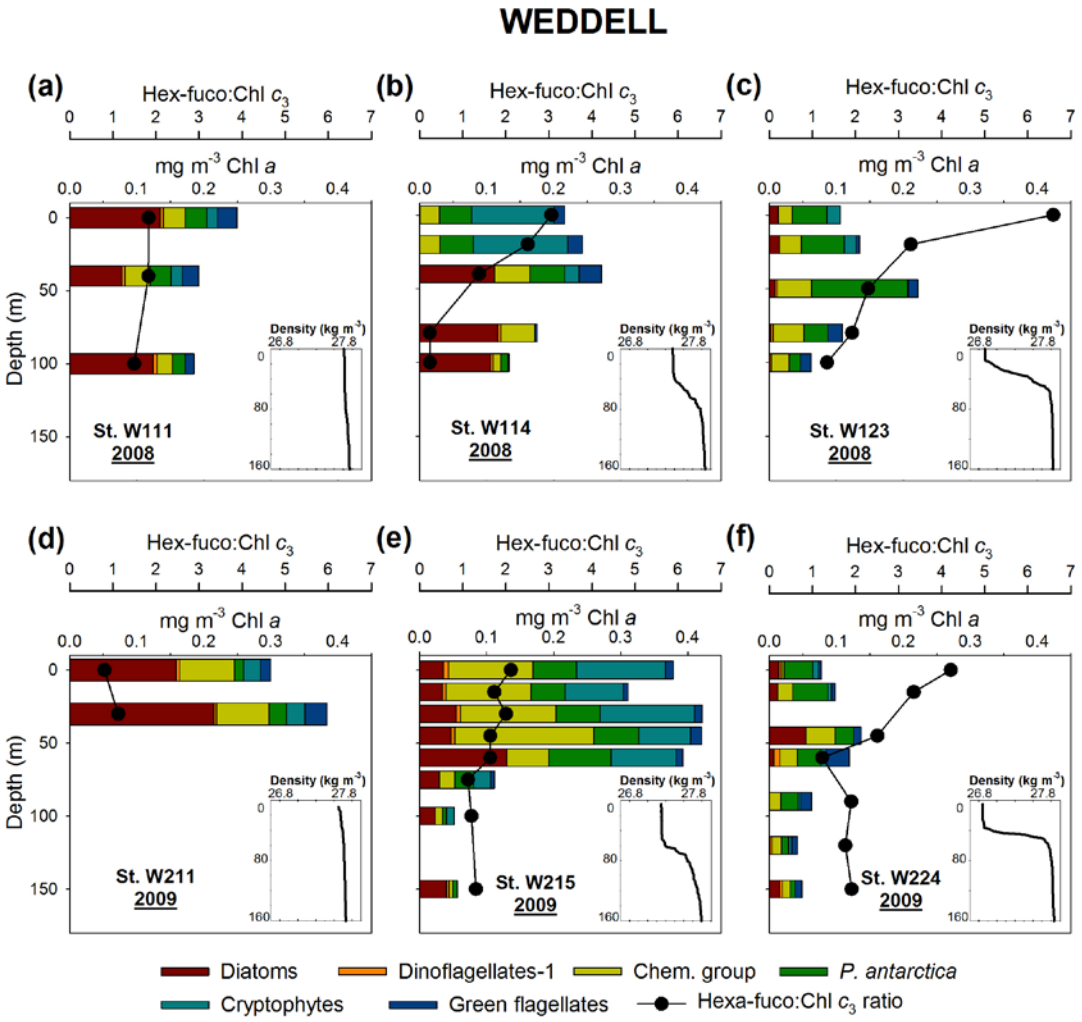

Figure 10: Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX (bars), and Hex-fuco:Chl c_3 ratio (marked line) at the selected stations from the Weddell Sea. The order of appearance of stations corresponds to the onshore-offshore gradient in 2008 (a-c) and 2009 (d-f) along with the Hex-fuco:Chl c_3 ratio profiles. Insets: density profiles of the respective stations (see Fig 1. for stations' locations).

Figure 10 shows vertical profiles of the community composition at six stations (Fig. 10a-f) and their density profiles (insets in Fig. 10). For station W211 (Fig. 10d), samples from only two depths (including surface) are available. In addition, the vertical distribution of the Hex-fuco:Chl c_3 ratio (Fe-

index) is also shown. The surface Hex-fuco:Chl c_3 ratio values increased, from approximately 2 at coastal station (Fig. 10a and d) to nearly 7 at the most offshore 2008 station (Fig. 10c), dominated by *P. antarctica*. At stations with intermediate ratio values at surface (station W114, Fig. 10b), a major contribution of cryptophytes was observed within the upper 20 m. Moreover, depth profiles showed that at stratified stations Hex-fuco:Chl c_3 ratios were higher at surface and decreased with depth (Fig. 10b,c,f). Although both statins were located in a similar position and showed a similar density profile, the intermediate stratified station W215 (Fig. 10e) showed a different biomass profile than the station W114 (Fig. 10b). Despite the similar biological pattern between sampling years, there was an evident interannual difference in the Weddell Sea region, as higher biomass was observed in 2009 (Fig. 10d-f) and was coupled with a lower Hex-fuco:Chl c_3 ratio.

Vicinity of James Ross Island (ROSS)

High Chl *a* concentration was generally recorded around the James Ross Island. On the other hand, at the Antarctic Sound stations (e.g., stations R118 and R210; see Fig. 1 for their location) surface Chl *a* was always below 0.5 mg m^{-3} (see Fig. 2). Figure 11 shows the vertical profiles of the phytoplankton community at six stations (Fig. 11a-f) that represent high (Fig. 11a and d), intermediate (Fig. 11b and e) and low (Fig. 11c and f) Chl *a* values during both years. Despite this relatively large biomass range, there was an absolute dominance of diatoms at all stations (>90 % contribution to total biomass). On a decreasing level of importance, the main diatom species were

Odontella weissflogii (> 70 µm in length), an assembly of moderately large centric diatoms (from 20 to 100 µm in diameter) and *Eucampia antarctica.* Areas with relatively high biomass were associated with a shallow UML, mainly comprising the stations nearest to land (e.g., Fig. 11a and d; stations R113 and R208, respectively). By contrast, relatively low biomass (Fig. 11c and f; stations R118 and R210, respectively) was observed at deep UML (insets in Fig. 11c and f) Antarctic Sound stations. Maximum and average Chl *a* levels in 2009 were twofold greater than those observed in 2008 (see Table 1).

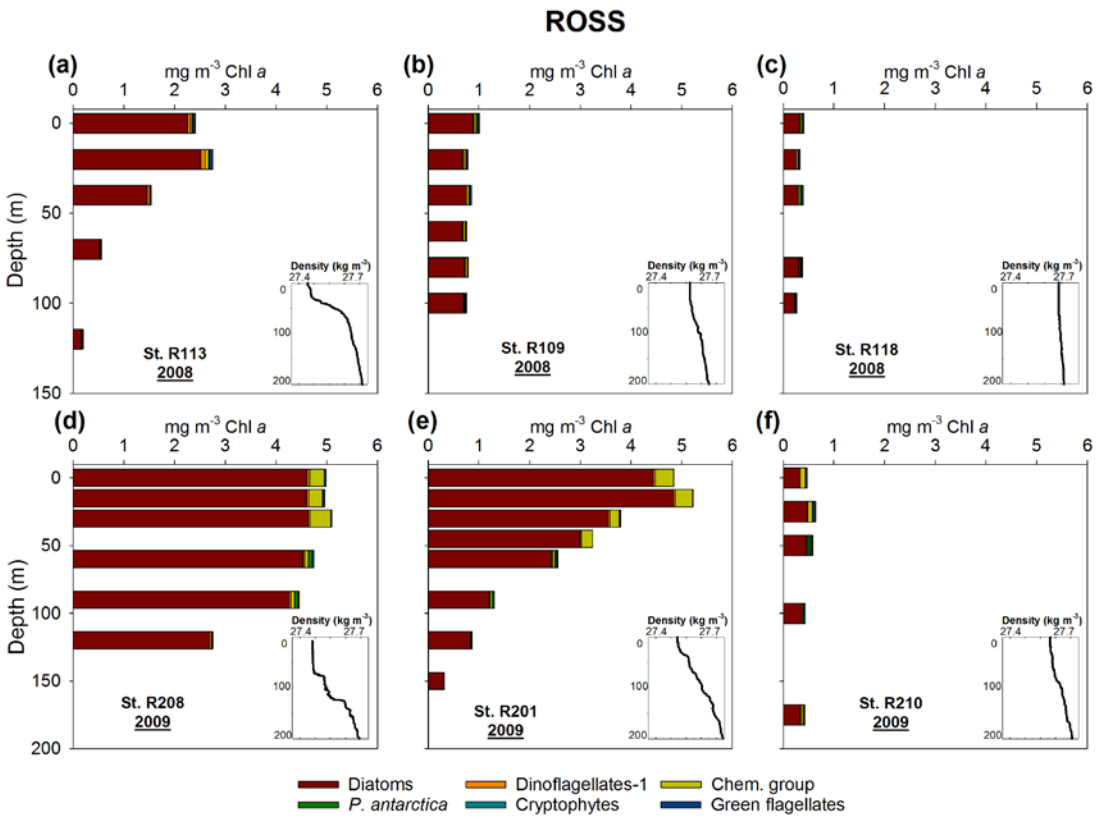

Figure 11: Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX, at the selected stations in the vicinities of James Ross Island (a-f). (a) Coastal station, 2008; (b) Non-coastal station, 2008; (c) Antarctic Sound station, 2008; (d) Coastal station, 2009; (e) Non-coastal station, 2009 and (f) Antarctic Sound station, 2009. Insets: density profiles of the respective stations (see Fig. 1 for stations' locations).

3.3.3. Other pigment indices

Pigment degradation products

The HPLC analysis allowed the separation, identification and quantification of three types of Chl *a* degradation products: chlorophyllide *a* (Chlide *a*), pheophytin *a* (Phytin *a*) and pheophorbide *a* (Phide *a*). Apart from the area around ROSS, where a typical diatom-bloom situation was observed, the concentration of the degradation products were always below 0.1 mg m^{-3}. The main degradation product of Chl *a* for the whole survey region was pheophorbide *a* (see Table 1). The concentrations of degradation products, particularly chlorophyllide *a*, were higher in the ROSS area than in other sampling areas. Figure 12 shows the linear relationships observed between degradation products and total Chl *a* in this region, and indicates a significant difference between both study years. In 2008 all degradation products were present at significantly higher concentrations than in 2009, with an average proportion (degradation product/total degradation products plus Chl *a*) of 11% for chlorophyllide *a*, 9% for pheophorbide *a* and 3% for pheophytin *a.* In 2009 the averages were 7%, 6% and 2%, respectively.

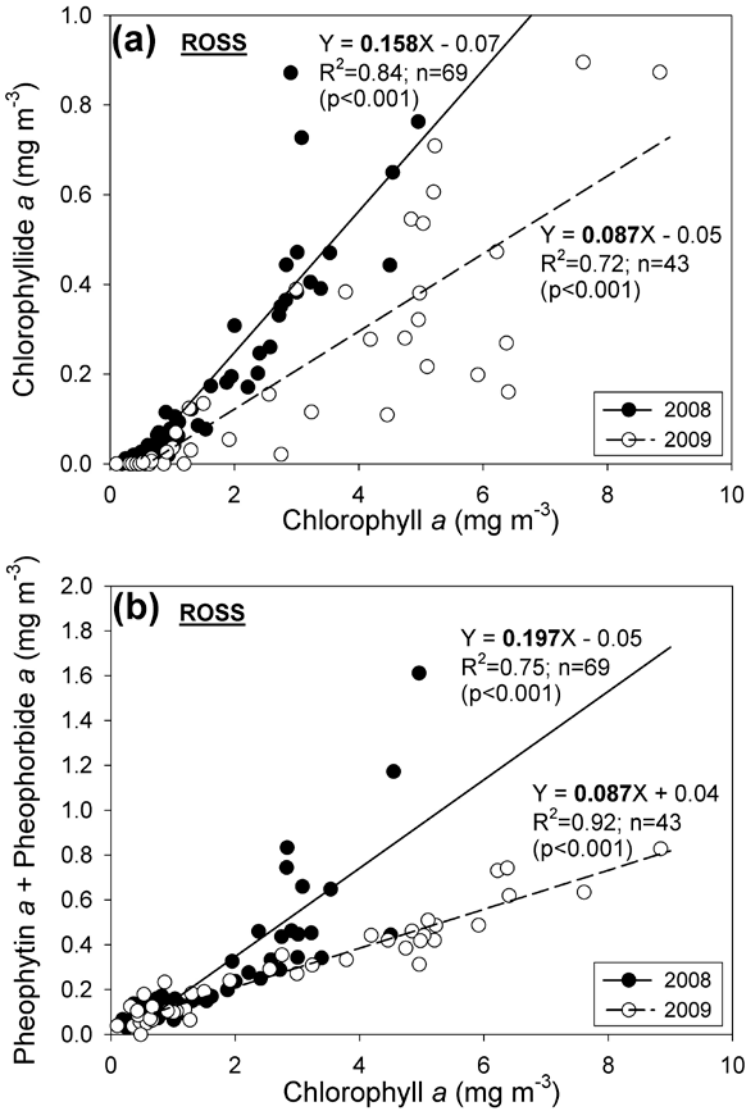

Figure 12: Relationship between chlorophyll *a* concentration and (a) chlorophyllide *a* concentration and (b) pheophytin *a* plus pheophorbide *a* concentration, for stations near James Ross Island.

Photosynthetic and photoprotective pigments

The array of phytoplankton pigments found in this study include photosynthetic and photoprotective carotenoids. The ratio of the sum of photoprotective carotenoids (PPC; alloxanthin, diadinoxanthin and diatoxanthin in our study) to the sum of total pigments (TP) was shown to indicate the

physiological adaptation of the phytoplankton community to the prevailing ambient light. An evident difference in those indices was found between samples taken during day and at nighttime in the diatom-dominated ROSS region. For instance, Figure 13 shows vertical profiles of PPC:TP for ROSS and DRAKE regions.

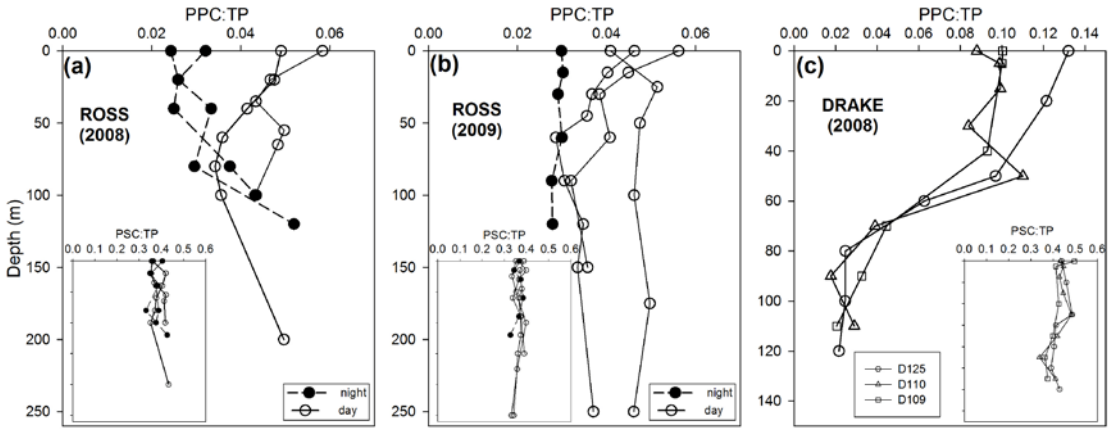

Figure 13: Vertical profiles of photoprotective carotenoids (PPC) to total pigments (TP) ratios for available night (R103 and R109) and day (R113 and R118) stations at Ross region in 2008 (a); night (R208) and day (R201, R210 and R212) stations at Ross region in 2009 (b) and stratified daytime stations in Drake Passage (c). Insets: Profiles of the PSC:TP ratios for the same stations. Note the different scales between main graphs and insets.

The PPC:TP ratios at the nighttime stations in ROSS were about twofold smaller than those at daytime, especially on the surface layer (Fig. 13a and b), which indication that relative PPC concentrations may change over the course of a day. In the DRAKE region, under stratified water column conditions and during daytime, the PPC:TP ratios within the upper mixed layer were five-times higher than those at or below the pycnocline (Fig. 13c). However, when examining the ratio of pooled photosynthetic carotenoids (PSC; 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, fucoxanthin and peridinin)

to TP (insets in Fig. 13), no noteworthy differences were found at stratified stations between day and nighttime stations or with depth variation.

3.4. Discussion

3.4.1. The application of CHEMTAX in the study of phytoplankton communities

Several studies in the western AP region have already suggested that both microscopy and HPLC techniques should be used together to complement one another [e.g., Rodríguez et al., 2002; Kozlowski et al., 2011]. A common feature over the study region was areas of low biomass characterized by nanoplanktonic cells. Since those small-sized organisms were generally not recognizable by light microscopy and were often difficult to preserve, the HPLC-CHEMTAX approach provides valuable information about the whole phytoplankton community, particularly for those small-size groups. The good relationship observed between HPLC-CHEMTAX and microscope derived biomass of representative taxonomic groups (diatoms and *P. antarctica*) (Fig. 5) also supports the reliability of the HPLC. Additionally, a high number of pigment samples analyzed during these oceanographic surveys, would be impractical to study by microscopic analysis. On the other hand, microscope observations complement the taxonomical information (to species or genus), which provides a better taxonomic resolution, particularly for large and recognizable organisms. The CHEMTAX software [Mackey et al., 1996] has been successfully used in many other worldwide investigations [e.g., Mackey et al., 1998; Schlüter et al.,

2000; Carreto et al., 2008; Wright et al., 2009; 2010]. However, regarding matrix optimization procedures, it would be advisable to apply different approaches when using the CHEMTAX tool, as either described by Latasa [2007] and Wright et al. [2009], and/or by using a combination of approaches in order to improve the results [Mendes et al., 2011; Schlüter et al., 2011; de Souza et al., 2011]. In the present study, the Wright's method was used to obtain the output data from CHEMTAX, as it is appropriate for regions with low concentrations of pigments [Wright et al., 2009], which was observed in the Weddell Sea and Drake Passage.

The output pigment ratios observed (see Table 2b and c) were generally equivalent to values available in the literature [e.g., Rodríguez et al., 2002; Kozlowski et al., 2011] for the AP region. The average Fuco:Chl *a* (diatom) output ratio was lower in Rodríguez et al. [2002] (0.425 in 1995/1996) as compared to our study (0.940 for 2008 and 0.822 for 2009), but it was still above the maximum value (0.714 ± 0.160 from 1995 to 2007) observed by Kozlowski et al. [2011]. The cryptophyte Allo:Chl *a* ratio (0.428 for 2008 and 0.362 for 2009) was also higher as compared with observations made by Rodríguez et al. (2002) (0.228 in 1995/1996), but again within the range (0.443 ± 0.125 from 1995 to 2007) observed by Kozlowski et al. [2011]. On the other hand, we have observed negligible variations between our study and results presented by both Rodríguez et al. [2002] and Kozlowski et al. [2011] regarding the Hex-fuco:Chl *a* and But-fuco:Chl *a* ratios of *P. antarctica*. Those slight differences found in pigment ratios between this study and literature data may be related to a light regime variation, nutrients availability and changes in algal populations [Schlütter et al., 2000]. Additionally, the different approaches

used by different authors to work with CHEMTAX can likely affect these final ratios. The output ratios for "chemotaxonomic group" varied between the two sampling years and also with the literature mentioned above. These differences are associated with the structure of "chemotaxonomic group", which is composed of different taxa. For instance, the high Chl c_3:Chl *a* ratio (0.501) observed in 2008 may be related to the presence of *Gymnodinium* spp. (detected by microscopy), since higher concentrations of Chl c_3 were registered only at stations with high dinoflagellates abundance. Moreover, the high abundance of c_3 -containing *Pseudonitzschia* spp. may have contributed to this ratio, particularly in the Bransfield Strait.

3.4.2. Phytoplankton communities in relation to oceanographic parameters

A great spatial variability (horizontal and vertical) in the phytoplankton community, both in biomass and composition, was found in the AP. This variation was mainly associated with the water column structure that can determine light availability and/or iron limitation within the UML. Stratification was associated with several physical processes in the whole study area, such as coastal ice melting (characteristic of ROSS region) and seasonal warming of surface layers (evident in WEDDELL and DRAKE regions). For instance, a remnant cold and salty Winter water is usually found below warmer and fresher Antarctic Surface Water commonly formed during summer [Gordon & Huber, 1984], particularly in offshore areas.

Our sampling period was the late summer, when the phytoplankton communities are result of the succession associated with timing and extent of ice melting during the summer [Garibotti et al., 2005 and references therein]. Although in the present study a temporal variation was not evaluated, the great spatial heterogeneity allowed the understanding of some processes related to the distribution of phytoplankton communities around the tip of the AP. For instance, the low phytoplankton biomass seen in WEDDELL may be associated with a post bloom stage, as frequent blooms are often observed in this region during summer [Sullivan et al., 1993; Park et al., 1999; Kang et al., 2001], while in ROSS, a clear diatom bloom situation was observed.

In ROSS, a reasonably well-stratified water column was observed due to ice melt and runoff from glaciers at James Ross Island, which are a likely source of iron that may have triggered the diatom-dominated (e.g., *Odontella weissflogii*) phytoplankton bloom. Moreover, a biological-physical gradient was observed, where stratified areas near land zones were associated with higher diatom biomass (see Fig. 11). This scenario was already described, highlighting the importance of a shallow UML depth [Mitchell & Holm-Hansen, 1991; Garibotti et al., 2005] and associated stratification as a result of ice melting on phytoplankton development. This feature has been observed predominantly in coastal areas, as these regions are apparently protected from strong winds [Ducklow et al., 2007]. Even though the lowest biomass levels in ROSS were measured in the Antarctic Sound area (associated with a deep UML), the phytoplankton composition was similar to other stations of the same region. This could be associated with advection processes in the Sound, which

prevented the accumulation of phytoplankton biomass [e.g., Moline & Prézelin, 1996].

In WEDDELL and DRAKE regions, the phytoplankton community was characterized by low biomass and dominated by flagellates, including *P. antarctica* and cryptophytes at the stratified offshore stations. In these situations stratification was probably a major physical feature affecting phytoplankton assemblages by a supposed limitation of Fe input into the upper surface layer, leading to development of a deep chlorophyll maximum [Ducklow et al., 2007]. Other factors such as senescence and/or grazing may have also contributed to the low biomass observed in those regions. Regarding the important contribution of *P. antarctica* at shallow UML stations, this pattern was not observed in other Antarctic regions such as the Ross Sea [Arrigo et al., 1999, 2000], where this organism was associated with deep UML and its dominance was linked to photophysiological abilities [Kropuenske et al., 2010; Mills et al., 2010]. In this study, this haptophyte was found in very low biomass and shallow UML layers and therefore was able to thrive under apparently low iron conditions. Those oligotrophic conditions (mainly in WEDDELL) may reflect the timing of our sampling period (late summer).

The BRANSFIELD region is hydrographically complex, comprising water masses that progressively change from Bellingshausen Sea to Weddell Sea influence [Sangrà et al., 2011 and references therein]. This could explain the great temporal (interannual) and spatial variability in phytoplankton biomass and composition. Briefly, higher biomass levels were recorded in 2009 mainly associated with diatoms and a shallower UML. At the northernmost part of this region (near Elephant Island), particularly in 2008, a low-biomass community

composed by small flagellates was observed, coupled with a deeper UML (presumably leading to light limitation) than in 2009 (see Fig. 7c and f). At coastal sites, diatoms and/or cryptophytes were the major groups contributing to phytoplankton biomass. On the other hand, the “chemotaxonomic group” was very important at the central channel (in the southernmost portion of the Strait). That group was represented mainly by *Gymnodinium* spp., which is known to contain carotenoids other than peridinin (Carreto et al., 2001). In this study, the high abundance of *Gymnodinium* spp. and other dinoflagellates was correlated with well-stratified water masses at the central channel in BRANSFIELD, as observed demonstrated in classical ecological theories [Margalef, 1958; Smayda & Reynolds, 2001]. Another interesting feature was the conspicuous dominance of cryptophytes at station B124, characterized by an intermediate stratification condition (see Fig. 8). One possible explanation for this particular area is the occurrence of a topographically induced upwelling of Weddell Sea water, observed by the temperature-salinity profile at that station (data not shown). Indeed, cryptophytes were a relatively important group at the WEDDEL region. A few studies have reported episodic upwelling caused by topographic characteristics in other regions close to AP [Ducklow et al., 2007 and references therein] as well as intrusions of Weddell Sea water from the southwest into the Bransfield Strait [Sangrà et al., 2011].

3.4.3. Pigments as indicators of community physiological state

Pigment information can be used not only as a taxonomic tool to describe the phytoplankton community but also as a proxy for physiological responses to

distinct environmental factors, such as nutrient stress, light availability and grazing pressure. This study tested the Hex-fuco:Chl c_3 ratio as an index of Fe-nutritional state of the phytoplankton community, based on the experimental work developed and validated by DiTullio et al. [2007] for *P. antarctica* cultures and on field studies about phytoplankton community affected by iron enrichment [e.g., Hoffmann et al., 2006; Wong & Crawford, 2006]. Under Fe-stress conditions, *P. antarctica* (present at most sampling stations, mainly in DRAKE and WEDDELL) was found to be able to convert fucoxanthin into 19'-hexanoyloxyfucoxanthin [Van Leeuwe & Stefels, 1998, 2007] and therefore increasing the Hex-fuco:Chl c_3 ratio [DiTullio et al., 2007]. Moreover, experimental in-situ studies [e.g., Hoffmann et al., 2006; Wong & Crawford, 2006] reported important shifts in phytoplankton communities inside a region under iron fertilization and thus in the phytoplankton pigments along the period of iron experiments. According to those studies, diatoms dominated (increase in fucoxanthin concentrations) when iron was supplied, while a nanoflagellate community, dominated mainly by haptophytes, declined. These Hex-fuco-containing nanoflagellates, presumably not stimulated by iron supply, were controlled by grazing pressure [Hoffmann et al., 2006]. Moreover, upon iron enrichment, there was a slight increase in Chl c_3 [Hoffmann et al., 2006; Wong & Crawford, 2006]. Although those studies associated Chl c_3 with haptophytes, the increase of this pigment could be also coupled with higher abundance of chl c_3-containing diatoms, such as *Pseudonitzschia* spp. [Wright & Jeffrey, 2006] observed in naturally iron-enriched environments. Iron enrichment would thus cause a change in the phytoplankton community followed by a decrease in the Hex-fuco:Chl c_3 ratio. The Hex-fuco:Chl c_3 ratio can therefore reflect the

physiological response of *P. antarctica* (in regions dominated by this species) to iron availability. Additionally, it can also provide information about changes in phytoplankton communities associated with this important micronutrient, regardless the presence of *P. antarctica*. In view of all this information, in this work we assume that the Hex-fuco:Chl c_3 ratio can provide a suitable index of the Fe-nutritional state of the whole phytoplankton community. Our results have shown that the ratio was highest (> 3), indicating Fe-limitation, in the Weddell Sea region and offshore Drake Passage, particularly at the surface layer, where the lowest biomass levels were recorded in both sampling years. A strong association was found between the pattern of Hex-fuco:Chl c_3 ratio and the Fe spatial distribution pattern previously reported for this region of the AP. For instance, in the northernmost sector of the studied region, which encompasses Drake Passage and the western Weddell Sea (where high Hex-fuco:Chl c_3 ratios were observed), a limitation of primary production and biomass associated to low iron concentration has been previously reported [Holm-Hansen & Hewes, 2004]. Additionally, Sañudo-Wilhelmy et al. [2002] described a coastal-offshore gradient in trace metal concentration (including Fe) in the AP region, from coastal waters with high metal concentrations to offshore waters, with low metal levels. This pattern was also evident in this study, particularly along the WEDDEL transect, from low (coast) to high (offshore) surface Hex-fuco:Chl c_3 ratio. This onshore-offshore gradient in Hex-fuco:Chl c_3 ratio was accompanied by changes in the phytoplankton community: a dominance of diatoms along with low Hex-fuco:Chl c_3 ratio was observed in coastal regions, a dominance of cryptophytes with intermediate Hex-fuco:Chl c_3 ratios was found in middle sites, and very low biomass (dominated by smaller flagellates such as

P. antarctica) was detected at far offshore stations, associated with the highest Hex-fuco:Chl c_3 ratios (see Fig. 10). These composition shifts are possibly related to competition for nutrient resources, especially iron, including different uptake physiological abilities of the distinct phytoplankton groups found across the WEDDELL transect. In the present work it was also observed a variation in the Fe-index between the study years, where Hex-fuco:Chl c_3 ratios were generally higher in 2008 (indicating a stronger limitation) and associated with lower biomass levels, as compared to 2009 (see Fig. 2).

Among other environmental factors controlling the phytoplankton community, grazing pressure must also be considered [Ross et al., 1998; Anadón et al., 2002]. Despite the lack of zooplankton data in this work, the relative content of Chl *a* degradation products can be used as a proxy for grazing pressure and for senescence of phytoplankton cells [Jeffrey et al., 1997]. Generally, low concentrations of these degradation products were observed (see Table 1) in all regions, except in ROSS, where a diatom bloom was found. Higher proportions of all those products were observed in this region in 2008 than in 2009, which may suggest that the 2008 diatom-bloom was in an advanced senescence stage and under higher grazing pressure relatively to the scenario found in 2009 (see Fig. 12).

Regarding the proportions of specific (photosynthetic or photoprotective) carotenoids over the total amount of pigments, considerable differences were found across vertical profiles of the sampling stations within the diatom-bloom at ROSS and at well-stratified offshore DRAKE stations (see Fig. 13). Contrasting differences were detected between the response of PPC and PSC relative to the irradiance variation. While a noteworthy difference in PPC:TP ratios were

observed between day (higher values) and night (lower values) stations at ROSS, no detectable differences were found between day and night PSC:TP ratios. In addition, the PPC:TP proportion was significantly higher in the upper surface layer than in depth at the well-stratified DRAKE stations, although this pattern was again not evident for the PSC:TP ratios. These results may be explained by the carotenoids' key functions in photosynthesis: (i) PSC have a significant role in extending the phytoplankton light-harvesting spectrum, thus ensuring optimal absorption efficiencies and (ii) PPC acts as a protector of microalgal cells against high irradiances that may damage the photosynthetic apparatus [Kirk, 1994]. Furthermore, the ratios of PPC:TP and PSC:TP have been considered remarkably robust for assessing the physiological state of a phytoplankton community [Barlow et al., 2008 and references therein]. Therefore, information on PPC:TP ratios can indicate the phytoplankton light histories (e.g., day vs. night, as in our study) and the degree of water column stability [Moline, 1998]. Nonetheless, the PSC:TP ratios did not show an apparent response to short-term light changes, associated with neither daily-varying light field, depth profiles nor degree of water column stratification. Our results support the assumption that photosynthetic pigments and respective ratios are rather adequate as taxonomic biomarkers.

3.5. Concluding remarks

This study shows that the spatial distribution of phytoplankton communities around the AP, particularly in the northernmost regions, is very complex and subject to several environmental factors, which may determine

their composition and succession stage. Diatoms were the main contributors to Chl *a* biomass in areas presumably affected by ice melting processes, as observed at ROSS. Ice melting processes probably enhance iron input into seawater, triggering the growth of large diatoms (both isolated cells and colonies). In open-ocean areas such as DRAKE and WEDDELL, where iron-limited conditions were observed in stratified waters, nanoflagellates replaced diatoms as the dominant phytoplankton group. Among flagellates, *P. antarctica* was the dominant organism. Cryptophytes were persistently found at intermediate stratification conditions and associated with intermediate Hex-fuco:Chl c_3 values, i.e., between diatom-dominated and offshore low biomass stations. At both BRANSFIELD and DRAKE coastal stations, many species of dinoflagellates (dominant taxa of the “chemotaxonomic group” that contain carotenoids rather than peridinin) were also important to total Chl *a* concentration. Based on the spatial distribution of phytoplankton community composition and associated environmental factors, it seems that flagellates may, in fact, replace diatoms under certain conditions (intermediate to strong stratification leading to iron limitation). Finally, this study highlights the usefulness of HPLC pigment data as biotic indicators of physiological responses to environmental conditions, such as Fe-nutritional state, ambient light and/or grazing pressure.

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References

Anadón R, Alvarez-Marques F, Fernandez E, Varela M, Zapata M, Gasol JM, Vaque D (2002). Vertical biogenic particle flux during austral summer in the Antarctic Peninsula area. Deep-Sea Res II 49, 883-901.

Arrigo KR, DiTullio GR, Dunbar RB, Robinson DH, VanWoert M, Worthen DL, Lizotte MP (2000). Phytoplankton taxonomic variability in nutrient utilization and primary production in the Ross Sea. J Geophys Res 105, 8827-8846.

Arrigo KR, Robinson DH, Worthen DL, Dunbar RB, DiTullio GR, VanWoert M, Lizotte MP (1999). Phytoplankton community structure and the drawdown of nutrients and CO_2 in the Southern Ocean. Science 283, 365-367.

Barlow R, Kyewalyanga M, Sessions H, van den Berg M, Morris T (2008). Phytoplankton pigments, functional types, and absorption properties in the Delagoa and Natal Bights of the Agulhas ecosystem. Est Coast Shelf Sci 79, 230-238.

Carreto JI, Montoya N, Akselman R, Carignan MO, Silva RI, Colleoni DAC (2008). Algal pigment patterns and phytoplankton assemblages in different water masses of the Río de la Plata maritime front. Cont Shelf Res 28, 1589-1606.

Carreto JI, Seguel M, Montoya NG, Clément A, Carignan MO (2001). Pigment profile of the ichthyotoxic dinoflagellate *Gymnodinium* sp. from a massive bloom in southern Chile. J Plankton Res 23, 1171-1175.

Castro CG, Rios AF, Doval MD, Perez FF (2002). Nutrient utilisation and chlorophyll distribution in the Atlantic sector of the Southern Ocean during Austral summer 1995-96. Deep-Sea Res II 49, 623-641.

Clarke A, Murphy EJ, Meredith MP, King JC, Peck LS, Barnes DKA, Smith RC (2007). Climate change and the marine ecosystem of the western Antarctic Peninsula. Phil Trans R Soc B 362, 149-166.

de Souza MS, Mendes CRB, Garcia VMT, Pollery R, Brotas V (2011). Phytoplankton community during a coccolithophorid bloom in the Patagonian shelf: microscopic and high-performance liquid chromatography pigment analyses. J Mar Biol Assoc UK (doi:10.1017/S0025315411000439).

DiTullio GR, Garcia N, Riseman SF, Sedwick PN (2007). Effects of iron concentration on pigment composition in *Phaeocystis antarctica* grown at low irradiance. Biogeochemistry 83, 71-81.

Ducklow HW, Baker K, Martinson DG, Quetin LB, Ross RM, Smith RC, Stammerjohn SE, Vernet M, Fraser W (2007). Marine pelagic ecosystems: the West Antarctic Peninsula. Phil Trans R Soc B 362, 67-94.

Garibotti I, Vernet M, Kozlowski W, Ferrario M (2003). Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: a comparison of chemotaxonomic and microscopic analyses. Mar Ecol Prog Ser 247, 27-42.

Garibotti IA, Vernet M, Smith RC, Ferrario ME (2005). Interannual variability in the distribution of the phytoplankton standing stock across the seasonal sea-ice zone west of the Antarctic Peninsula. J Plankton Res 27, 825-843.

Gordon AL, Huber BA (1984). Thermohaline stratification below the Southern Ocean sea ice. J Geophys Res 89, 641-648.

Hasle GR, Syvertsen EE (1996). Marine diatoms. In: Tomas CR (Ed), Identifying Marine Diatoms and Dinoflagellates. Academic Press Inc., London, pp. 5-385.

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999). Biovolume calculation for pelagic and benthic microalgae. J Phycol 35, 403-424.

Hoffmann LJ, Peeken I, Lochte K, Assmy P, Veldhuis M (2006). Different reactions of Southern Ocean phytoplankton size classes to iron fertilization. Limnol Oceanogr 51, 1217-1229.

Holm-Hansen O, Hewes CD (2004). Deep chlorophyll-a maxima (DCMs) in Antarctic waters. I. Relationships between DCMs and the physical, chemical, and optical conditions in the upper water column. Polar Biol 27, 699-710.

Jeffrey SW, Mantoura RFC, Wright SW (1997). Phytoplankton pigments in oceanography: guidelines to modern methods. UNESCO, Paris.

Kang SH, Kang JS, Lee S, Chung KH, Kim D, Park MG (2001). Antarctic phytoplankton assemblages in the marginal ice zone of the northwestern Weddell Sea. J Plankton Res 23, 333-352.

Karl DM, Holm-Hansen O, Taylor GT, Tien G, Bird DF (1991). Microbial biomass and productivity in the western Bransfield Strait, Antarctica during the 1986-87 austral summer. Deep-Sea Res I 38, 1029-1055.

Kirk JTO (1994). Light and photosynthesis in aquatic ecosystems, 2nd edn. Cambridge University Press, Cambridge.

Kozlowski WA, Deutschman D, Garibotti I, Trees C, Vernet M (2011). An evaluation of the application of CHEMTAX to Antarctic coastal pigment data. Deep-Sea Res I 58, 350-364.

Kropuenske LR, Mills MM, van Dijken GL, Alderkamp AC, Berg GM, Robinson DH, Welschmeyer NA, Arrigo KR (2010). Strategies and rates of photoacclimation in two major Southern Ocean phytoplankton taxa: *Phaeocystis antarctica* (Haptophyta) and *Fragilariopsis cylindrus* (Bacillariophyceae). J Phycol 46, 1138-1151.

Latasa M (2007). Improving estimations of phytoplankton class abundances using CHEMTAX. Mar Ecol Prog Ser 329, 13-21.

Mackey DJ, Higgins HW, Mackey MD, Holdsworth D (1998). Algal class abundances in the western equatorial Pacific: estimation from HPLC measurements of chloroplast pigments using CHEMTAX. Deep-Sea Res I 45, 1441-1468.

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996). CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144, 265-283.

Margalef R (1958). Temporal succession and spatial heterogeneity in phytoplankton. In: Buzzati-Traverso AA (Ed), Perspectives in marine biology. University of California Press, Los Angeles, pp. 323-349.

Menden-Deuer S, Lessard EJ (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45, 569-579.

Mendes CR, Cartaxana P, Brotas V (2007). HPLC determination of phytoplankton and microphytobenthos pigments: comparing resolution and sensitivity of a C18 and a C8 method. Limnol Oceanogr: Methods 5, 363-370.

Mendes CR, Sá C, Vitorino J, Borges C, Garcia VMT, Brotas V (2011). Spatial distribution of phytoplankton assemblages in the Nazaré submarine canyon region (Portugal): HPLC-CHEMTAX approach. J Marine Syst 87, 90-101.

Mills MM, Kropuenske LR, van Dijken GL, Alderkamp AC, Berg GM, Robinson DH, Welschmeyer NA, Arrigo KR (2010). Photophysiology in two Southern Ocean taxa: photosynthesis of *Phaeocystis antarctica* (Prymnesiophyceae) and *Fragilariopsis cylindrus* (Bacillariophyceae) under simulated mixed-layer irradiance. J Phycol 46, 1114-1127.

Mitchell BG, Holm-Hansen O (1991). Observations and modeling of the Antarctic phytoplankton crop in relation to mixing depth. Deep-Sea Res 38, 981-1007.

Moline MA (1998). Photoadaptive response during the development of a coastal Antarctic diatom bloom and relationship to water column stability. Limnol Oceanogr 43, 146-153.

Moline MA, Claustre H, Frazer TK, Schofield O, Vernet M (2004). Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. Glob Change Biol 10, 1973-1980.

Moline MA, Prézelin BB (1996). Palmer LTER 1991–1994: longterm monitoring and analyses of physical factors regulating variability in coastal Antarctic phytoplankton biomass, in situ productivity and taxonomic composition over subseasonal, seasonal and interannual time scales. Mar Ecol Prog Ser 145, 143-160.

Montagnes DJS, Berges JA, Harrison PJ, Taylor FJR (1994). Estimating carbon, nitrogen, protein, and chlorophyll *a* from volume in marine phytoplankton. Limnol Oceanogr 39, 1044-1060.

Montes-Hugo M, Doney SC, Ducklow HW, Fraser W, Martinson D, Stammerjohn SE, Schofield O (2009). Recent changes in phytoplankton communities associated with rapid regional climate change along the western Antarctic Peninsula. Science 323, 1470-1473.

Park MG, Yang SR, Kang SH, Chung KH, Shim JH (1999). Phytoplankton biomass and primary production in the marginal ice zone of the northwestern Weddell Sea during austral summer. Polar Biol 21, 251-261.

Prézelin BB, Hofmann EE, Mengelt C, Klinck JM (2000). The linkage between Upper Circumpolar Deep Water (UCDW) and phytoplankton assemblages on the west Antarctic Peninsula continental shelf. J Mar Res 58, 165-202.

Rodriguez F, Varela M, Zapata M (2002). Phytoplankton assemblages in the Gerlache and Bransfield Straits (Antarctic Peninsula) determined by light microscopy and CHEMTAX analysis of HPLC pigment data. Deep-Sea Res I 49, 723-747.

Ross RM, Quetin LB, Haberman KL (1998). Interannual and seasonal variability in short-term grazing impact of *Euphausia superba* in nearshore and offshore waters west of the Antarctic Peninsula. J Mar Syst 17, 261-273.

Sangrà P, Gordo C, Hernández-Arencibia M, Marrero-Díaz A, Rodríguez-Santana A, Stegner A, Martínez-Marrero A, Pelegrí JL, Pichon T (2011). The Bransfield current system. Deep-Sea Res I 58, 390-402.

Sañudo-Wilhelmy SA, Olsen KA, Scelfo JM, Foster TD, Flegal AR (2002). Trace metal distributions off the Antarctic Peninsula in the Weddell Sea. Mar Chem 77, 157-170.

Schlüter L, Henriksen P, Nielsen TG, Jakobsen HH (2011). Phytoplankton composition and biomass across the Southern Indian Ocean. Deep-Sea Res I 58, 546-556.

Schlüter L, Møhlenberg F, Havskum H, Larsen S (2000). The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios. Mar Ecol Prog Ser 192, 49-63.

Scott FJ, Marchant HJ (2005). Antarctic marine protists. Australian Biological Resources Study and Australian Antarctic Division, Canberra.

Smayda TJ, Reynolds CS (2001). Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. J Plankton Res 23, 447-461.

Sournia A (1978). Phytoplankton manual. Muséum National d'Histoire Naturelle, UNESCO, Paris.

Steig EJ, Schneider DP, Rutherford SD, Mann ME, Comiso JC, Shindell DT (2009). Warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. Nature 457, 459-463.

Sullivan CW, Arrigo KR, McClain CR, Comiso JC, Firestone J (1993). Distributions of Phytoplankton Blooms in the Southern-Ocean. Science 262, 1832-1837.

Turner J, Colwell SR, Marshall GJ, Lachlan-Cope TA, Carleton AM, Jones PD, Lagun V, Reid PA, Iagovkina S (2005). Antarctic Climate Change during the last 50 years. Int J Climatol 25, 279-294.

Utermöhl H (1958). Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt Int Ver Theor Angew Limnol 9, 1-38.

Van Leeuwe MA, Stefels J (1998). Effects of iron and light stress on the biochemical composition of Antarctic *Phaeocystis* sp. (Prymnesiophyceae). II. Pigment composition. J Phycol 34, 496-503.

Van Leeuwe MA., Stefels J (2007). Photosynthetic responses in *Phaeocystis antarctica* towards varying light and iron conditions. Biogeochemistry 83, 61-70.

Wong CS, Crawford DW (2006). Evolution of phytoplankton pigments in an in-situ iron enrichment experiment in the subarctic NE Pacific. Deep-Sea Res II 53, 2152-2167.

Wright SW, Ishikawa A, Marchant HJ, Davidson AT, van den Enden RL, Nash GV (2009). Composition and significance of picophytoplankton in Antarctic waters. Polar Biol 32, 797-808.

Wright SW, Jeffrey SW (2006). Pigment markers for phytoplankton production. In: Volkmann JK (Ed.), Marine Organic Matter: Biomarkers, Isotopes and DNA. Spring-Verlag, Berlin, pp. 71-104.

Wright W, Thomas DP, Marchant J, Higgins HW, Mackey MD, Mackey DJ (1996). Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorisation program. Mar Ecol Prog Ser 144, 285-298.

Wright SW, van den Enden RL (2000). Phytoplankton community structure and stocks in the East Antarctic marginal ice zone (BROKE survey, January March 1996) determined by CHEMTAX analysis of HPLC pigment signatures. Deep-Sea Res II 47, 2363-2400.

Wright SW, van den Enden RL, Pearce I, Davidson AT, Scott FJ, Westwood KJ (2010). Phytoplankton community structure and stocks in the Southern Ocean (30–801E) determined by CHEMTAX analysis of HPLC pigment signatures. Deep-Sea Res II 57, 758-778.

Zapata M, Rodriguez F, Garrido JL (2000). Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. Mar Ecol Prog Ser 195, 29-45.

Zhou M, Nordhausen W, Huntley M (1994). ADCP measurements of the distribution and abundance of Euphausiids near the Antarctic Peninsula in Winter. Deep-Sea Res I 41, 1425-1445.

CAPÍTULO IV

Cryptophytes dominated diatoms in the Bransfield Strait (Antarctic Peninsula) in the late summer of 2010.

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ABSTRACT

Recent global warming, which results in glacial meltwater runoff, consequently reduces surface water salinity around the Antarctic Peninsula. This predicament has increased the occurrence and abundance of certain phytoplankton groups, such as cryptophytes. The dominance of this group over diatoms affects grazers such as antarctic krill, which preferably feed on diatoms. By using three late summer's data sets (2008-2010) from the Bransfield Strait, we observed changes in the dominant phytoplankton group using HPLC pigment analysis and confirmed by microscopy. Multivariate statistical analyses indicate that the dominance of diatoms, mainly in 2008 and 2009, was associated with deeper upper mixed layer (UML), high salinity and warmer sea surface temperature. On the other hand, cryptophytes, which were dominant in 2010, appeared at shallower UML, lower salinity and colder sea surface temperature. The low diatom biomass observed in the summer of 2010 was associated with high nutrient concentrations, particularly silicate, and low chlorophyll *a* (summer monthly average calculated from satellite images). The observed interannual variability in the dominance of phytoplankton groups reflected a delayed seasonal succession cycle of phytoplankton, which was, in turn, associated with a cold summer and a late ice retreat process in the region. This delay resulted in a drastic decrease of primary producers' biomass in 2010, which may have impacted regional food web interactions.

Keywords: Antarctic Peninsula, Bransfield Strait, Phytoplankton succession, Cryptophytes, Diatoms.

4.1. Introduction

The Antarctic Peninsula (AP) is among world regions most susceptible to global climate change [Turner et al., 2005; Steig et al., 2009]. Consequences of these changes are not yet fully understood. Regional environmental changes in the AP have been altering the biomass and composition of primary producers, particularly phytoplankton [Garibotti et al., 2005; Ducklow et al., 2007; Montes-Hugo et al., 2009]. However, as large spatial and seasonal/interannual variations of physical variables and, consequently, biological communities have been observed in the region, it is not known if changes that are currently being observed in such dynamic communities are natural temporal/spatial variations or result from recent climate change. As phytoplankton supports oceanic food webs and plays a key role on the AP marine ecosystem's resilience, changes in its abundance and composition may have a direct effect on the regional ecosystem.

The three main phytoplankton taxonomic groups in coastal regions of the AP are diatoms, haptophytes (primarily *Phaeocystis antarctica*) and cryptophytes [Rodriguez et al., 2002; Garibotti et al., 2003, 2005; Kozlowski et al., 2011]. Phytoplankton blooms around the AP are typically associated with the development of a shallow mixed layer, which keeps phytoplankton within adequate light levels and iron availability [e.g., Prézelin et al., 2000]. These blooms are usually dominated by diatoms and/or *P. antarctica*. Nevertheless, some studies highlight the increasing importance of cryptophytes in the AP region that prevail over diatoms, particularly in ice melting areas [Moline & Prézelin, 1996; Moline et al., 2004]. As diatoms are more efficiently grazed by

Antarctic krill than cryptophytes, the shift from a diatom to a cryptophyte-dominated community will affect food-web trophic interactions [Haberman et al., 2003]. These phytoplankton community's changes around the AP waters have been associated with a recent increase in temperature together with dominance of salps instead of krill as main consumers [e.g., Moline et al., 2004; Montes-Hugo et al., 2009]. Organisms from higher trophic levels, such as penguins and seals, preferably consume krill rather than salps [Loeb et al., 1997]. Consequently, shifts in the phytoplankton community composition may anticipate negative feedbacks on the ecology of these consumers.

This study investigates environmental factors that may trigger changes in phytoplankton communities and lead to a dominance of either diatoms or cryptophytes in the Bransfield Strait, around the tip of the AP.

4.2. Methods

Twenty stations in the Bransfield Strait (Figure 1a) were sampled in the late summers of 2008 (21 February to 4 March), 2009 (25 February to 1 March) and 2010 (16 to 21 February), as part of the SOS-CLIMATE (Southern Ocean Studies for Understanding Global-CLIMATE Issues) project. Both physical data (conductivity, temperature and salinity) and water samples were collected using a combined Sea-Bird CTD/Carrousel 911+system® equipped with 24 five-liter Niskin bottles. Density (kg m^{-3}) was calculated based on temperature, salinity and pressure data in order to evaluate the water column physical structure. The upper mixed layer depth (UMLD) was determined as the depth where a change of 0.05 kg m^{-3} occurred over a 5 m depth interval (adapted from Mitchell &

Holm-Hansen [1991]). Water column stability (hereafter referred to as stability) was estimated using vertical density variations, as a function of the buoyancy or Brunt-Väisälä frequency (N^2). Average stability values (between 0 and 100 m) were used in the statistical analyses.

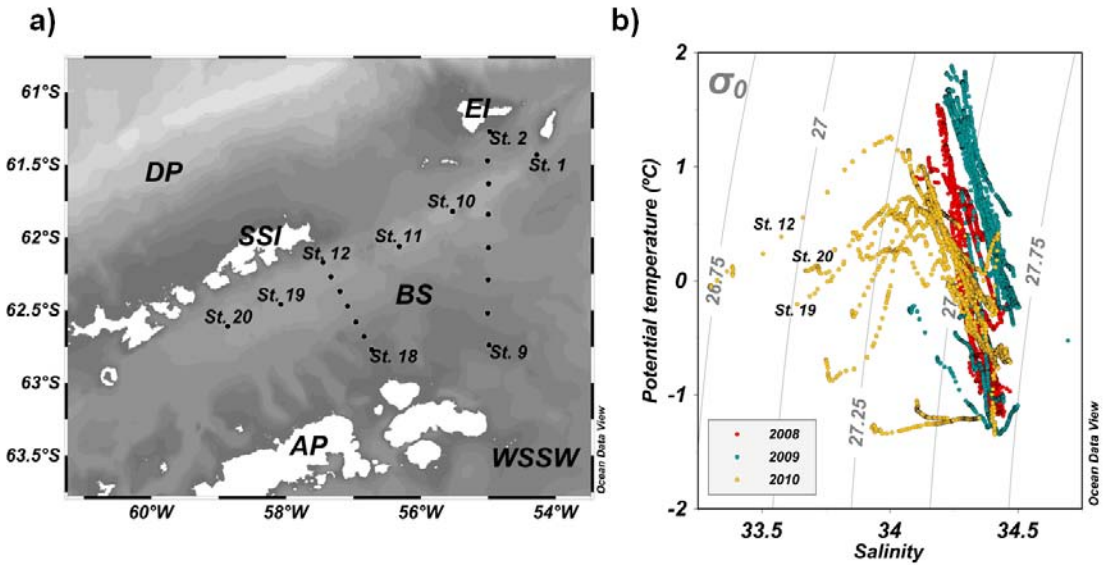

Figure 1: (a) Study area and station's location during SOS-CLIMATE 2008, 2009 and 2010 summer cruises. Abbreviations are as follows: Drake Passage (DP), Elephant Island (EI), South Shetland Islands (SSI), Bransfield Strait (BS), Weddell Sea Shelf Water (WSSW) and Antarctic Peninsula (AP). **(b)** T/S diagram (temperature and salinity data in the upper 200 m layer) from all stations sampled during the three years (2008, 2009 and 2010). Stations near the SSI clearly indicating ice-melting conditions are labeled (stations 12, 19 and 20).

Surface water samples were filtered on cellulose acetate membrane filters to determine dissolved inorganic nutrients (nitrate, nitrite, ammonium, phosphate and silicate). Nutrients were analyzed on board, following the processing recommendations in Aminot & Chaussepied [1983], and absorbance values were measured in a FEMTO® spectrophotometer. Seawater samples (0.5-2 L) were filtered onto Whatman GF/F filters (nominal pore size of 0.7 µm and 25 mm in diameter) and immediately stored in liquid nitrogen for HPLC

pigment analysis. Pigments were extracted with 2 mL of 95% cold-buffered methanol (2% ammonium acetate) for 30 min at -20 °C, in the dark. Samples were sonicated (Bransonic, model 1210, w: 80, Hz: 47) for 1 min at the beginning of the extraction period and then centrifuged at 1100 g for 15 min, at 4 °C. Extracts were filtered (Fluoropore PTFE membrane filters, 0.2 µm pore size) and immediately injected in the HPLC instrument. Method procedures for HPLC analyses (using a monomeric C8 column with a pyridine-containing mobile phase) are fully described in Mendes et al. [2007].

Three marker carotenoids were used for determining distributions of the major phytoplankton taxa: fucoxanthin (FUCO) for diatoms, 19'-hexanolyoxyfucoxanthin (HEX-FUCO) for haptophytes (primarily *P. antarctica*), and alloxanthin (ALLO) for cryptophytes. From the class-specific accessory pigments and total chlorophyll *a* (CHL-*a*), the percentage contribution of each taxonomic group to the overall biomass was calculated using the ChemTax software [Mackey et al., 1996; Kozlowski et al., 2011]. Pigment-based estimates were verified and confirmed by microscope analyses.

In order to compare environmental variables over different years, a one-way analysis of variance (ANOVA) was performed, followed by the Tukey method for multiple comparisons among data sets. Data were logarithmically transformed when necessary to comply with the assumptions of ANOVA. Relationships between the phytoplankton groups' biomass and environmental variables were explored through a Canonical Correspondence Analysis (CCA).

4.3. Results

The mean surface water temperature from the 20 stations showed significant differences ($p < 0.05$) between 2009 (1.08 ± 0.87 ºC) and 2010 (0.16 ± 0.71 ºC). An intermediate temperature was observed in 2008 (0.54 ± 0.87 ºC), but not significantly different from the other two years (Figure 1b). Salinity was very similar in 2008 and 2009 in the upper layer (< 200 m), yet notably different from 2010, when the lowest and most variable surface salinity values were observed (see Figure 1b). This salinity pattern observed in 2010 caused a noteworthy stratification, particularly at stations close to the South Shetland Islands. Salinity varied between 34.1 and 34.6, which together with data from the T/S diagrams suggests that Weddel Sea water influenced the region during the three sampling years [García et al., 2002].

Table 1: Concentration of the main photosynthetic pigments detected in this study and ratios of degradation products to chlorophyll *a*. CHL-*a* = chlorophyll *a*; FUCO = fucoxanthin; ALLO = alloxanthin; HEX-FUCO = 19'-hexanoyloxyfucoxanthin; CHLIDE-*a* = chlorophyllide *a*; PHE-*a* = pheopigments *a* (pheophorbide *a* + pheophytin *a*). Different superscript labels (a, b) between years indicate significant differences ($p<0.05$, Tukey method).

PIGMENT / RATIO	2008	2009	2010
	average and range of surface concentrations (mg m^{-3})		
CHL-*a*	0.52 (0.12-1.08) ^{a}	0.94 (0.35-1.97) ^{b}	1.07 (0.38-3.78) ^{b}
FUCO	0.42 (0.10-0.96) ^{a}	0.61 (0.10-1.42) ^{a}	0.21 (0.08-0.32) ^{b}
ALLO	0.01 (0.00-0.05) ^{a}	0.01 (0.00-0.03) ^{a}	0.14 (0.00-0.97) ^{b}
HEX-FUCO	0.07 (0.02-0.14) ^{a}	0.02 (0.01-0.06) ^{b}	0.11 (0.03-0.25) ^{a}
	average and range of surface ratios		
CHLIDE-*a* :CHL-*a*	0.03 (0.00-0.09) ^{a}	0.02 (0.00-0.07) ^{a}	0.01 (0.00-0.04) ^{a}
PHE-*a* :CHL-*a*	0.17 (0.04-0.38) ^{a}	0.13 (0.04-0.23) ^{a}	0.02 (0.01-0.05) ^{b}

High interannual variations were also observed in surface nutrient concentrations. Dissolved inorganic nitrogen (DIN) ranged from 16.6 to 47.8 µM, with the lowest values recorded in 2008 (24.1 ± 4.6 µM) and highest in 2010 (41.6 ± 3.5 µM). Silicates varied from 23.1 to 79.9 µM, with maximum values in 2010 (69.2 ± 6.4 µM), and phosphates varied between 0.3 and 3.4 µM, with minimum values in 2008 (0.8 ± 0.3 µM).

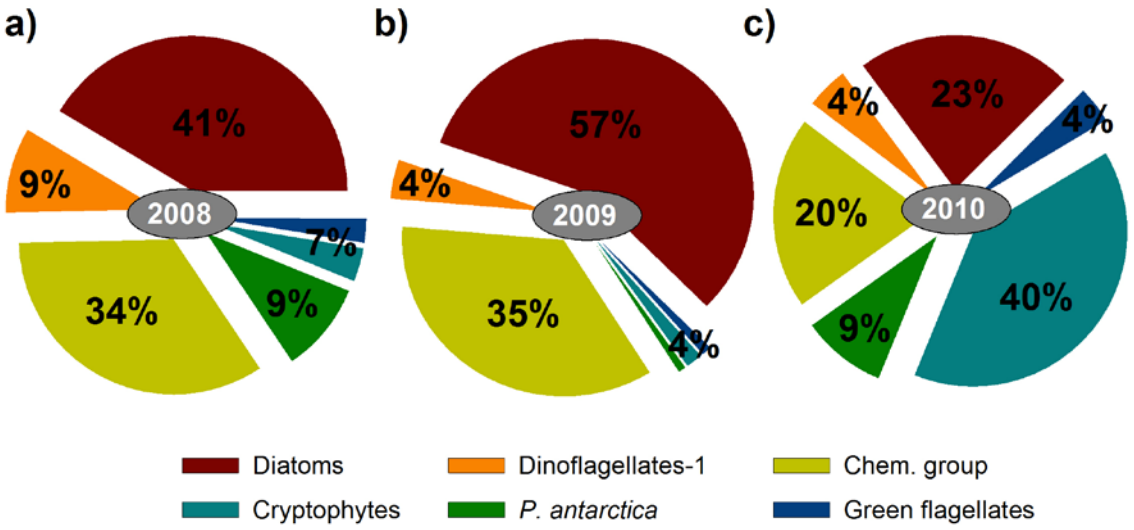

Figure 2: Average percentage contribution of phytoplankton groups (CHEMTAX-allocated) to total chlorophyll *a* in **(a)** 2008, **(b)** 2009 and **(c)** 2010. Dinoflagellates-1 = peridinin-containing dinoflagellates; Green flagellates = flagellates bearing chlorophyll *b*; *P. antarctica* = *Phaeocystis antarctica*; Chem. group = a group including peridinin-lacking autotrophic dinoflagellates and other algal groups such as parmales and chrysophytes.

Significantly lower CHL-*a* values (used as phytoplankton biomass index) were recorded in 2008 (ranging from 0.12 to 1.08 mg m^{-3}) (Table 1), while the highest value was observed in 2010 (3.78 mg m^{-3}; station 12). FUCO, ALLO and HEX-FUCO were the main carotenoids observed in this study, and their concentrations displayed different interannual patterns. FUCO was the major carotenoid in the first two years and its concentration reached values higher

than 1 mg m^{-3} in 2009. The lowest concentration of FUCO was observed in 2010, while ALLO was the main carotenoid in most stations sampled during in this year, particularly in the surface layers. The HEX-FUCO concentrations ranged from 0.01 to 0.25 mg m^{-3}, and values were relatively lower in 2009 (see Table 1). Pheopigments *a* (PHE-*a*):CHL-*a* ratios (used as a relative index of grazing) was significantly lower in 2010 ($p<0.05$), and it was always below 0.05 (Table 1). The maximum values (~ 0.4) were observed in 2008. On the other hand, chlorophyllide *a* (CHLIDE-*a*):CHL-*a* ratios (used as an index of cells senescence) did not show significant differences among years (Table 1).

The relative contributions of the main phytoplankton groups to total CHL-*a* are shown in Figure 2. Diatoms were the dominant group both in 2008 (Figure 2a) and 2009 (Figure 2b), with a relatively higher value in 2009 accompanied by a decrease in the contributions of all nanoplanktonic groups (e.g., *P. antarctica*, cryptophytes and green flagellates). In 2010, diatoms were mainly replaced by cryptophytes (Figure 2c). Figure 2 also shows the important contribution of the "chemotaxonomic group" for the phytoplankton community in all years. This group is an assemblage consisting of peridinin-lacking autotrophic dinoflagellates (e.g., *Gymnodinium* spp.), other algal groups such as parmales and chrysophytes, and CHL-c_3-containing diatoms (e.g., *Pseudonitzschia* spp.).

A multivariate analysis of the phytoplankton groups in the three years showed a strong association with water physical and chemical properties (see CCA diagram in Figure 3). The first axis of the CCA (63.5% explanation) reveals a notable separation between diatoms and cryptophytes, mainly associated with salinity, UMLD, temperature, DIN and silicate. The second axis (29.1%

explanation) indicates that most flagellates (e.g., *P. antarctica*) are positively correlated with water column stability and negatively correlated with phosphate concentration (see Figure 3).

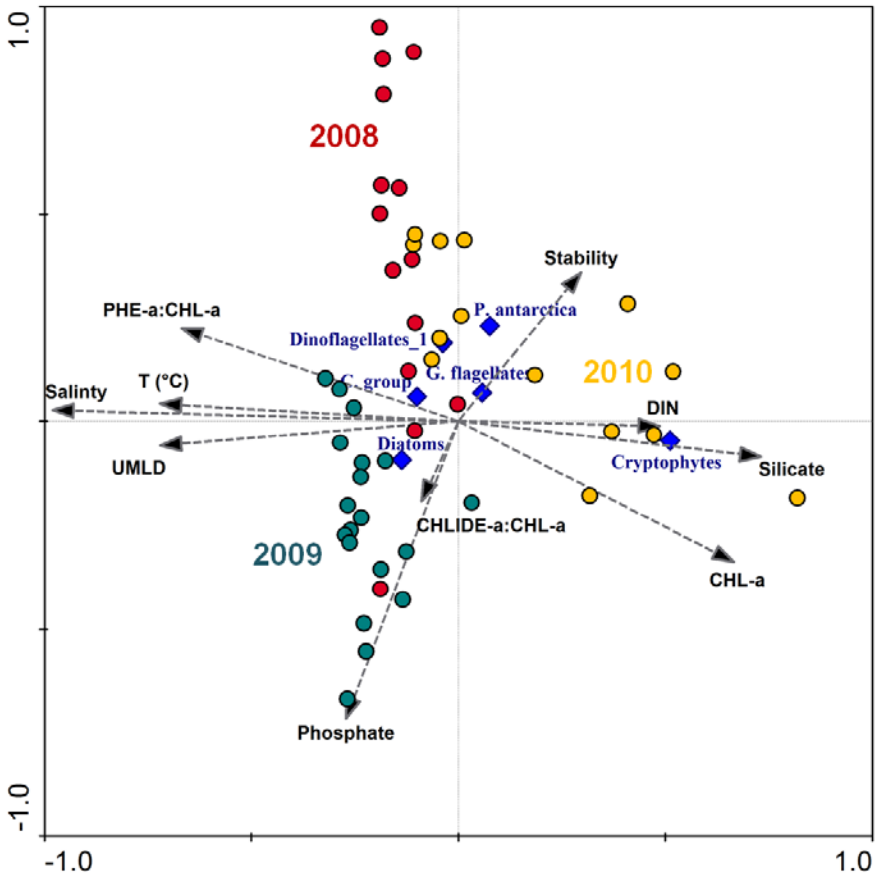

Figure 3: Canonical Correspondence Analysis ordination diagram of absolute contributions of different phytoplankton groups at sea surface. The first two ordination axes represent 76.3% of the total phytoplankton group's variance and 92.6% of phytoplankton groups-environment relationships. Arrows indicate environment variables (water column stability (Stability), upper mixed layer depth (UMLD), and sea surface water values of temperature (T (ºC)), salinity (Salinity), chlorophyll *a* (CHL-a), pheopigments *a*:chlorophyll *a* ratio (PHE-a:CHL-a), chlorophyllide *a*:chlorophyll *a* ratio (CHLIDE-a:CHL-a) and dissolved inorganic nitrogen (DIN), phosphate and silicate). Blue diamond's refer to absolute contributions of phytoplankton groups (Diatoms; C_group = chemotaxonomic group; Dinoflagellates_1 = dinoflagellates with peridinin; G. flagelates = Green flagellates; P. antarctica = *Phaeocystis antarctica*; Cryptophytes). Stations are separated according to sampling year (red circles = 2008; green circles = 2009; yellow circles = 2010).

4.4. Discussion

Several studies have been addressing the issue of seasonal succession of phytoplankton communities in the Antarctic Peninsula region, particularly during the Austral summer. Most studies report that the timing of sea ice retreat is critical for the progression of phytoplankton seasonal cycles [e.g., Moline & Prézelin, 1996; Garibotti et al., 2005]. Diatom blooms are found in the early summer, when the sea ice retreat is progressing. Later, flagellate blooms, such as cryptophytes, replace diatoms [Ducklow et al., 2007]. In a final stage of succession, the community is dominated by diatoms and unidentified phytoflagellates [Moline & Prézelin, 1996; Garibotti et al., 2005]. In the present study, sampling was always performed during the late summer. Consequently, the observed phytoplankton community was a result of the succession associated with the timing and extent of sea ice melting during the whole summer.

Cryptophytes emerged as the dominant phytoplankton group associated with lower salinity, shallower mixed layer and stronger stratification (see CCA results in Figure 3), which are typical oceanographic characteristics of glacial ice melting conditions. Particularly, high cryptophytes' biomass in the low salinity surface layers was associated with strong water column stratification, which was observed at some nearshore stations in 2010 (Figure 4). As cryptophytes respond to changes in water column salinity [Moline & Prézelin, 1996; Moline et al., 2004], taxonomic changes of the phytoplankton community may reflect variations in the timing, duration and amount of the annual fresh water input. Other factors, such as water column structure, have also been

suggested as possible triggers for the replacement of diatoms by cryptophytes [e.g., Mitchell & Holm-Hansen, 1991]. Freshwater input causes a strong stratification, and the prolonged occurrence of this condition can lead to severe nutrient limitation in surface layers, particularly iron. This probably favors opportunistic small-sized and motile species, such as cryptophytes, which can still grow in very low iron concentrations [Gerringa et al., 2000], contrasting with diatoms, which require relatively high iron levels. Although the association of cryptophytes with stratification/low salinity has been already discussed [Moline & Prézelin, 1996; Moline et al., 2004], the connection of this group with iron availability is still vague, due to the difficulty in measuring this trace metal in seawater [Lancelot et al., 2009].

In the present work, results from 2010 show cryptophytes dominance under evident glacial ice melting (surface salinity in nearshore waters below 33.8; Dierssen et al. [2002]) which contribute to stratification, but may not be a significant source of iron. According to Klunder et al. [2011], vertical mixing and upwelling are the most important iron supply mechanisms to the upper surface mixed layer in Antarctic regions. In 2010, when cryptophytes were the dominant group, very low relative levels of PHE-*a*, degradation products associated with grazing processes [Jeffrey, 1974], were observed, suggesting that grazing activities were less intense in that year. This possible shift in the primary producers' community, namely diatoms to cryptophytes, may have caused a negative impact on the Antarctic Peninsula marine ecosystem, due to lower efficiency of local grazers, such as krill, on these nanoflagellates [Haberman et al., 2003]. These low grazing rates may have also contributed to sustaining the high biomass levels (CHL-*a*) associated with cryptophytes in late summer 2010.

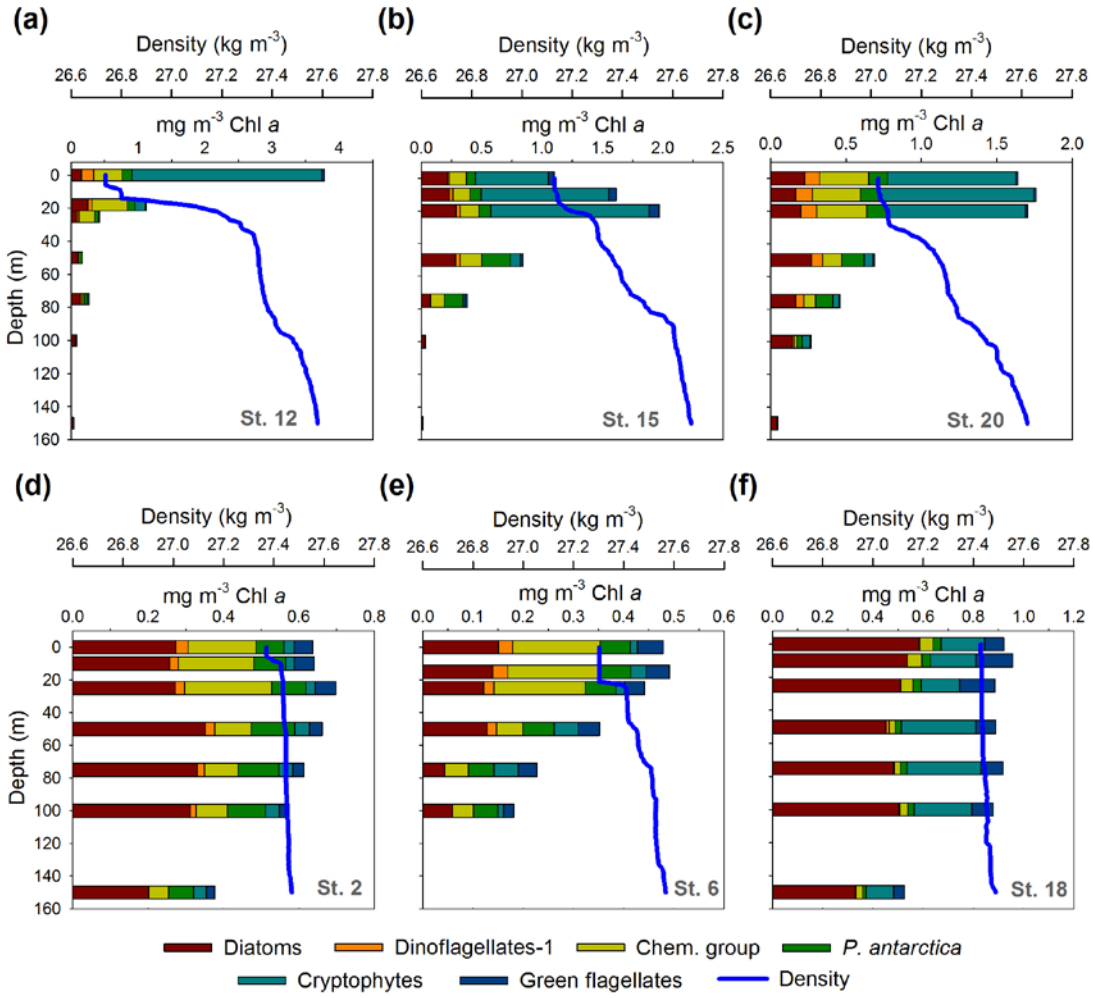

Figure 4: Depth distribution of phytoplankton groups' biomass (as chlorophyll *a* concentration) calculated by CHEMTAX, at the six selected stations in the Bransfield Strait in 2010, and respective density vertical profiles. Cryptophytes were found in the upper layer, above the pycnocline (a, b and c). Contrarily, diatoms appeared in deeper upper mixed layer (d, e and f). Dinoflagellates-1 = peridinin-containing dinoflagellates; Green flagellates = flagellates with chlorophyll *b*; *P. antarctica* = *Phaeocystis antarctica*; Chem. group = a group including peridinin-lacking autotrophic dinoflagellates and other algal groups such as parmales and chrysophytes. See Figure 1a for station locations. Note the different scales in chlorophyll *a* concentration.

Based on results of in-situ data (T/S diagram in Figure 1b), ice melting was more evident in 2010 than in the two previous sampling years. However, the study period (late summer) does not represent the conditions of the whole season. The summer of 2010 was shown to be much colder than previous summers, and preceded by a relatively cold winter (Figure 5), which resulted in

a great accumulation of ice around the AP (Figure 6). In contrast to 2008 and 2009, when phytoplankton blooms were more intense during the austral summer, particularly around the South Shetland Islands (see Figure 6), the outcome of the cold and icy conditions in 2010 was a lower phytoplankton biomass (CHL-*a* estimated by remote sensing). The higher nutrient concentrations recorded in February 2010 also indicate low consumption and therefore low biomass accumulation, as observed in the Bransfield Strait. Silicate concentrations, which are almost only taken up by diatoms, were approximately twice the concentrations observed in the two previous years. Therefore, the natural diatom bloom that normally precedes cryptophyte development in early summer (seasonal succession) under sea ice melting [Moline & Prézelin, 1996; Garibotti et al., 2005] must have been less intense in 2010.

Based on this interpretation, we conclude that the phytoplankton community sampled in the late summer in the study region was in a delayed stage of its seasonal succession cycle during the coldest year (2010), and in an advanced stage as expected for late summer during the warmer years (2008 and 2009). Moreover, although diatoms dominated the phytoplankton community in 2008 and 2009, the biomass levels were relatively lower in 2008. In this first year, a larger contribution of nanoflagellates was also observed, together with lower phosphate concentrations (see CCA in Figure 3). These results indicate that the phytoplankton community in 2008 was probably in a later stage of its seasonal cycle.

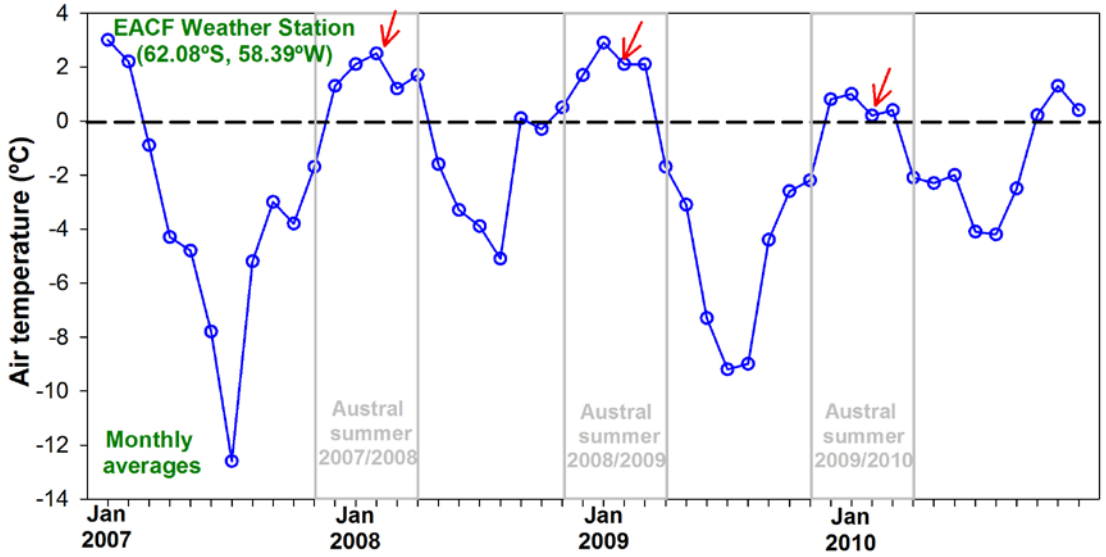

Figure 5: Monthly averages of air temperature recorded at the EACF Weather Station (62.08°S, 58.39°W) located on King George Island, South Shetland Islands. Red arrows refer to sampling times.

A possible reason for the delay in the phytoplankton seasonal succession in 2010 comparing to 2008 and 2009 (as stated above) might be a lower degree of sea ice melting, resulting from relatively lower air temperatures in the summer 2010 (monthly average air temperatures below 1 ºC; see Figure 5). These environmental conditions probably arrested the development of the initial diatom bloom in the first stage of natural succession and delayed the start of the second stage, which is replacement of diatoms by cryptophytes.

The probable cause of the atypical cold temperatures in late 2009/beginning of 2010 was the moderate-to-strong El Niño episode (source from U.S. National Oceanic and Atmospheric Administration, NOAA). Previous studies show that direct impacts of the El Niño-Southern Oscillation (ENSO) are consistent with Antarctic sea ice variability [e.g., Yuan, 2004; Stammerjohn et al., 2008]. Besides the effects in the Pacific basin [Trenberth & Hoar, 1996], the increasing frequency of ENSO events have also caused critical disturbances in

the timing and intensity of sea ice melting in Antarctica [Yuan, 2004; Stammerjohn et al., 2008]. As observed in this study, such disturbances can lead to decrease in the phytoplankton biomass levels in the region, which ultimately can have cascading effects in the whole ecosystem. Moreover, this study also shows that not only warmer but also colder temperatures affect the regular functioning of the phytoplanktonic primary production in the AP.

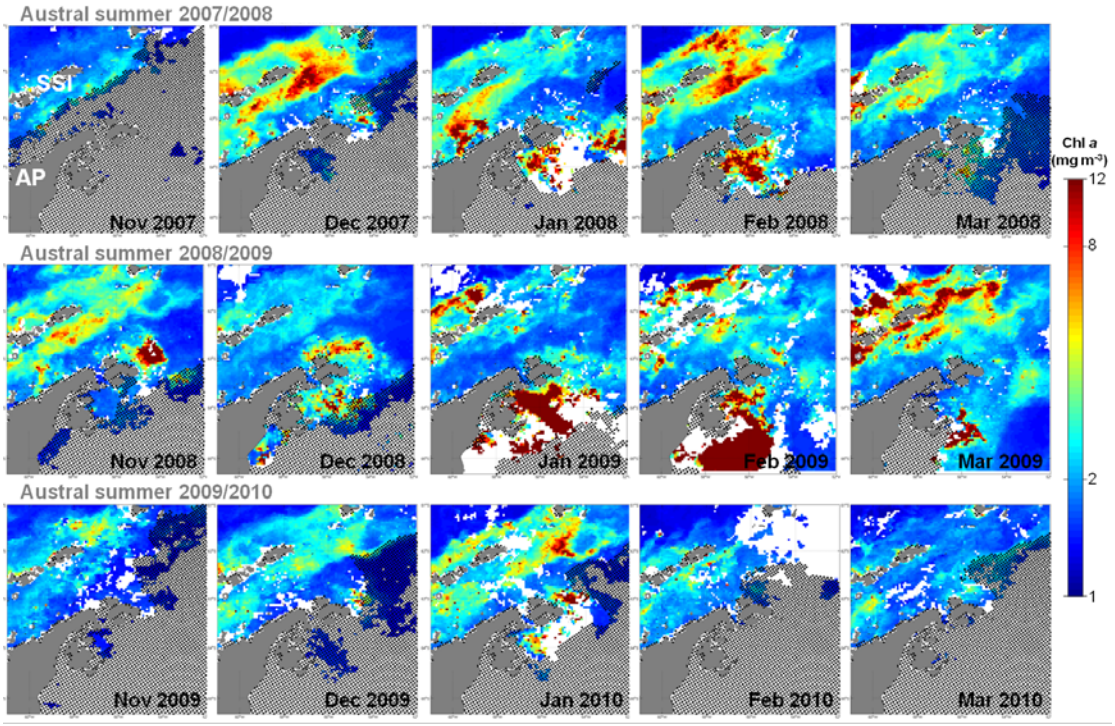

Figure 6: Remote sensing of chlorophyll *a* concentration data were derived from monthly composites of MODIS-Aqua satellite images. Level 3 (L3) Standard Mapped Image (SMI) images were obtained from http://oceancolor.gsfc.nasa.gov at 4 km resolution. Daily images of sea ice concentration were used for calculating mean monthly images of the study area. The selected period was November to March from 2007 to 2010. Data were collected from the AMSR-E sensor (AQUA platform), with a spatial resolution of approximately 6x4 km at 89 Ghz. The Artist Sea Ice (ASI) algorithm was applied to retrieve the ice concentration between 0% and 100% (Spreen et al., 2008). Hemispherical (6.25 km grid) sea ice concentration (ASI algorithm) daily maps were provided by the Institute of Environment Physics, University of Bremen, and used in this work (www.iup.physik.uni-bremen.de). SSI = South Shetland Islands; AP = Antarctic Peninsula**.**

4.5. Concluding remarks

This study concludes that the interannual variation observed in late summer phytoplankton composition in the Bransfield Strait represented a temporal displacement of the seasonal phytoplankton succession. The low temperatures recorded during the whole summer of 2010 did not allow a normal development of high biomass phytoplankton blooms (presumably diatoms), which are typical in this region. This departure from temporal norms certainly impacted all other trophic levels during that summer. Future studies should focus on broader spatial and finer temporal scale surveys in order to better understand how phytoplankton community responds to environmental factors. Such phytoplankton monitoring procedures are vital to fully understanding the function of marine food webs, particularly in regions extremely sensitive to global climate change, as the Antarctic Peninsula region.

Acknowledgments

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References

Aminot A, Chaussepied J (1983). Manuel dês Analyses Chimiques en Milieu Marin, C.N.E.X.O., Brest, p. 230.

Dierssen HM, Smith RC, Vernet M (2002). Glacial meltwater dynamics in coastal waters west of the Antarctic peninsula. PNAS 99: 1790-1795.

Ducklow HW, Baker K, Martinson DG, Quetin LB, Ross RM, Smith RC, Stammerjohn SE, Vernet M, Fraser W (2007). Marine pelagic ecosystems: the West Antarctic Peninsula. Phil. Trans. R. Soc. B 362: 67-94.

García MA, Castro CG, Ríos AF, Doval MD, Rosón G, Gomis D, López O (2002). Water masses and distribution of physico-chemical properties in the Western Bransfield Strait and Gerlache Strait during Austral summer 1995/96. Deep Sea Res., Part II, 49: 585-602.

Garibotti IA, Vernet M, Kozlowski WA, Ferrario ME (2003). Composition and biomass of phytoplankton assemblages in coastal Antarctic waters: a comparison of chemotaxonomic and microscopic analyses. Mar. Ecol. Prog. Ser. 247: 27-42.

Garibotti IA, Vernet M, Ferrario ME (2005). Annually recurrent phytoplanktonic assemblages during summer in the seasonal ice zone west of the Antarctic Peninsula (Southern Ocean). Deep Sea Res., Part I, 52: 1823-1841.

Gerringa LJA, de Baar HJW, Timmermans KR (2000). A comparison of iron limitation of phytoplankton in natural oceanic waters and laboratory media conditioned with EDTA. Mar. Chem. 68: 335-346.

Haberman KL, Ross RM, Quetin LB (2003). Diet of the Antarctic krill (*Euphausia superba* Dana): II. Selective grazing in mixed phytoplankton assemblages. J. Exp. Mar. Biol. Ecol. 283: 97-113.

Jeffrey SW (1974). Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar. Biol. 26: 101-110.

Klunder MB, Laan P, Middag R, de Baar HJW, van Ooijen JC (2011). Dissolved iron in the Southern Ocean (Atlantic sector). Deep Sea Res., Part I, 58: 2678-2694.

Kozlowski WA, Deutschman D, Garibotti I, Trees C, Vernet M (2011). An evaluation of the application of CHEMTAX to Antarctic coastal pigment data, Deep Sea Res., Part I, 58: 350-364.

Lancelot C, de Montety A, Goosse H, Becquevort S, Pasquer B, Vancoppenolle M (2009). Spatial distribution of the iron supply to phytoplankton in the Southern Ocean: a model study. Biogeosciences 6: 2861-2878.

Loeb V, Siegel V, Holm-Hansen O, Hewitt R, Fraser W, Trivelpiece W, Trivelpiece S (1997). Effects of sea-ice extent and krill or salp dominance on the Antarctic food web. Nature 387: 897-900.

Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996). CHEMTAX – a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser. 144: 265-283.

Mendes CR, Cartaxana P, Brotas V (2007). HPLC determination of phytoplankton and microphytobenthos pigments: comparing resolution and sensitivity of a C18 and a C8 method. Limnol. Oceanogr.: Methods 5: 363-370.

Mitchell BG, Holm-Hansen O (1991). Observations and modeling of the Antarctic phytoplankton crop in relation to mixing depth. Deep Sea Res., Part I, 38: 981-1007.

Moline MA., Prézelin BB (1996). Palmer LTER 1991–1994: longterm monitoring and analyses of physical factors regulating variability in coastal Antarctic phytoplankton biomass, in situ productivity and taxonomic composition over subseasonal, seasonal and interannual time scales. Mar. Ecol. Prog. Ser. 145: 143-160.

Moline MA, Claustre H, Frazer TK, Schofield O, Vernet V (2004). Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. Glob. Change Biol. 10: 1973-1980.

Montes-Hugo M, Doney SC, Ducklow HW, Fraser W, Martinson D, Stammerjohn SE, Schofield O (2009). Recent changes in phytoplankton communities associated with rapid regional climate change along the western Antarctic Peninsula. Science 323: 1470-1473.

Prézelin BB, Hofmann EE, Mengelt C, Klinck JM (2000). The linkage between Upper Circumpolar Deep Water (UCDW) and phytoplankton assemblages on the west Antarctic Peninsula continental shelf. J. Mar. Res. 58: 165-202.

Rodriguez F, Varela M, Zapata M (2002). Phytoplankton assemblages in the Gerlache and Bransfield Straits (Antarctic Peninsula) determined by light microscopy and CHEMTAX analysis of HPLC pigment data. Deep Sea Res., Part II, 49: 723-747.

Steig EJ, Schneider DP, Rutherford SD, Mann ME, Comiso JC, Shindell DT (2009). Warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. Nature 457: 459-463.

Stammerjohn SE, Martinson DG, Smith RC, Yuan X, Rind D (2008). Trends in Antarctic annual sea ice retreat and advance and their relation to El Niño Southern Oscillation and Southern Annular Mode variability. Journal of Geophysical Research, 113, C03S90.

Trenberth KE, Hoar TJ (1996). The 1990–1995 El Niño-Southern Oscillation Event: Longest on Record. Geophysical Research Letters 23: 57-60.

Turner J, Colwell SR, Marshall GJ, Lachlan-Cope TA, Carleton AM, Jones PD, Lagun V, Reid PA, Lagovkina S (2005). Antarctic Climate Change during the last 50 years. Int. J. Climatol. 25: 279-294.

Yuan X (2004). ENSO-related impacts on Antarctic sea ice: A synthesis of phenomenon and mechanisms. Antarcic Science 16: 415-425.

CAPÍTULO V

Conclusões

5.1. Conclusões

Neste trabalho foram efectuados estudos em dois ecossistemas bastante diferentes: uma região temperada fortemente influenciada por fenómenos de afloramento (zona costeira de Portugal) e uma região polar (região em redor da Península Antártica). Uma grande particularidade que diferenciou os dois ecossistemas foi a sua disponibilidade em (macro) nutrientes. Na Tabela 1 encontram-se os valores médios das concentrações superficiais dos principais macronutrientes analisados neste estudo. Valores mais reduzidos foram registados na região do canhão da Nazaré, sendo que as concentrações mais altas observadas nesta região da costa Portuguesa (e em situação de "upwelling") nunca atingiram os valores mínimos detectados para a região da Península Antártica, excepto no ano de 2008 onde se registaram concentrações de fosfatos (na região da Península Antártica) relativamente baixos.

Tabela 1: Concentrações superficiais de clorofila *a* e dos principais macronutrientes determinados neste trabalho (média e mínimo/máximo). N total = nitrato+nitrito+amónia.

		Clorofila *a* (mg m^{-3})	**Fosfato ($P\text{-}PO_4$)** (µM)	**Sílica ($Si\text{-}SIO_2$)** (µM)	**N total ($N\text{-}NO_x$)** (µM)
Canhão Nazaré	**2006**	0.68 (0.10 - 4.33)	0.20 (0.08 - 0.8)	1.45 (0.31 - 8.11)	2.15 (0.21 - 7.77)
Península Antártica	**2008**	0.73 (0.04 - 4.50)	0.82 (0.26 - 2.98)	45.2 (23.1 - 65.9)	23.7 (14.1 - 40.2)
	2009	1.29 (0.02 - 7.61)	2.59 (1.50 - 3.46)	54.3 (42.0 - 64.9)	28.5 (19.2 - 36.4)
	2010	1.06 (0.12 - 3.78)	1.99 (1.56 - 2.21)	70.4 (52.9 - 92.0)	40.1 (30.7 - 48.6)

No que diz respeito à Península Antártica, o ano de 2010 destacou-se por ter apresentado concentrações médias quase duas vezes mais elevadas de sílica e principalmente de azoto, relativamente a 2008 e 2009 (nos fosfatos este padrão não foi evidente). Estas elevadas concentrações estão relacionadas, tal como descrito e discutido no capítulo IV desta dissertação, com um menor consumo associado a baixas biomassas fitoplantónicas registadas durante o verão de 2010.

As concentrações superficiais de clorofila *a* (usada como índice de biomassa) na Antártica variaram entre 0.02 e 7.61 mg m^{-3}, com as maiores concentrações a serem registadas junto à ilha James Ross (Península Antártica) e as menores nas regiões mais oceânicas e afastadas da Península. Na região do canhão da Nazaré, os valores de clorofila *a* variaram entre 0.10 e 4.33 mg m^{-3}, com os maiores valores registados junto à costa e os mais baixos nas estações mais oceânicas. Outros pigmentos, para além da clorofila *a*, foram também detectados em concentrações relevantes e com grande heterogeneidade na sua distribuição espacial, para ambas as regiões em estudo. Na região do canhão da Nazaré foi possível o registo de estações costeiras com máximos de peridinina (associados à presença de dinoflagelados) e outras, também junto à costa, com máximos de fucoxantina (pigmento principal das diatomáceas). Em estações menos costeiras, principalmente a norte do canhão, a 19'-hexanoyloxyfucoxantina (pigmento exclusivo das primnesiófitas) foi um dos pigmentos dominantes. Nas estações mais oceânicas, onde se registaram baixas concentrações de todos os pigmentos, a zeaxantina (indicador de cianobactérias) foi um pigmento preponderante.

Na região da Península Antártica a fucoxantina surgiu como o pigmento dominante nas regiões onde se registaram os valores de biomassa mais elevados (Estreito de Bransfield e junto à ilha James Ross), enquanto nas regiões da Passagem de Drake e do mar de Weddell a sua dominância foi substituída por outros pigmentos como a 19'-hexanoyloxyfucoxantina e/ou a aloxantina (exclusivo de criptófitas). Além desta diferenciada distribuição espacial na região da Península Antártica, os três anos consecutivos de amostragem no Estreito de Bransfield possibilitaram a observação de uma mudança temporal na dominância dos principais pigmentos onde a fucoxantina, dominante nos dois primeiros anos (2008 e 2009), foi substituída por uma dominância de aloxantina na maioria das estações amostradas em 2010. Estas variações, espaciais e temporais, na distribuição dos principais pigmentos fitoplanctónicos são um reflexo das adaptações das comunidades do fitoplâncton às modificações físico-químicas registadas nos ecossistemas em estudo.

Com a aplicação e optimização do CHEMTAX foi possível fazer uma quantificação, com base nos dados de pigmentos, da contribuição dos principais grupos taxonómicos para o total de clorofila *a*. Como complemento, o uso das análises microscópicas permitiu uma maior resolução taxonómica (ao nível da espécie ou gênero), que por sua vez foi importante na determinação de espécies-chave, quer sob o ponto de vista de contribuição para a biomassa quer sob o ponto de vista ecológico. Por outro lado, a utilização de índices pigmentares permitiu avaliar alguns processos fisiológicos das comunidades de fitoplâncton em resposta a determinadas condições ambientais (disponibilidade de ferro, luz e/ou herbivoria). Esta informação serviu para uma melhor

compreensão dos complexos processos responsáveis pela dinâmica do fitoplâncton ao redor da Península Antártica (ver capítulo III para maiores pormenores).

As Figuras 1 e 2 representam, esquematicamente, os processos responsáveis pela distribuição das comunidades de fitoplâncton nos dois ecossistemas em estudo – costa de Portugal e Península Antártica, respectivamente –, cumprindo assim os objetivos propostos inicialmente.

Na região da costa de Portugal, canhão da Nazaré (Fig. 1), verificou-se uma clara resposta das comunidades do fitoplâncton ao aumento de nutrientes resultante da condição de afloramento, proporcionado por um regime de ventos favorável.

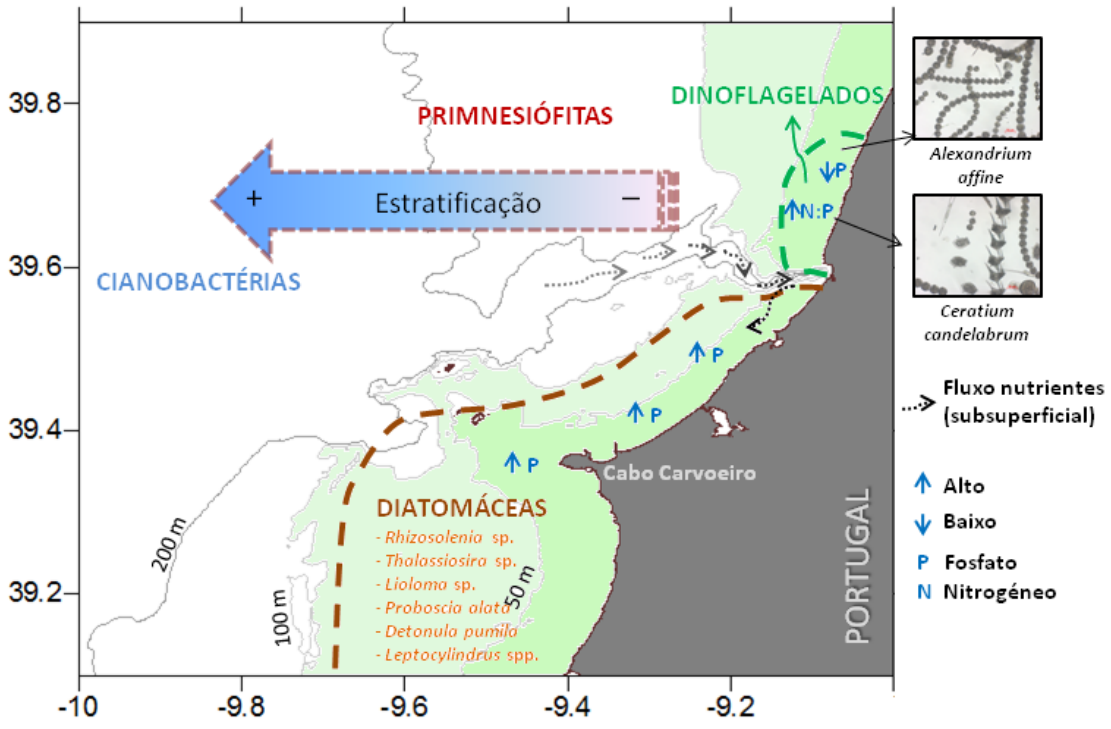

Figura 1: Esquema conceitual dos processos físico-químicos responsáveis pela distribuição das comunidades de fitoplâncton na região do Canhão da Nazaré, costa de Portugal. As zonas em verde correspondem às regiões com maiores concentrações de clorofila *a*. O tracejado castanho delimita a região de domínio das diatomáceas e o tracejado verde a região dominada pelos dinoflagelados.

Foram observados maiores valores de clorofila *a* junto à costa (regiões em verde na Fig. 1) associados a águas mais frias e ricas em nutrientes. No entanto, a presença do canhão submarino determinou um diferencial Norte-Sul na circulação dos nutrientes, que proporcionou uma injecção de maiores quantidades de nutrientes (em especial de fosfatos) para a região costeira a Sul do canhão, favorecendo o desenvolvimento de diatomáceas nesta área (ver Fig. 1). Os dinoflagelados, por sua vez, competindo com as diatomáceas por nutrientes e, provavelmente, mais bem adaptados a regimes com menores concentrações de fosfatos, estabeleceram-se numa região a norte da cabeceira do canhão, onde este nutriente apresentava níveis muito baixos. Esta região foi caracterizada por um forte florescimento de um dinoflagelado tóxico formador de cadeias longas – *Alexandrium affine* –, em conjunto com outros dinoflagelados como *Ceratium candelabrum*, *Ceratium furca*, *C*eratium *fusus*, *Dinophysis acuta* e *Dinophysis caudata*. O domínio de formas em cadeias facilita a permanência destes organismos em regiões de mistura da coluna de água costeira sob influência das águas frias provenientes do processo de afloramento. Nas regiões mais afastadas da costa, fora da região de afloramento, observou-se um aumento da estratificação, menores concentrações de nutrientes e um total domínio de primnesiófitas, com um incremento na contribuição das cianobactérias (para o total de clorofila *a*) nas estações mais oceânicas.

Os factores preponderantes na distribuição da biomassa e das comunidades de fitoplâncton ao redor da Península Antártica foram a disponibilidade em ferro, inferido neste trabalho através de um índice pigmentar (19'-hexanoyloxyfucoxantina:clorofila c_3), a estrutura da coluna de água e o

grau de degelo (também com influencia na estrutura da coluna) na época das amostragens. Os maiores valores de biomassa registaram-se nas estações mais costeiras e próximas à Península, que apresentaram uma coluna de água mais misturada e uma maior disponibilidade em ferro, e foram associadas ao predomínio de diatomáceas (Fig. 2). Em zonas mais oceânicas (mar do Weddell e Passagem de Drake) verificou-se um aumento da estratificação (principalmente condicionada por degelo), provavelmente restringindo os níveis de ferro na camada eufótica, limitando a biomassa e favorecendo os organismos nanoplanctónicos, como as criptófitas e/ou a *Phaeocystis antarctica* (Fig. 2).

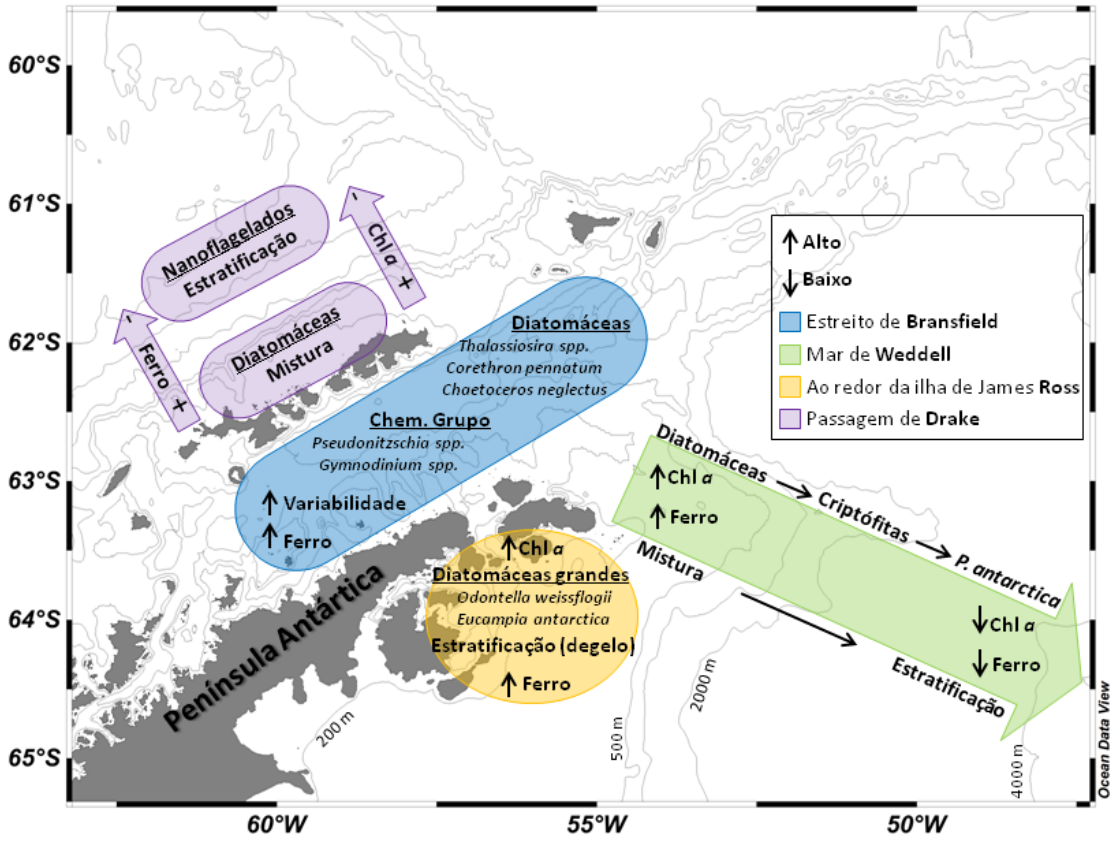

Figura 2: Esquema representativo da distribuição das comunidades de fitoplâncton na região da Península Antártica.

A região do Estreito de Bransfield apresentou uma grande variabilidade espacial e temporal na distribuição das comunidades do fitoplâncton devido, em parte, a uma influência repartida de águas provenientes do mar de Weddell e do mar de Bellingshausen. Por outro lado, foi possível associar os momentos do início do degelo marinho, para a região do Estreito de Bransfield, com as variações observadas nas comunidades de fitoplâncton no final do verão austral (período em que se realizaram as amostragens). Tal como já observado por diversos autores para outras regiões ao redor da Península Antártica (ver capítulo IV), uma alteração no início e/ou intensidade do degelo marinho causa um desfasamento (atraso ou antecipação) na natural sucessão fitoplanctónica da região. No ano de 2010, onde se verificaram temperaturas do ar atipicamente frias durante o verão austral, o início/intensidade do degelo (gatilho fundamental para o desenvolvimento dos primeiros florescimentos de diatomáceas) foi de tal forma atrasado que resultou em valores de biomassa bastante baixos durante todo o verão austral de 2010. Além disso, este processo resultou num domínio de criptófitas em detrimento de diatomáceas nesse mesmo ano, com presumíveis implicações para toda a cadeia trófica marinha da região. A grande variabilidade (espacial e temporal) dos factores ambientais, em estrita associação com as comunidades de fitoplâncton observada para a região da Península Antártica, suscita a necessidade de um processo de monitorização mais intenso, com escalas temporais e espaciais mais abrangentes, de forma a detectar os efeitos de possíveis alterações climáticas no ecossistema marinho desta importante região do Globo terrestre.

Em síntese, o presente trabalho produziu um conjunto de informação científica relevante para a melhor compreensão da dinâmica do fitoplâncton em dois ecossistemas de extrema importância ecológica, nomeadamente:

- Demonstrou a importância de estruturas geomorfológicas, como o canhão da Nazaré, na variabilidade espacial dos processos de afloramento costeiro e, consequentemente, na distribuição das comunidades de fitoplâncton;

- Associou o aparecimento de florescimentos de algas potencialmente tóxicas, em sistemas influenciados por processos de afloramento, com a dinâmica da distribuição dos nutrientes nas camadas superficiais da coluna de água;

- Testou e sugeriu o uso de alguns índices pigmentares na avaliação de determinados processos ecológico-fisiológicos envolvendo as comunidades de fitoplâncton na região da Península Antártica: indicadores de limitação por ferro e por luz, e taxas de herbivoria e/ou senescência celular;

- Evidenciou a disponibilidade de luz e/ou ferro, associados maioritariamente com a estrutura da coluna de água, como os factores determinantes na distribuição espacial das comunidades fitoplantónicas na região em redor da Península Antártica;

- Comprovou a forte influência do grau e tempo do degelo durante o verão austral (factor susceptível às alterações climáticas) sobre a biomassa e composição das comunidades do fitoplâncton em ecossistemas Antárticos.

“A criança que fui agora chora na estrada.
Deixei-a ali quando vim ser quem sou.
Mas hoje, vendo que o que sou é nada,
quero ir buscar quem fui onde ficou”.
(Fernando Pessoa)