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EFFECT OF THE LIGHT FIELD ON THE OPTICAL PROPERTIES OF PHYTOPLANKTON

by

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Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
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November, 1999

DALHOUSIE UNIVERSITY

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ABSTRACT

The pigment composition, absorption, and fluorescence excitation spectra of phytoplankton were analysed in two different environments of the North Atlantic: the Central North Atlantic, and the Labrador Sea. The distribution of different groups of algae could be explained, in great part, by photoadaptation (i.e., evolutionary adaptation of different species to have different pigment composition). A background of small cells was more-or-less uniformly distributed in the whole study area. Large cells contributed, on average, most of the total biomass.The coefficients of variation for absorption and fluorescence excitation spectra were of similar magnitude for the small and for the large size-fractions of phytoplankton. Both photoadaptation and photoacclimation (i.e., temporary changes in pigment concentrations in a given species) contributed to variations in the optical characteristics of phytoplankton. This was especially evident for the fluorescence excitation spectra, which varied not only with the concentration of photoprotective pigments (photoacclimation to high light), but also with the arrangement of the photosynthetic apparatus, which differed with the species present. Laboratory experiments were carried out with three species of phytoplankton grown at different light levels. In the diatom *Thalassiosira weissflogii* all types of pigments serve both photosystems, and as a consequence the shapes of the absorption and fluorescence excitation spectra were similar, except for the effect of photoprotective pigments at high light. In the cyanophyte *Synechococcus* sp., where different pigments served each individual photosystem, the shapes of the absorption and fluorescence spectra showed marked differences even at low light. The diatom *Chaetoceros* sp. represented an intermediate case where part of the difference between absorption and fluorescence excitation could be attributed to pigments associated exclusively to photosystem I. These results are presented in an ecological and physiological context.

LIST OF ABBREVIATIONS AND SYMBOLS

Notation	Quantity [Units]
A	Coefficient used in the quadratic equation to correct absorption on a filter for the pathlength amplification factor [dimensionless].
$a_{ca}(\lambda)$	Absorption coefficient of chlorophyll-*a* *in vivo* at wavelength λ [m^{-1}].
$a_{cc}(\lambda)$	Absorption coefficient of chlorophyll-*c* *in vivo* at wavelength λ [m^{-1}].
$a_i^*(\lambda)$	Specific absorption coefficient of pigment i at wavelength λ [$m^{-2}\,mg^{-1}$].
$a_{pp}(\lambda)$	Absorption coefficient of photoprotective pigments *in vivo* at wavelength λ [m^{-1}].
$a_{ps}(\lambda)$	Absorption coefficient of photosynthetic pigments *in vivo* at wavelength λ [m^{-1}].
$\alpha + \beta$	$\alpha + \beta$-carotenes.
$a_p(\lambda)$	Absorption coefficient of particles in suspension at wavelength λ [m^{-1}].
$a_{pf}(\lambda)$	Absorption coefficient of particles on a filter at wavelength λ [m^{-1}].
$a_{ph}(\lambda)$	Absorption coefficient of phytoplankton at wavelength λ [m^{-1}].
$a_{ph}^n(\lambda)$	Absorption coefficient of phytoplankton at wavelength λ normalised to 1 at 623 nm or at 545 nm (as indicated in the text) [dimensionless].
$a_{PP}(\lambda)$	Absorption coefficient of photoprotective pigments at wavelength λ [m^{-1}].
$a_{PP}^n(\lambda)$	Absorption coefficient of photoprotective pigments at wavelength λ normalised to 1 at 545 nm [dimensionless].
$a_r(\lambda)$	Absorption coefficient of phytoplankton at wavelength λ reconstructed from sum of absorptions by individual pigments [m^{-1}].
B	Coefficient used in the quadratic equation to correct absorption on a filter for the pathlength amplification factor [dimensionless].
β	Pathlength amplification factor [dimensionless].
bu	19-butanoyloxyfucoxanthin.
ca	Chlorophyll-*a*.

cb	Chlorophyll-*b*.
c_3	Chlorophyll-c_3.
cc	Chlorophylls-$c_1 + c_2$.
$C_s(\lambda)$	Ratio of the fluorescence excitation spectra of phytoplankton on a filter to that in suspension [dimensionless].
$C_x(\lambda)$	Spectrum of the excitation light of the spectrofluorometer normalised to 1 at 468 nm [dimensionless].
$D(\lambda)$	Optical density of particles at wavelength λ [dimensionless].
$D_d(\lambda)$	Optical density of detritus in suspension [dimensionless].
$D_{df}(\lambda)$	Optical density of detritus on a filter [dimensionless].
$D_p(\lambda)$	Optical density of particles in suspension [dimensionless].
$D_{pf}(\lambda)$	Optical density of particles on a filter [dimensionless].
$D_{ph}(\lambda)$	Optical density of phytoplankton on a filter [dimensionless].
DCM	Depth of the chlorophyll maximum [m^{-1}].
Δa^n_{ph}	Difference between the absorption spectrum of a phytoplankton species grown at HL and that of the same species grown at LL. both spectra normalised to 1 at 545 nm [dimensionless].
Δa^n_{pp}	Difference between the absorption spectrum of photoprotective pigments from a phytoplankton species grown at HL and that of the same species grown at LL. both spectra normalised to 1 at 545 nm [dimensionless].
Δf^n_c	Difference between the fluorescence excitation spectrum of a phytoplankton species grown at HL and that of the same species grown at LL. both spectra normalised to 1 at 545 nm [dimensionless].
d+t	Diadinoxanthin+diatoxanthin.
$f(\lambda)$	Fluorescence excitation spectrum. i.e. emission of phytoplankton at 730 nm. when excited at a wavelength λ [relative units].
$f^n(\lambda)$	Fluorescence excitation spectrum of phytoplankton corrected by $C_x(\lambda)$. smoothed and normalised to 1 at 545 nm [dimensionless].
$f_c(\lambda)$	Fluorescence excitation spectrum of phytoplankton corrected for distortions produced by the incident light ($C_x(\lambda)$). and for differences between

	fluorescence on a filter and that in suspension ($C_s(\lambda)$) [relative units].
$f_c^n(\lambda)$	Corrected fluorescence excitation spectrum of phytoplankton normalised to 1 at 600 nm or at 545 nm (as indicated in the text) [dimensionless].
$f_{sm}(\lambda)$	Fluorescence excitation spectrum of phytoplankton corrected by $C_x(\lambda)$ and smoothed by running average every 5 nm [relative units].
$f_{xc}(\lambda)$	Fluorescence excitation spectrum corrected for distorsions produced by the incident light ($C_x(\lambda)$) [relative units].
fu	Fucoxanthin.
G	Phytoplankton fraction greater than 2 μm in size.
he	19-hexanoyloxyfucoxanthin.
HL	Highest light level used to grow a phytoplankton species.
I_0	Irradiance at the surface [μmol quanta $m^{-2} s^{-1}$].
I_z	Irradiance at depth z. here defined as percentage of I_0 [dimensionless].
L	Phytoplankton fraction less than 2 μm in size.
LHCI	Light harvesting complex I.
LHCII	Light harvesting complex II.
LHCs	Light harvesting complexes.
LL	Lowest light level used to grow a phytoplankton species.
NO_3^-	Nitrate concentration [μM].
pe	Peridinin.
p_i	Concentration of pigment i [mg m^{-3}].
PP	Photoprotective pigments.
PS	Photosynthetic pigments.
PSI	Photosystem I.
PSII	Photosystem II.
PSU	Photosynthetic unit.
q	Exponential coefficient of the equation used to define the shape of the absorption spectrum of detritus [dimensionless].
RCI	Reaction center I.
RCII	Reaction center II.

RCs	Reaction centers.
σ_t	(specific gravity of sea-water - 1) x 1000 [dimensionless].
S(439)	Ratio of $a^n_{ph}(439)$ to $f^n_c(439)$, both normalised to 1 at 545 nm [dimensionless].
S(676)	Ratio of $a^n_{ph}(676)$ to $f^n_c(676)$, both normalised to 1 at 545 nm [dimensionless].
T	Total phytoplankton sample; i.e., all sizes.
va	Divinyl-chlorophyll-*a*.
vb	Divinyl-chlorophyll-*b*.
X	Volume of sea-water filtered divided by the area of the filter [m].
ze	Zeaxanthin.

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CHAPTER 1

General Introduction

In this thesis, I present the results of a study on the effect of the light field on the optical properties of phytoplankton. Variations in pigment composition, absorption and fluorescence excitation spectra are examined in natural samples collected in two contrasting environments in the North Atlantic (the Central North Atlantic and the Labrador Sea). I also present results of laboratory experiments carried out with different species of phytoplankton grown at different irradiances to elucidate in more detail some of the interpretations made from observations of the field study. In this introductory chapter, I provide some background material relevant to the study, and outline the rationale which led to the work presented here.

Initially I will examine why it is important to improve our understanding of the interactions between phytoplankton and their light environment.

Approximately half of the primary production of the earth occurs in the ocean (Longhurst *et al.*, 1995). Aquatic photosynthesis, thus, plays an important role in the global carbon cycle. The presence of unicellular algae in the ocean has drawn the attention of researchers since early times (reviewed in Mills, 1989), and over the years, considerable effort has been put into studying the geographical distribution of different species (Marshall and Cohn, 1983; Hulburt, 1983), the dynamics of species successions (Margalef, 1963*a*; *b*; 1978) and of seasonal bloom formation (Riley, 1942; Sverdrup, 1953), as well as the rates of production of phytoplankton communities (Talling, 1957; Ryther and Yentsch, 1957; Platt and Jassby, 1976). These ecological studies of phytoplankton communities have been complemented by a broad range of physiological studies, including those of adaptation of different species to specific environments and their acclimation to temporary changes in their environment (reviewed in Richardson *et al.*, 1983; Larkum and Barrett, 1983; Prézelin and Boczar, 1986; Falkowski and La Roche, 1991).

Many factors work together to determine the distribution and abundance of different groups of phytoplankton in the ocean, for example nutrient availability, the stability of the water column, the light field, and grazing pressure (Margalef, 1978; Kiørboe, 1993). Differences in the ability of different species to acclimate to their light environment may be an important evolutionary strategy (Glover *et al.*, 1985; Richardson *et al.*, 1983; Bidigare *et al.*, 1990*a*; Falkowski and LaRoche, 1991). There is also some evidence that a uniform concentration of small cells constitutes the major part of the phytoplankton population in the open ocean, which is masked by the presence of larger populations of bigger cells in other areas, such as coastal or frontal regions (Malone, 1980*a*; Yentsch and Spinrad, 1987; Yentsch, 1990).

Phytoplankton pigments, which are prime components of the photosynthetic system, are also active components of the light environment by virtue of their optical properties (absorption, scattering and fluorescence). In other words, the pigment composition and concentration of algal cells in the water can modify the quality and quantity of the underwater light field (Jerlov, 1976). Phytoplankton are subjected to a dynamic physical and chemical environment. Vertical mixing can affect some algal cells directly (e.g., via mechanical damage caused by excessive turbulence; Pollingher and Zemel, 1981; Thomas *et al.*, 1995). Vertical mixing also changes the light field to which the cells are exposed, and their distribution relative to that of nutrients by moving the cells vertically through the water column (Margalef, 1978; Cullen, 1982; Kiørboe, 1993). Several studies have focused on the effect of vertical mixing on the photoacclimation of phytoplankton (e.g., Falkowski, 1983; Lewis *et al.*, 1984*a*;*b*; Cullen and Lewis, 1988) and in turn the effect of this photoacclimation on primary production (e.g., Platt *et al.*, 1982; Fasham and Platt, 1983; Geider and Platt, 1986; Mitchell *et al.*, 1991).

Other authors have suggested that *in vivo* absorption and *in vivo* fluorescence excitation spectra can be used to discern, at least at a class level, the algal composition in the ocean (e.g., SooHoo *et al.*, 1986; Hoepffner and Sathyendranath, 1992; Johnsen *et al.*, 1994*a*; Yentsch and Yentsch, 1979; Poryvkina *et al.*, 1994). It has

also been suggested that both absorption (Morel, 1978; 1991; Kyewalyanga *et al.*, 1997) and fluorescence excitation spectra (Sakshaug *et al.*, 1991; Babin *et al.*, 1995; Sosik and Mitchell, 1995) can be used as proxies for the action spectrum of photosynthesis for modelling primary production in the ocean. Despite the huge effort that has been put into understanding and parameterising the effect of light conditions on the phytoplankton distribution and their photosynthetic capabilities, not all the questions are exhaustively or unequivocally answered. There is still a great deal to learn about how the light field influences species distribution in the ocean, and about physiological differences in the fluorescence characteristics of different species.

1.1 Background

1.1.1 Phytoplankton pigments

Of all the pigments present in phytoplankton cells, chlorophyll-*a* (or its substitute divinyl-chlorophyll-*a* in prochlorophytes) is the only 'true' photosynthetic pigment, i.e. it is an essential part of the reaction centers (RCs) of the photosynthetic units (PSUs) where light energy is finally converted into photochemical products. Therefore, chlorophyll-*a* is an ubiquitous pigment in algae, with the exception of prochlorophytes which contain the substitute divinyl-chlorophyll-*a*. A small portion, <0.5 %, of the chlorophyll-*a* is located in the RCs (Prézelin and Boczar, 1986), where it takes part in photochemistry. Algae, however, possess several accessory pigments, i.e., pigments not involved in photochemistry. There are three main types of accessory pigment: accessory chlorophylls (including most of the chlorophyll-*a* and divinyl-chlorophyll-*a*; chlorophylls *b*, c_1, c_2, c_3 and divinyl-chlorophyll-*b*); carotenoids (a large variety of carotenes and xanthophylls), and phycobilins (phycoerythrin, phycocyanin, allophycocyanin, phycoerythrocyanin; reviewed in Prézelin, 1981; Rowan, 1989). The main function of accessory pigments is to harvest light energy which is transferred to the RCs. A second function carried

out by specialised carotenoids is to protect the RCs from photodamage at excessively high irradiance (this subject is discussed in more detail in section 1.1.2). Each pigment has a particular absorption spectrum, which in turn is modified by interaction with the proteins to which it is bound within the PSUs.

Most of the accessory pigments are located in the light-harvesting complexes (LHCs), which along with the two photosystems, photosystem I (PSI) and photosystem II (PSII), make up the photosynthetic unit (PSU) of phytoplankton cells. Each taxonomic group of phytoplankton is characterised by its pigment composition, which confers on the alga its characteristic absorption spectrum.

1.1.2 Factors affecting the pigment composition and the *in vivo* absorption of phytoplankton

Light absorption by photosynthetic pigments is mostly limited to the visible range of the solar spectrum ($\sim$ 400 - 700 nm; although there is evidence that certain species have UV-absorbing compounds; Yentsch and Yentsch, 1982; Carreto *et al.*, 1989; Vernet *et al.*, 1989; Marchant *et al.*, 1991). This restriction to absorb in the visible may be because of the conditions under which photosynthesis first evolved on earth. The first photosynthetic organisms evolved in the ocean, about $2 - 3 \times 10^9$ years ago. At that time the ozone layer had not yet been formed, indeed its existence could only have followed after enough O_2 had been produced by photosynthesis (Larkum and Barrett, 1983; Gordon *et al.*, 1985). The ozone layer protects organisms from the harmful effects of UV-radiation (Karentz, 1989), but prior to its creation algae would have been forced to live at sufficient depth from the sea surface, so that absorption of UV-radiation by water (Smith and Baker, 1981) would act as a shield. Water also absorbs IR-radiation, however, leaving the window between the UV- and the IR-radiation (i.e., the visible range) available for the photosynthetic organisms (Yentsch, 1960; Larkum and Barrett, 1983; Gordon *et al.*, 1985). Even in this spectral window, however, not all the wavelengths were (or are) equally available, since the spectral composition of light under water changes with depth because of the absorption and scattering by the water itself and by the

inorganic and organic matter present in particulate or dissolved form (Kirk, 1994). Thus, algae evolved under conditions of variable spectral-light composition, and in response developed different pigments to enable them to capture light within the range of available wavelengths (Yentsch, 1960). The first proof that algae utilize different wavelengths of light with different efficiencies for photosynthesis was given by Engelmann (1883), who proposed the idea of chromatic adaptation. This theory was then used to explain the distribution of seaweeds in the littoral zones by Levring (1947; 1968) who suggested that, according to the 'complementary' pigment composition, green algae would be more adapted to live and photosynthesize on the upper eulittoral, brown algae more adapted to the lower eulittoral and upper sublittoral, and red algae to the deep sublittoral. In the case of phytoplankton, living in an environment where magnitude and spectral quality of light change simultaneously in a dynamic way, it is difficult to separate the effects of chromatic from intensity adaptation. Nevertheless, some studies have addressed the effect of chromatic adaptation in different populations of phytoplankton (Wood, 1985; Glover *et al.*, 1986; Takahashi *et al.*, 1989; Bidigare *et al.* 1990*a*).

Most of the studies dealing with light adaptation of phytoplankton have focused on the adaptation to changes in light intensity, usually called photoadaptation (reviewed in Richardson *et.al.*, 1983). Later on, Falkowski and La Roche (1991) proposed a distinction between: 'photoacclimation' referring to temporary changes in the intracellular concentration of pigments of a given species induced by changes in the light climate; and 'photoadaptation' referring to the genotypical capability of different species to adapt to different light environments by virtue of their pigment composition. Although these authors, as well as many others, focused their attention on the effect of light intensity alone, they do not rule out the simultaneous effect of spectral changes in the light composition.

Different processes of photoacclimation occur at different time scales. 'Short-term' photoacclimation induces rapid (time scale of minutes) physiological responses, which are manifested in changes in, e.g., *in vivo* fluorescence (Cullen and

Lewis, 1988; Ibelings *et al.*, 1994), and the proportion of carotenoids involved in the xanthophyll-cycle (Demers *et al.*, 1991). These rapid responses are beyond the scope of this study. 'Long-term' photoacclimation (time scale of hours to days) to low-light intensity causes an increase in the size of the PSUs, and an increase in the number of PSUs (reviewed in Prézelin, 1981 and Falkowski and La Roche, 1991). Both processes imply an increase in the intracellular concentration of pigments leading to an increased capacity to capture light. An increase in intracellular pigment concentrations results in an intensification of the 'packaging effect' or self-shading among molecules, which in turn produces a decrease in the specific absorption coefficient (absorption per unit pigment concentration), especially in large cells, (Duysens, 1956; Sathyendranath *et al.*, 1987; Berner *et al.*, 1989). Thus, a decrease in light induces an increase in pigment concentration, which increases cellular light absorption, but decreases intracellular light absorption per pigment molecule. In the case of photoacclimation to high-light intensity, the reverse processes occur, i.e., intracellular pigment concentrations decrease, both by dilution through growth and by actual degradation (Falkowski, 1984). At the same time, at high-light intensities, there is an increase in the concentration of 'photoprotective' carotenoids (Dubinsky *et al.*, 1986; Demers *et al.*, 1991). These photoprotective carotenoids produce a quenching of the absorbed radiation, that is, they dissipate the energy absorbed instead of transmitting it to the photosystems. A well-known case of the photoprotective function in carotenoids is that of the pigments involved in the xanthophyll-cycle. This cycle, first described in green plants (Hager, 1975; Demmig-Adams, 1990), is also present in chromophyte algae (algae containing chlorophyll-*c*). For chromophytes, it involves the conversion of diadinoxanthin into diatoxanthin through a fast ($<$ 1 hour) de-epoxidation reaction when algae are exposed to a high light intensity; the reverse reaction (epoxidation) occurs when algae are exposed to low-light intensity (Demers *et al.*, 1991; Olaizola and Yamamoto, 1994). Both carotenoids (diadinoxanthin and diatoxanthin) are inactive, that is, they do not transfer energy to the photosystems, but diatoxanthin is more efficient at preventing the formation

of triplet chlorophyll-*a* (and eventually singlet oxygen) than diadinoxanthin. When algae are continuously exposed to high light, on a longer time scale, the total intracellular concentrations of both, diadinoxanthin and diatoxanthin, as well as other photoprotective carotenoids (not involved in the xanthophyll-cycle; e.g., β-carotene, zeaxanthin, astaxanthin) increase (Prézelin, 1981; Dubinsky *et al.*, 1986; Kana *et al.*, 1988; Partensky *et al.*, 1993; Bidigare *et al.*, 1993; Moore *et al.*, 1995).

Changes in pigment concentrations due to photoacclimation will result in changes in the absorption spectrum of the cell (Neori *et al.*, 1984; SooHoo *et al.*, 1986; Sakshaug *et al.*, 1991; Johnsen and Sakshaug, 1993), because of changes in the relative proportion of different pigments and because of the effect of intracellular packaging of pigments (discussed in the next paragraph). Changes in absorption spectra also occur with variations in phytoplankton species (since each has its specific suite of pigments). Photoacclimation and species composition together result in much of the variation observed in the absorption spectra of phytoplankton in different regions and at different depths in the ocean (e.g., Prieur and Sathyendranath, 1981; Mitchell and Kiefer, 1988*b*; Yentsch and Phinney, 1989; Hoepffner and Sathyendranath, 1992).

The *in vivo* absorption spectrum of phytoplankton is not exclusively determined by their pigment composition however, but also by other factors that affect the optical characteristics of the cells. For example, the efficiency of light absorption by any compound inside a particle is lower than the efficiency of absorption of the same material dispersed in solution (Duysens, 1956; Morel and Bricaud, 1981; Kirk, 1994). This is called the "packaging effect" and it increases as a function of the cell-size and the intracellular concentration of pigments (Morel and Bricaud, 1981; Sathyendranath *et al.*, 1987). The degree of packaging is linked to the morphological arrangement of the PSUs inside the cell (i.e., the number and arrangement of photosystems and LHCs in the thylakoids, the number and arrangement of thylakoids in the chloroplasts, and the number and arrangement of chloroplasts in the cell), which can in turn be affected by physiological factors such as nutrient stress and

photoacclimation (Berner *et al.*, 1989; Dubinsky, 1992; Sosik and Mitchell, 1991; and as reviewed in Geider and Osborne, 1992). In addition, the optical characteristics of phytoplankton may also be influenced by: 1) the presence of chemical components other than pigments, and the way in which they are arranged inside the cell (e.g., presence of vacuoles; Berner *et al.*, 1989; Stephens, 1995); 2) the structure and composition of the cell-wall (e.g., calcium carbonate in coccolithophorids; Ackelson *et al.*, 1988; Gieskes, 1991); and 3) the shape of the cells and the presence of multicellular organisations (e.g., chain, cluster-like colonies; Gieskes, 1991).

1.1.3 *In vivo* fluorescence of phytoplankton

Not all of the light absorbed by the PSUs is used in photosynthesis: in fact, less than 18 % of the energy absorbed is used in the fixation of carbon; about 1 - 3 % is re-emitted as fluorescence; and the rest is dissipated as heat (Kirk, 1994). The absorption of a photon produces a change in the electronic state of a pigment molecule, which passes from the ground state to an excited higher-energy state. To return to its more stable ground-state the molecule has to release the extra energy. Most accessory pigments transfer energy to the subantennae of the photosystems, which finally transfer it to the reaction centers. In the case of PSII, if the energy is not transferred to the reaction centers within an appropriate time (of the order of 5 nanoseconds; Prézelin, 1981) it can be released as fluorescence. Part of this energy is dissipated in transitions through different substates at the excited electronic level, and the rest is then re-emitted as fluorescence (reviewed in Clayton, 1980; and Geider and Osborne, 1992).

The energy re-emitted is less than that absorbed, because of the loss of energy through the substate transitions. In fact, this transitional dissipation acts as a buffer, in the sense that the light re-emitted will always be of approximately the same energy, irrespective of the energy of the photon absorbed (i.e., its wavelength). The re-emitted light corresponds to the minimum energy of the excited state, which for chlorophyll-*a* has a spectrum of emission in the red ($\sim$ 650 - 800 nm) with a maximum at $\sim$ 680 nm and a secondary peak at $\sim$ 730 nm. That is to say, if

we record, in a spectrofluorometer, a number of emission spectra of chlorophyll-*a*, exciting the molecules with different wavelengths of incident light within the visible range, the shape of the emission spectrum will not change, but the intensity of the emitted fluorescence will vary, however, depending on the wavelength of the excitation light.

If we now record another spectrum fixing the emission monochromator to receive light at 680 nm, and allow the excitation monochromator to scan light from ~ 400 to 600 nm, we can see the efficiency with which photons of different wavelengths excite the chlorophyll-*a* molecules. This fluorescence 'excitation' spectrum will resemble the absorption spectrum of chlorophyll-*a*. Note that the fluorescence excitation spectrum can be also recorded fixing the emission wavelength at 730 nm (secondary peak of emission), having the advantages of being able to obtain the whole visible spectrum (400 - 700 nm; without running into a conflict between excitation and emission signals in the red region of the spectrum; Neori *et al.*, 1988), and also of minimising the problem of re-absorption of fluorescence (since absorption at 730 nm is extremely low; Hoftsraat *et al.*, 1992). Chlorophylls and phycobilins are able to fluoresce when isolated in pure solutions, carotenoids, on the other hand, are not fluorescent. In a living cell, however, all the pigments in the LHCs, regardless of their ability to fluoresce, will transfer the captured light energy to chlorophyll-*a* (Duysens, 1951; Butler, 1978). As a result, the *in vivo* fluorescence excitation spectrum of an alga (emission at 730 nm) represents the fluorescence of the chlorophyll-*a* excited not only by its own spectrum of absorption but also by the spectra of absorption of all the ('active') accessory pigments in the cell combined (Yentsch and Yentsch, 1979; Johnsen and Sakshaug, 1993). Hence, the *in vivo* fluorescence excitation spectrum of a cell should generally resemble its *in vivo* absorption spectrum.

In fact, however, there may be significant differences between the two spectra. First of all, PSI dissipates most of its excess of absorbed energy through non-photochemical processes; so that, the *in vivo* fluorescence excitation spectrum

of phytoplankton is largely (>95%) due to fluorescence of chlorophyll-*a* from PSII (Prézelin, 1981; Kiefer and Reynolds, 1992). Thus, the *in vivo* fluorescence excitation spectrum will differ more-or-less from the *in vivo* absorption spectrum according to the distribution of LHCs between the two photosystems. For example, if all types of LHC transfer energy to both photosystems then the shape of the fluorescence excitation spectrum will approximate the shape of the total *in vivo* absorption spectrum of the alga (as seen in e.g., dinoflagellates and diatoms; Neori *et al.*, 1986). On the other hand, if most of the chlorophyll-*a* of the cell is located in LHCs transferring energy to PSI, the fluorescence excitation spectrum of the alga will be biased towards the sum of absorption spectra of the accessory pigments of LHCs transferring energy to PSII alone (as seen in e.g., red algae, cryptophytes, cyanophytes; Neori *et al.*, 1986). Another source of variation between the *in vivo* fluorescence excitation and the *in vivo* absorption spectrum is caused by the presence of photoprotective carotenoids, which, as explained earlier, absorb light but do not transfer the energy to chlorophyll-*a* (Mitchell and Kiefer, 1988*b*; Nelson and Prézelin, 1990; Kirk, 1994; Johnsen and Sakshaug, 1993; Moore *et al.*, 1995). In this sense they are 'inactive' pigments.

1.2 Objectives

In summary, several factors (including photoadaptation, photoacclimation, and the composition of cell-sizes in the population) determine the variability in the optical properties of phytoplankton. Furthermore, when comparing the absorption and fluorescence properties of phytoplankton, it has to be recognised that the presence of photoprotective pigments, as well as the distribution of photosynthetic pigments between PSI and PSII can contribute to the observed differences between these two optical properties. If indeed it is true that there is a constant concentration of small cells in the ocean, and if photoacclimation has a negligible influence on optical properties in these cells, then we might postulate that it would be the variability in

the optical properties of the large phytoplankton cells that would lead to variations in these properties overall.

One of my main goals in this thesis was to improve our understanding of the roles of taxonomic composition and photoacclimation (as indicated by changes in pigment composition and packaging effect of a given species) in determining the variation in optical characteristics of phytoplankton in nature. A second goal is to understand how the composition of the PSU changes with species and growth conditions, and how this is related to differences between the absorption and fluorescence characteristics of phytoplankton.

The study area was the North Atlantic. The specific objectives of the study were as follows:

1- To examine the distribution of different pigments in the North Atlantic in relation to variations in the species composition of the phytoplankton communities and the photoadaptation of these groups to different light regimes, as well as the photoacclimation of the cells to different levels of irradiance.

2- To investigate the effect of the changes in species distribution and photoacclimation on the optical characteristics (absorption and fluorescence) of phytoplankton.

3- To study the contributions of different size-classes to the total phytoplankton biomass and overall optical properties.

4- To assess the role of photoprotective pigments in determining differences between the shapes of the absorption and fluorescence excitation spectra in natural samples of phytoplankton.

5- To investigate possible causes of differences between the shapes of the absorption and fluorescence excitation spectra in different species of phytoplankton cultured under different light levels.

In Chapter 2, an overview is presented of the pigment distribution and optical characteristics of phytoplankton in two contrasting environments of the North

Atlantic: the Central North Atlantic and the Labrador Sea. The strategies of photoadaptation of the most abundant phytoplankton groups in these areas, are discussed, along with the trends observed in photoacclimation with depth. Particular emphasis is placed on interpreting the contribution of the small-size fraction of phytoplankton to the total pigment concentration and the absorption and fluorescence spectra, as well as the variation observed in their spectral optical characteristics.

In Chapter 3, I focus on one particular aspect of the variability observed in the optical characteristics of phytoplankton in the North Atlantic: namely, the difference between the shapes of the absorption and fluorescence excitation spectra. Possible causes of differences between the two types of spectra in the natural environment, such as changes in the concentrations of photoprotective pigments (e.g., photoacclimation), differences in the distribution of pigments between photosystems (e.g., species composition) and variations in environmental variables (e.g., irradiance, nutrients, density) are examined.

In Chapter 4, I investigate further possible causes of differences between absorption and fluorescence excitation spectra using algal cultures. Three different species of phytoplankton, which are known to have different types of photosynthetic apparatuses, were grown at various levels of irradiance, which is known to induce changes in concentrations of photoprotective pigments. The species used in the laboratory studies were comparable to some of the major species encountered during the field study.

In Chapter 5, a general discussion of the ecological and physiological relevance of the results reported in the thesis is presented, and some final conclusions are made.

CHAPTER 2

Variability in Pigment Composition and Optical Characteristics of Phytoplankton in the Labrador Sea and the Central North Atlantic

2.1 Introduction

The wide diversity observed in the composition of phytoplankton communities in the ocean is the result of species-dependent differences in their ability to grow under different environmental conditions, such as nutrient availability, light field, stability of the water column, and grazing pressure (see Margalef, 1978; Kiørboe, 1993). Among these factors, the effect of light quality and quantity on phytoplankton growth has received special attention, since light is the driving force for photosynthesis. Phytoplankton possess taxon-specific suites of pigments arranged in pigment-protein complexes in their photosynthetic apparatuses (Porra *et al.*, 1997). Each one of these pigment-complexes absorbs in a particular region of the light spectrum; hence, according to its pigment composition, each phytoplankton species should have a characteristic absorption spectrum (see Prézelin, 1981; Porra *et al.*, 1997), which may be subject to some modification according to environmental conditions. Chromatic adaptation, that is, adaptation of a given species to particular spectral light conditions, has been reported to occur in the ocean (Glover *et al.*, 1986; Takahashi *et al.*, 1989, Bidigare *et al.*, 1990*a*). Algae are also able to change the amount of pigment per cell according to the intensity of irradiance to which they are exposed (reviewed in Richardson *et al.*, 1983; Falkowski and La Roche, 1991). In the pelagic environment, the magnitude and spectral quality of light change simultaneously, making it difficult to separate chromatic from intensity adaptation.

In this study I make a distinction between the terms photoadapatation and photoacclimation following Falkowski and La Roche (1991). The term photoadaptation will be used to refer to the evolutionary, genetic, adaptation of different species to have particular suites of pigments according to the light regimes in which they grow; hence, it involves adaptation to both light quality and intensity. The term photoacclimation, on the other hand, will be used to refer to the temporary (time scale comparable to a generation time) changes in the pigment composition of a given species in response to changes in light climate. Photoacclimation could also refer to fast (order of minutes) changes in the organisation of photosynthetic apparatus, for example state transitions (Bonaventura and Myers, 1969) and the xanthophyll cycle (Demers *et al.*, 1991), but processes on such short time scales are beyond the scope of this study.

Another factor affecting the absorption of light by phytoplankton, besides the pigment composition, is the packaging effect, that is, the reduction in absorption efficiency of a substance enclosed in a particle in comparison with the absorption efficiency of the same substance in solution (Duysens, 1956). In phytoplankton the packaging effect is a function of the diameter of the cell and the intracellular concentration of pigments (Morel and Bricaud, 1981; Sathyendranath*et al.*, 1987). It is now well known that the phytoplankton community in the subtropical open ocean is dominated by small cells (picoplankton; Johnson and Sieburth, 1979; Platt and Li, 1987; Chisholm *et al.*, 1988). It has also been suggested that this community of small-size phytoplankton is homogeneously distributed in the ocean forming a background on which superimposed larger cells on coastal and nutrient-rich waters (Malone, 1980*a*; Yentsch and Spinrad, 1987; Yentsch, 1990).

The optical characteristics of phytoplankton depend on both the species composition (including size-composition) and photoacclimation status of the cells. Hence, studies on pigment composition and optical characteristics of phytoplankton provide some insight as to why different groups of phytoplankton are distributed the way they are, and on their ability to adjust to a variable light environment. Information

about the characteristics of phytoplankton absorption is a necessary input to models of primary production (Morel, 1978; Sathyendranath and Platt, 1989; Kyewalyanga *et al.*, 1997). Algorithms used to retrieve phytoplankton biomass by remote sensing are based on the relationship between ocean-colour, which is strongly influenced by phytoplankton absorption, and chlorophyll-*a* concentration (Gordon and Morel, 1983; Sathyendranath and Platt, 1989; IOCCG, 1998). Therefore, knowledge of the optical characteristics of different groups of algae, and their photoacclimation status in the ocean, is useful for interpretation of ocean-colour data.

In this chapter, I present the results of a study of absorption and fluorescence characteristics of phytoplankton and their pigment composition, in two contrasting environments of the North Atlantic: the Central North Atlantic, and the Labrador Sea. The main objectives of this work are: 1) To examine if pigment composition of the phytoplankton varies according to the magnitude and spectral quality of the light fields to which they are exposed. 2) To study the average concentrations of different pigments relative to the concentration of total chlorophyll-*a* in the area, in order to enable the estimation of the relative contribution to chlorophyll-*a* of different algal groups over large spatial scales. 3) To assess changes in pigment composition with depth, resulting from photoacclimation. 4) To investigate relationships between changes in pigment composition (due to differences in species composition or as result of photoacclimation status) and optical characteristics of phytoplankton (absorption and fluorescence). 5) To study the contribution of small cells (<2 μm) to total biomass (as chlorophyll-*a*) and to total absorption and fluorescence at 440 nm (wavelength of maximum response to irradiance) in the different locations and on average for the whole area. 6) To investigate the spectral variability in the optical properties of the two size-classes (<2 μm and >2 μm). The aim of these last two objectives is to assess the homogeneity in the pigment composition and optical characteristics of the small-size fraction of phytoplankton.

2.2 Materials and Methods

2.2.1 Sampling

Samples were collected during two cruises: the first cruise, during spring of 1993, occupied stations near the coast of Morocco and along a transect from the Canary Islands to Nova Scotia; and the second, during the spring of 1994, occupied stations on the Newfoundland Shelf and along a transect from Greenland to Southern Labrador (Figure 2.1). During these cruises, samples for the determination of nutrient and pigment concentrations, *in vivo* absorption and *in vivo* fluorescence spectra were taken at 7 depths from 8-l Niskin bottles attached to a rosette sampler, except for some of the stations of the Canary cruise, where the samples were taken using a submersible pumping system. The sampling depths were selected based on the fluorescence profile provided by the *in situ* fluorometer. Temperature and salinity profiles were obtained from a Guildline CTD (Model 8705). Seawater (0.25 to 1.5 l) was filtered, at a pressure <35 kPa, through GF/F filters which were stored in liquid N_2 for later analysis of pigment content; another set of duplicate samples (0.25 to 1.5 l) was filtered, at a pressure <35 kPa, through GF/F filters and these were read immediately, first with the spectrofluorometer, and then with the spectrophotometer. All the procedures, including filtration and handling of samples during measurements, were carried out under dim light to avoid photodegradation of the pigments. Some artifacts from the filtration procedure, which can potentially cause degradation of pigments, and hence affect the optical characteristics of phytoplankton, have been reported (Stramski, 1990). The amount of degradation products in these field samples, however, were extremely low, which would indicate that no substantial pigment degradation occurred during filtration. Sea-water samples, taken in duplicate, from three depths (surface, the depth of the chlorophyll maximum, DCM, and one chosen depth below the DCM) were size-fractionated. That is to say, in addition to the "total" sample filtered through GF/F glass-fibre-filter, another sample was first filtered through a 2 μm Nuclepore filter and the

Figure 2.1. Map showing the location of the sampling stations. Asterisks indicate the stations where size-fractionation was performed.

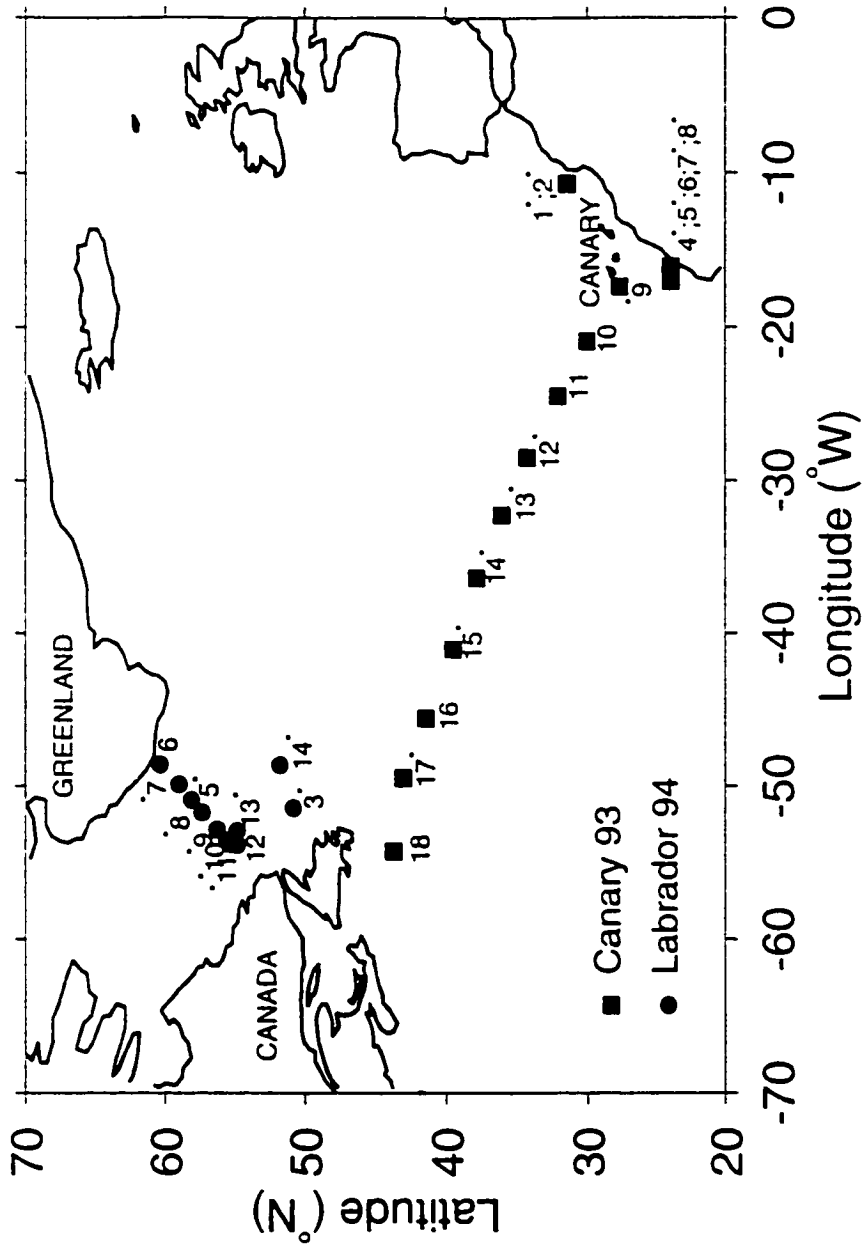
GREENLAND
CANADA
CANARY
Canary 93
Labrador 94
Latitude (°N)
Longitude (°W)
70
60
50
40
30
20
0
-10
-20
-30
-40
-50
-60
-70

Figure 2.1

filtrate was then filtered through a GF/F filter to represent the "small" (<2 μm) fraction. Samples of 250 - 500 ml of sea-water from these three selected depths were filtered through 0.6 μm Nuclepore filters for the determination of fluorescence emission by phycoerythrin.

2.2.2 Pigment determination

Pigments were analysed in the laboratory following the method described by Head and Horne (1993). Pigments identified and quantified by this method are the tetra-pyrroles: chlorophyll-*a*, chlorophyll-*b*, chlorophyll-c_1+c_2, chlorophyll-*c3*, chlorophyllide-*a*, several types of phaeophorbide-*a*-like pigments, phaeophytin-*a*, divinyl-phaeophytin-*a*, phaeophytin-*b*, and divinyl-phaeophytin-*b*; and the carotenoids: peridinin, 19-butanoyloxyfucoxanthin, fucoxanthin, 19-hexanoyloxyfucoxanthin, alloxanthin, diadinoxanthin, diatoxanthin, zeaxanthin, prasinoxanthin, α-carotene and β-carotene. This method is not able to separate zeaxanthin from lutein, i.e. they have the same retention time. Although there is evidence suggesting that zeaxanthin dominates over lutein in the ocean (Everitt *et al.*, 1990), the presence of zeaxanthin, when not supported by data on species composition, should be interpreted with caution. Divinyl-chlorophyll-*a* and divinyl-chlorophyll-*b* were identified by the presence of the corresponding divinyl-phaeophytins, after acidifying and running the samples a second time. The precision of the HPLC determination of pigments was checked by running triplicate samples of a culture of *Chaetoceros* sp.: the coefficients of variation were: 0.9 % for chlorophyll-*a*, 1.7 % for chlorophyll-$c_1 + c_2$, 1.8 % for fucoxanthin, 7.4 % for diadinoxanthin, and 11.3 % for β-carotene, which is found in very small concentrations.

The relative abundance of water-soluble phycoerythrin was determined on board following the method of Wyman (1992). Cells retained on a 0.6 μm Nuclepore filter were resuspended in 50% glycerol and after approximately 5 minutes the fluorescence emission at 570 nm (excitation light fixed at 520 nm) was measured on a SPEX-Fluorolog F111A spectrofluorometer.

2.2.3 Fluorescence excitation spectra

The *in vivo* fluorescence excitation measurements were made on board using a SPEX - Fluorolog F111A spectrofluorometer. This instrument was equipped with a red-sensitive photomultiplier Hamamatsu R-928. The blank consisted of a clean GF/F filter, through which a volume of prefiltered seawater comparable to that of the samples had been filtered; for some oligotrophic stations distilled water was used for the blank filter since prefiltered seawater showed a fluorescence signal larger than that observed at other stations (coming probably from small cells passing through the GF/F filters). Filters were wetted with filtered seawater before reading. The emission monochromator was set at 730 nm (Neori *et al.*, 1988; Hofstraat *et al.*, 1992), and the excitation spectra were recorded between 380 and 700 nm. The excitation and emission slits were set at either 2 nm or 5 nm bandpass, according to the intensity of the signal. The data were recorded in a computer connected to the spectrofluorometer. A technical problem, probably related to the highly scattering properties of the filters (GF/F), allowed stray light to enter the emission detector and distorted the signal in the red part of the spectrum. Therefore, only the blue-green part of the spectra, from 400 to 600 nm, was considered for the analysis.

The fluorescence excitation spectra ($f(\lambda)$) were later corrected for distortions produced by the spectrum of the source (xenon) lamp, and the geometrical configuration of the instrument, according to the method of Culver *et al.* (1994): the excitation light incident at the sample position was measured with a radiometer (Biospherical Instruments QSL-100 4π collector) every 2 nm, and this spectrum was in turn corrected for spectral variations in the efficiency of the radiometer (information provided in the specifications of the radiometer). The average of three such spectra, normalised to 1 at 468 nm (maximum emission of the xenon lamp), was then interpolated every 1 nm, and used as the excitation-correction-spectrum ($C_x(\lambda)$) for the measurements. The accuracy of this correction procedure was tested by comparing the fluorescence excitation spectrum of pure chlorophyll-*a* in 90%

acetone divided by $C_x(\lambda)$ with its corresponding absorption spectrum, after normalising both spectra to 1 at 664 nm. The two spectra agreed (Figure 2.2). Further testing using a culture of *Thalassiosira* sp. (isolated from a seawater sample from the Labrador Sea), showed slight but consistent differences in the shape between normalised spectra obtained in suspension and on the filter (results not shown). Therefore, the ratio of the spectra on the filter to that in suspension was used as a shape-correction-factor ($C_s(\lambda)$), to correct the spectra of the samples. Thus, the corrected fluorescence excitation spectra ($f_c(\lambda)$) were obtained as:

$$f_c(\lambda) = f(\lambda)/[C_x(\lambda)\, C_s(\lambda)].$$

No corrections were made for re-absorption of fluorescence (Mitchell and Kiefer, 1988*a*) since volumes of water filtered were adjusted according to the concentration of phytoplankton in the sample, to avoid high absorbances. Furthermore, fluorescence emission was measured at 730 nm, which minimises the problem of re-absorption of fluorescence (Hofstraat *et al.*, 1992).

The corrected fluorescence excitation spectra of phytoplankton were smoothed using a 5 nm running average. Finally the averages of the two replicate samples were computed and used for the analysis. The mean coefficient of variation for the duplicate values of $f_c(440)$ from 21 stations at the depth of the chlorophyll maximum was 17.9%±15.1: for the same samples the mean coefficient of variation for the duplicate values of $f_c(439)$ normalised to their corresponding values of $f_c(545)$ (later used in estimations of the shapes of absorption and fluorescence spectra) was 3.9%±2.9. More details on the process of the fluorescence measurements as well as the values of the correction factors ($C_x(\lambda)$ and $C_s(\lambda)$) are given in Appendix A.

2.2.4 Absorption spectra

The *in vivo* absorption spectra of total particulate matter were measured on GF/F glass-fiber filters (Yentsch, 1962; Kiefer and SooHoo, 1982; Mitchell and Kiefer, 1984; Kishino *et al.*, 1985) using a Beckman DU-64 spectrophotometer. Absorption was measured immediately after the fluorescence measurement. The

Figure 2.2. Comparison of the absorption and fluorescence excitation spectra of chlorophyll-*a* in 90% acetone. The two spectra are normalised to 1 at 664 nm.

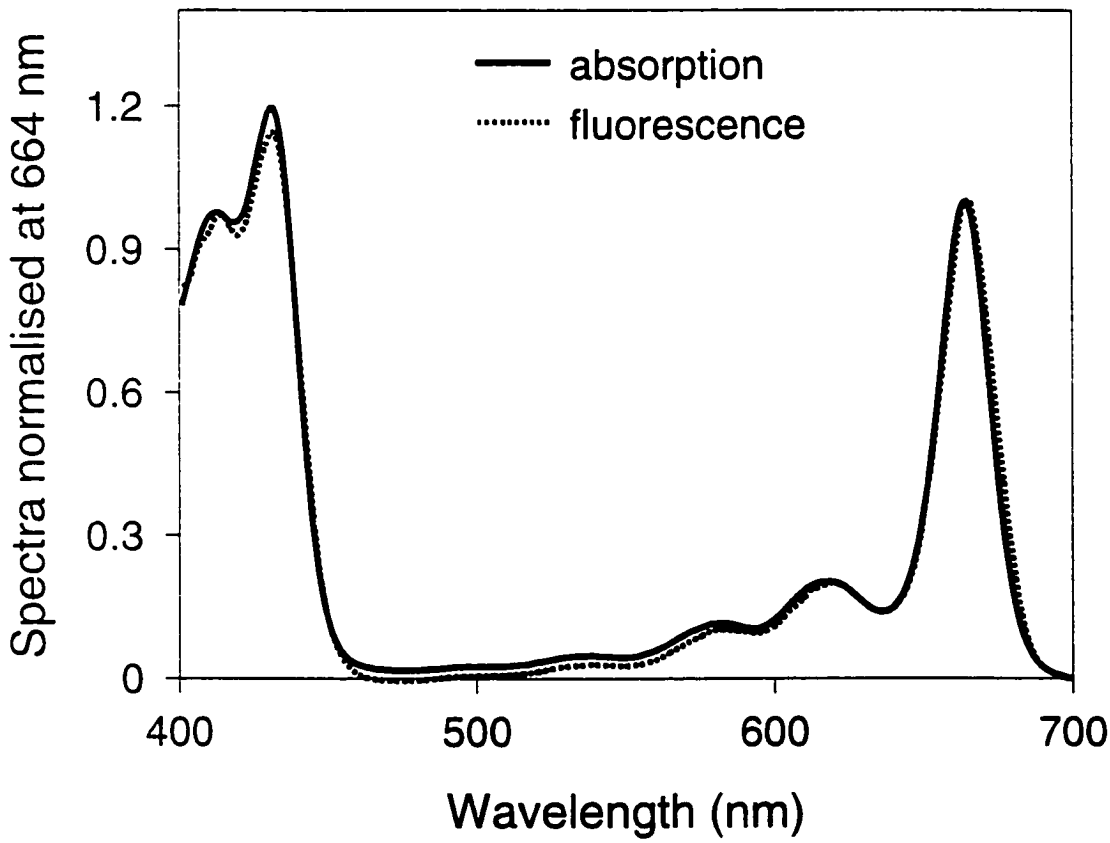
absorption
fluorescence
Spectra normalised at 664 nm
1.2
0.9
0.6
0.3
0
400
500
600
700
Wavelength (nm)

Figure 2.2

filters were placed in a special sample holder close to the photomultiplier to avoid loss of forward-scattered light (Mitchell and Kiefer, 1984). The filters were wetted with filtered seawater before reading, and the blank was the same as that used for the fluorescence measurements. The optical density or absorbance (D) was recorded between 350 and 750 nm. The value measured at 750 nm was subtracted from the rest of the spectrum, assuming that this optical density was due to non-pigmented substances and that its effect was spectrally neutral (Sathyendranath *et al.*, 1987; Bricaud and Stramski, 1990).

The values of absorption measured on filters were corrected for the pathlength amplification factor (Butler, 1962; Kiefer and SooHoo, 1982), which is defined as the ratio of the optical pathlength of light in a diffusing material to the geometric thickness of the material. To correct for the pathlength amplification the quadratic equation proposed by Hoepffner and Sathyendranath (1992) was used. This is very similar to the equation proposed by Mitchell (1990) and Cleveland and Weidemann (1993). In the samples where prochlorophytes were present, the coefficients of the quadratic equation were adjusted to those specifically calculated for prochlorophytes by Moore *et al.* (1995). That is, the portion of optical density corresponding to prochlorophytes was estimated from the ratio of divinyl-chlorophyll-*a* to total chlorophyll-*a*. This portion of the optical density was then corrected by the pathlength amplification factor using the coefficients given by Moore *et al.* (1995), the remaining portion of the optical density was corrected using the coefficients given by Hoepffner and Sathyendranath (1992).

Many studies have dealt with this subject (Mitchell and Kiefer, 1988*a*; Mitchell, 1990; Bricaud and Stramski, 1990; Hoepffner and Sathyendranath, 1992; Cleveland and Weidemann, 1993; Moore *et al.*, 1995; Tassan and Ferrari, 1995; Roesler, 1998), although, the issue of what is the best approach to estimate the optical density of particles in suspension from measurements on a filter is still controversial. An exhaustive analysis of the problem of the pathlength amplification factor is beyond the scope of this thesis. However, it is recognised that such an analysis is long overdue

since many studies of optical characteristics of phytoplankton rely on measurements of optical density made on filters. Recently Roesler (1998) has demonstrated, theoretically, that this amplification would increase the optical density of the particles on the filter by a factor of two. The demonstration was based on the assumption that the pathlength enhancement is solely due to the filter and does not depend on the type or on the amount of particles retained on the filter. Roesler (1998) attributed the variation observed in the pathlength amplification factor to errors associated with the measurements of optical density of phytoplankton in suspension, and to variability in the absorption spectrum of the glass-fiber filters. In this study an effort was made to treat the blanks in the same way as the samples, i.e.: approximately the same volume of filtered seawater (or distilled water in a few cases) was filtered through the blank as the volume of seawater filtered on the sample; both filters, blank and sample, were equally wetted, by placing them on a petri dish with a paper tissue saturated with filtered seawater, before measuring the optical density. This eliminates some of the sources of errors identified by Roesler (1998). On the other hand, the approach proposed by Roesler (1998) contradicts the findings of Tassan and Ferrari (1995), who reported that the scattering properties of particles retained on the filter can affect the value of optical density measured. Similarly, Moore *et al.* (1995) found significant differences in the pathlength amplification factor of different sizes of phytoplankton (especially in prochlorophytes, < 1 μm). The quadratic equation, used to estimate the optical density of phytoplankton in suspension from that on a filter, might produce, according to Roesler (1998), an underestimation of optical density at low values of optical density and an overestimation at high values of optical density. Admittedly, some errors in the absorption spectra of phytoplankton must be associated with the correction applied for the pathlength amplification factor in the present study. It is difficult at the moment to evaluate the extent of such errors.

The absorption of particles on the filter, $a_{pf}(\lambda)$, was obtained as: $a_{pf}(\lambda) = 2.3\,D(\lambda)$, where 2.3 is the conversion factor for transforming decimal logarithms to natural logarithms.

Corrections were made for detrital absorption using the theoretical approach proposed by Hoepffner and Sathyendranath (1993), which assumes an exponential shape for detrital absorption (Roesler *et al.*, 1989; Bricaud and Stramski, 1990). This method of parameterising detrital absorption (Hoepffner and Sathyendranath, 1993) has the advantage of not being affected by absorption of pigments difficult to extract from the filtered sample (e.g., phaeopigments and phycobilins).

Finally, the absorption spectra of phytoplankton, $a_{ph}(\lambda)$, were obtained and the average of the two replicate samples was computed and used in the analysis. The mean coefficient of variation for the duplicate values of $a_{ph}(440)$ from 21 stations at the depth of the chlorophyll maximum was 6.9%±7.3; for the same samples the mean coefficient of variation for the duplicate values of $a_{ph}(439)$ normalised to their corresponding values of $a_{ph}(545)$ (later used in estimations of the shapes of absorption and fluorescence spectra) was 2.9%±2.2.

The proportion of $a_{ph}(\lambda)$ due solely to photoprotective pigments (PP; see section 2.3.2) was estimated using the reconstruction method described by Bidigare *et al.* (1990*b*) as modified by Babin *et al.* (1996). This method is based on using a known shape for the specific absorption spectrum of PP and scaling its magnitude according to the concentration of PP in the sample (Bidigare *et al.*, 1990*b*). This PP absorption, $a_{PP}(\lambda)$, is then divided by the total phytoplankton spectrum reconstructed using the specific coefficients and concentrations of all pigments present in the sample, and multiplied by the total measured phytoplankton spectrum (Babin *et al.*, 1996; see section 4.2.5). The percentage contribution by PP to the total measured phytoplankton absorption at 440 nm was estimated as $\%a_{PP}(440)=100{*}a_{PP}(440)/a_{ph}(440)$. More details on the process of the absorption measurements, including all the equations used to convert raw values of optical

density to absorption of phytoplankton (e.g., corrections for the pathlength amplification factor and detrital absorption) are given in Appendix A.

2.2.5 Other measurements

PAR (photosynthetically available radiation, 400 - 700 nm) was measured on deck with a radiometer LI-COR LI-190 with a 4 π collector. CTD (conductivity, temperature, density) data were obtained using a Guildline CTD (Model 8705). Nitrate concentrations were measured using an Alpkem RFA autoanalizer. Picoplankton composition was determined by flow cytometric analysis following the method described in Li (1994). PAR and CTD data were kindly provided by E. Horne, nutrients by J. Anning, and flow cytometric data by W.K.W. Li.

2.3 Results

2.3.1 Distribution of phytoplankton biomass in relation to the hydrographic characteristics of the area

To get a general understanding of the environmental differences in the two areas of study, I analysed the main features of the distribution of phytoplankton biomass (as chlorophyll-*a* concentration) in the context of the hydrographic features.

Canary cruise: Some information on pigment and main hydrographic characteristics of the Canary cruise has been reported (Olaizola *et al.*, 1996; Waser *et al.*, in press). The data, necessary for the interpretation of the results, are also shown here. The concentration of chlorophyll-*a* (Figure 2.3), was low (<0.2 mg m^{-3}) on the east side of the transect (close to Canary Islands) and much higher in the west (The Grand Banks of Newfoundland). The depth of the chlorophyll maximum (DCM) was greater on the east side ($\sim$ 80 - 100 m) and shallower towards the west ($\sim$ 20 - 30 m). This distribution of phytoplankton abundance followed the distribution of nitrate concentration in the 0 to 100 m depth range (Figure 2.4), which showed extremely low values (<0.02 μM) at the surface in the warm subtropical waters (Figure 2.4) and a deep nitracline which showed increasing concentrations at shallower depth towards the west.

Figure 2.3. Vertical distribution of main phytoplankton pigments (mg m^{-3}) found during the Canary transect.

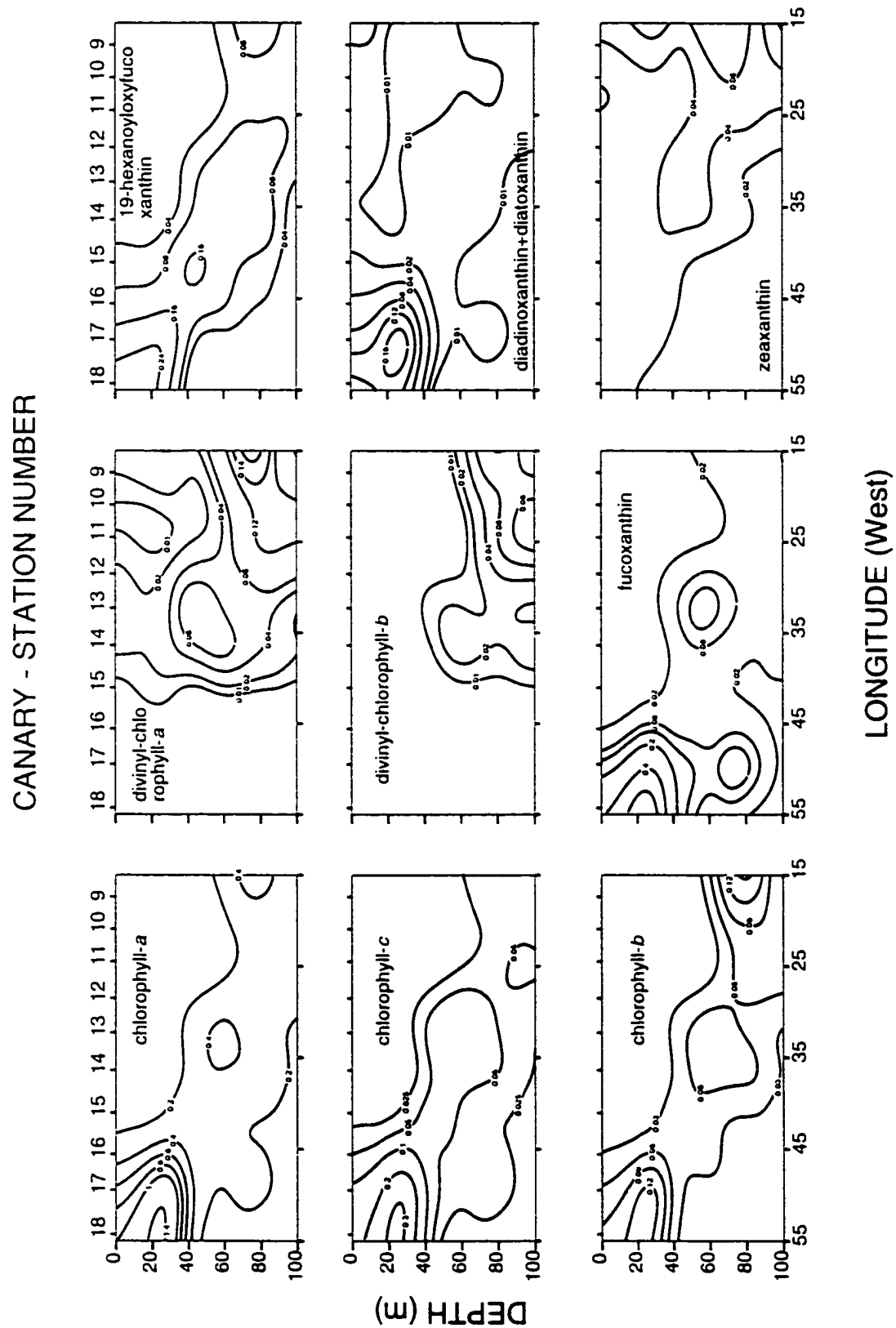
CANARY - STATION NUMBER
DEPTH (m)
LONGITUDE (West)
chlorophyll-a
divinyl-chlorophyll-a
19-hexanoyloxyfuco xanthin
chlorophyll-c
divinyl-chlorophyll-b
diadinoxanthin+diatoxanthin
chlorophyll-b
fucoxanthin
zeaxanthin

Figure 2.3

Figure 2.4. Vertical distribution of nitrate concentration (NO_3^-) in μM, temperature (Temp.) in °C. and density as σ_t. for the Canary and Labrador transects.

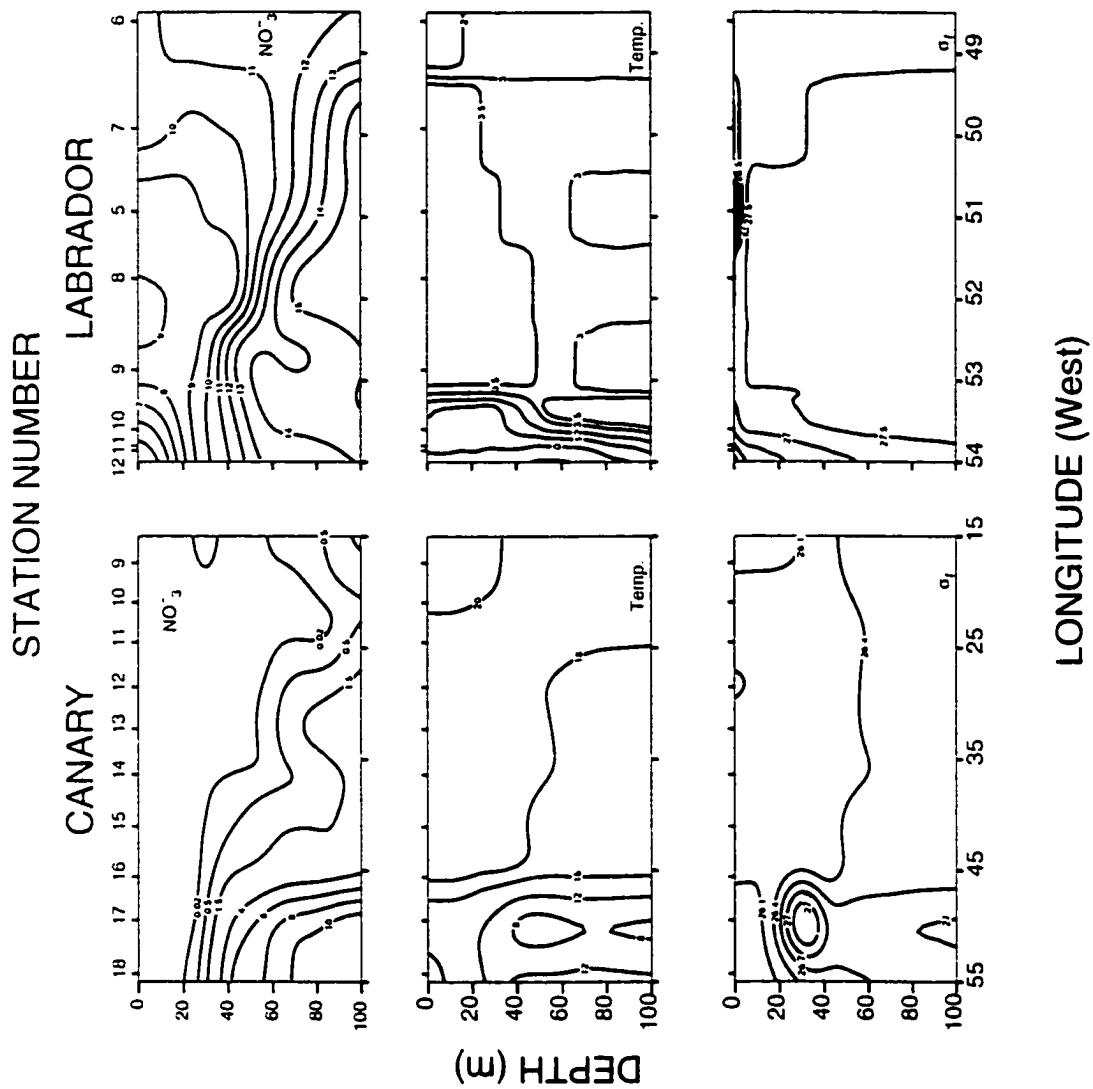
STATION NUMBER
CANARY
LABRADOR
NO_3^-
Temp.
σ_t
DEPTH (m)
LONGITUDE (West)

Figure 2.4

Labrador cruise: Chlorophyll-*a* concentrations were higher in the top ~40 m of the water column, and reached maximum values close to the coasts of Labrador (> 4 mg m^{-3}) and Greenland (> 0.8 mg m^{-3}; Figure 2.5). Nitrate concentrations throughout the 0 to 100 m depth range were high for the whole section (>5 μM; Figure 2.4). The hydrography of the Labrador Sea is complex (Lazier and Wright, 1993; Reynauld *et al.*, 1995), and it has been reported that bloom formation in this area does not depend entirely on stratification, but also on the bathymetry and the dynamics of the ice melt (Head *et al.*, *in press*). The temperature section showed the lowest values, uniformly distributed with depth, close to the two coastal areas, and a thermocline at ~ 20 - 40 m in the centre of the transect. The stability of the water column was high in the upper few meters along the transect, as shown in the σ_t profile, due to the melt of ice in the two coastal areas and subsequent advection into the central region.

2.3.2 Distribution of main pigments

The pigment composition provides an overview of the distribution of the main taxonomic groups along the transects. In this work, what is referred to as chlorophyll-*a* is the sum of chlorophyll-*a* and divinyl-chlorophyll-*a*; similarly chlorophyll-*b* is the sum of chlorophyll-*b* and divinyl-chlorophyll-*b*. A detailed description of pigments present in different taxonomic groups can be found in the literature (Rowan, 1989; Jeffrey and Vesk, 1997). The main phytoplankton pigments (besides chlorophyll-*a*), which could be used to infer algal composition in these samples are listed in Table 2.1. Carotenoids can be divided into photosynthetic (PS) and photoprotective (PP) types (see Siefermann-Harms, 1987). In these samples the most commonly found photosynthetic carotenoids were: fucoxanthin, peridinin, 19-hexanoyloxyfucoxanthin, and 19-butanoyloxyfucoxanthin, and the most common photoprotective carotenoids were: zeaxanthin, diadinoxanthin, diatoxanthin, α-carotene and β-carotene. The concentrations of diadinoxanthin and diatoxanthin are reported as the sum (diadinoxanthin+diatoxanthin) since the time of sampling was longer than that of the xanthophyll cycle (Demmig-Adams,

Figure 2.5. Vertical distribution of main phytoplankton pigments (mg m^{-3}) found during the Labrador transect.

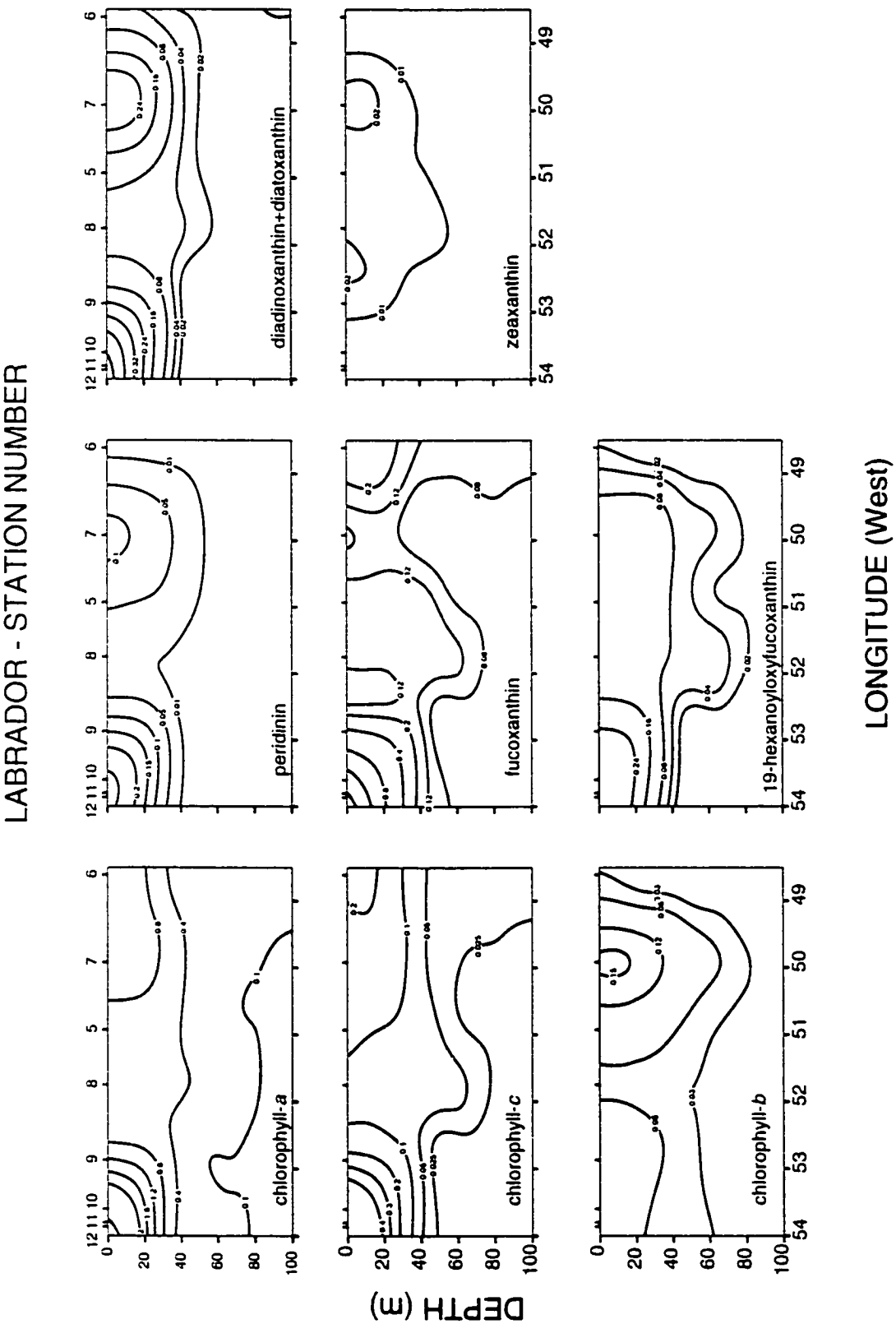
LABRADOR - STATION NUMBER
LONGITUDE (West)
DEPTH (m)
chlorophyll-a
peridinin
diadinoxanthin+diatoxanthin
chlorophyll-c
fucoxanthin
zeaxanthin
chlorophyll-b
19-hexanoyloxyfucoxanthin

Figure 2.5

Table 2.1 Main pigments found in the samples which could be used to identify main phytoplankton groups present in the two cruises (from Rowan, 1983; Jeffrey and Vesk, 1997).

Pigments	Phytoplankton groups					
	diatoms	dinoflagellates	prymnesiophytes	chlorophytes	prochlorophytes	cyanophytes
chlorophyll-*c*	+	+	+			
chlorophyll-*c*3			+			
chlorophyll-*b*				+	+	
divinyl-chlorophyll-*a*					+	
divinyl-chlorophyll-*b*					+	
fucoxanthin	+		+			
19-hexanoyloxyfucoxanthin			+			
19-butanoyloxyfucoxanthin			+			
peridinin		+				
diadinoxanthin	+	+	+			
diatoxanthin	+	+	+			
zeaxanthin*				+	+	+
phycobilins						+

* zeaxanthin here represents the sum of zeaxanthin+lutein (since they elute at the same time in the method used).

1990; Demers *et al.*, 1991; Olaizola and Yamamoto, 1994), which rapidly (< 1 hour) converts one of these pigments into the other according to the intensity of irradiance. The amounts of α- and β-carotenes are also reported together as the sum (α+β-carotenes), since these pigments were found in very low concentrations. The distributions of the most abundant pigments are shown in Figures 2.3 and 2.5.

Canary: Chlorophyll-*c* followed the pattern of distribution of chlorophyll-*a*. Chlorophyll-*b* distribution was also similar to that of chlorophyll-*a*, but it showed a more conspicuous deep maximum on the east side of the transect (Figure 2.3). Divinyl-chlorophylls-*a* and -*b* were restricted to warm subtropical waters with concentrations increasing with increase in depth. Divinyl-chlorophyll-*b* was only detected below $\sim$ 40 m. The distribution of fucoxanthin was similar to that of chlorophyll-*a*, whereas 19-hexanoyloxyfucoxanthin was more widespread. Peridinin (distribution not shown for Canary cruise) was only detected at one location (station 17) on the Newfoundland Shelf. The diadinoxanthin+diatoxanthin combination had its highest concentrations close to the surface, with a maximum in the west. Zeaxanthin showed high concentrations in the east decreasing towards the west side of the transect.

Labrador: The distribution of chlorophyll-*c* followed that of chlorophyll-*a*, while chlorophyll-*b* showed a marked maximum at station 7 extending to the centre of the transect (Figure 2.5). Divinyl-chlorophylls were not detected at any of the stations in this transect. Fucoxanthin distribution was similar to chlorophyll-*a*, and 19-hexanoyloxyfucoxanthin was again, as in the Canary cruise, widely spread along the transect. Peridinin occurred in the top $\sim$ 40 m of the water column along the transect, with maximum concentrations at the surface near the Labrador shelf and a secondary maximum at the surface at station 7. The distribution of diadinoxanthin+diatoxanthin followed that of chlorophyll-*a*, but showed a maximum at station 7. Zeaxanthin was found at low levels (< 0.02 mg m^{-3}) close to the surface, in the central section of the Labrador Sea.

2.3.3 Distribution of main phytoplankton groups at the DCM

The predominant algal groups at the depth of the chlorophyll maximum, DCM, (Table 2.2; see stations marked with an asterisk on Figure 2.1) were inferred from the ratios of the main pigments to total chlorophyll-*a* (data not shown). This information was later used to interpret changes in optical characteristics of phytoplankton in the different size-fractions at the DCM. A more detailed study of the picoplankton distribution at stations from the Canary cruise using flow cytometer data was reported by Li (1995). Here flow cytometer data were used as auxiliary information to confirm the presence of cyanophytes.

Note that in Table 2.2 (and subsequently in figures reporting data on size-fractionation) stations are ordered following their approximate biogeochemical location (Longhurst, 1998). Thus, for the Canary cruise: stations 8 and 7 were in South Morocco Coastal waters; stations 5, 4, 1, 2, 9, 12, 13, 14, and 15 are typical of the Subtropical Gyres (East and West); and station 17 corresponds to The Grand Banks of Newfoundland. On the Labrador cruise: stations 14 and 3 were on the Newfoundland Shelf; stations 13, 11, and 10 were located on the Labrador Shelf; and stations 9, 8, 5, and 7 were in the Central Labrador Sea (or Polar domain; Longhurst, 1998).

During the Canary cruise the phytoplankton populations at stations 1, 2, 4, 5, 7, 9, 12, 13 and 14 were composed of a mixture, in different proportions, of: prochlorophytes, cyanophytes, prymnesiophytes and probably chlorophytes and diatoms. At stations 4, 5, 7, and 9 cyanophytes seemed to be the dominant group. At stations 1 and 2 prochlorophytes were dominant, and at stations 13 and 14 prymnesiophytes dominated the phytoplankton community. Station 15 showed a mixture of prymnesiophytes, cyanophytes and probably diatoms, and prymnesiophytes were the dominant group. Station 8 (off Morocco) was dominated by diatoms and prymnesiophytes, and station 17 (on the Newfoundland Shelf) was dominated by diatoms, dinoflagellates and prymnesiophytes.

Table 2.2 Main phytoplankton groups found in the whole sample at the DCM at selected stations from the two cruises (see Fig. 1). Different groups were identified by their characteristic pigment composition determined by HPLC, and by flow cytometry in the particular case of cyanophytes.

Phyto. group	Canary stations											
	8	7	5	4	1	2	9	12	13	14	15	17
cyanophytes		++	++	++	+	+	++	+	+	+	+	
prochlorophytes		+	+	+	++	++	+	+	+	+		
chlorophytes*		+?	+?	+?	+?	+?	+?	+?	+?	+?		
prymnesiophytes	++	+	+	+	+	+	+	+	++	++	++	++
diatoms	++?	+?	+?	+?	+?	+?	+?	+?	+?	+?	+?	++?
dinoflagelates												++

Phyto. group	Labrador stations								
	14	3	13	11	10	9	8	5	7
cyanophytes	+ L	+L	+L	+L	+L	+L	+L	+L	+L
prochlorophytes									
chlorophytes								++	++
prymnesiophytes	+	+	+	+	+	++	++	++	++
diatoms	++?	++?	++?	+?	++?	+?	+?	+?	+?
dinoflagelates			+	+	+	+		+	+

The code used in the table are as follows:

++ : abundant +? : probably present, but lacks pigments exclusive to particular algal types

\+ : present ++? : probably present in abundance, but lacks pigments exclusive to particular algal types

+L : present in low concentrations

* Note that chlorophytes cannot be distinguished from prochlorophytes when both groups are present in the sample.

In the Labrador Sea, all stations showed a mixture of diatoms, prymnesiophytes and chlorophytes, plus dinoflagellates at stations 5, 7, 9, 10, 11, and 13. Flow cytometry and phycoerythrin fluorescence also indicated that cyanophytes were present, although in only minor concentrations in comparison with the values found at subtropical stations during the Canary cruise. Stations in the central Labrador Sea (5, 7, 8 and 9) seemed to be dominated by prymnesiophytes and also chlorophytes (stations 5 and 7), while stations located on the Labrador and Newfoundland shelves (3, 10, 11, 13, 14) seemed to have been dominated by diatoms, although it cannot be discounted that some of the fucoxanthin might have been from prymnesiophytes (Stuart *et al.* submitted.).

2.3.4 Abundance of main pigments relative to chlorophyll-*a* at the surface and at the chlorophyll maximum

To assess the contribution of different phytoplankton groups to the total chlorophyll-*a* in the area, I calculated the average values of the concentrations of different pigments relative to chlorophyll-*a*, and the percentage of occurrence of these pigments in the samples (stations marked with an asterisk on Figure 2.1). To examine the effect of photoacclimation with depth, ratios were calculated separately for two depths: the surface, and the depth of the chlorophyll maximum, DCM (Figure 2.6).

Chlorophyll-*c* was more abundant and widely spread (100 % occurrence) both at the surface and at the DCM than chlorophyll-*b*, and the ratio of both these pigments to chlorophyll-*a* was higher at the DCM than at the surface. Divinyl-chlorophyll-*a* was present in $\sim$ 28 and 38 % of the samples (at the surface and at the DCM respectively) and was slightly more abundant at the surface than at the DCM. On the other hand, divinyl-chlorophyll-*b* was present in $\sim$ 14 and 33 % of the samples (at the surface and at the DCM respectively) and was hardly detectable at the surface.

Figure 2.6. Average of the ratios of main phytoplankton pigments to chlorophyll-*a* for all samples at the surface and at the depth of the chlorophyll maximum (DCM) from 21 selected stations (see Figure 2.1). The percentage occurrence of these pigments in the total number of samples is shown in the bottom panel. ca: chlorophyll-*a*; cc: chlorophylls-$c_1 + c_2$; cb: chlorophyll-*b*; va: divinyl-chlorophyll-*a*; vb: divinyl-chlorophyll-*b*; fu: fucoxanthin; he: 19-hexanoyloxyfucoxanthin; d+t: diadinoxanthin+diatoxanthin; ze: zeaxanthin; pe: peridinin; PS: sum of photosynthetic carotenoids (fu+he+pe); PP: sum of photoprotective carotenoids (ze+(d+t)).

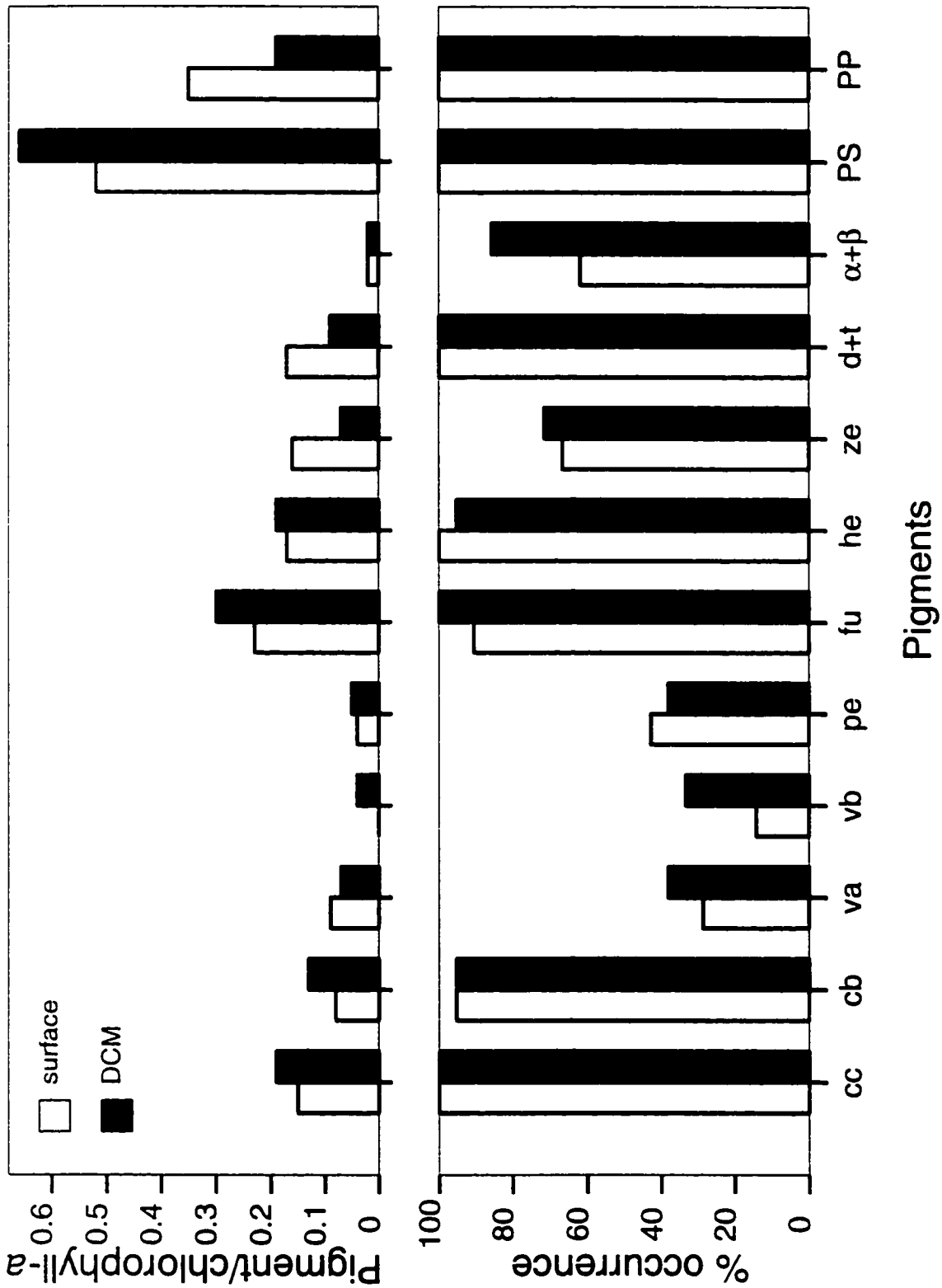
surface
DCM
Pigment/chlorophyll-a
0
0.1
0.2
0.3
0.4
0.5
0.6
% occurrence
0
20
40
60
80
100
cc
cb
va
vb
pe
fu
he
ze
d+t
α+β
PS
PP
Pigments

Figure 2.6

Among the photosynthetic carotenoids, fucoxanthin was the most abundant, followed by 19-hexanoyloxyfucoxanthin and finally peridinin. In all cases the ratios were higher at the DCM than at the surface. 19-hexanoyloxyfucoxanthin and fucoxanthin seemed to have been the most extensively distributed photosynthetic carotenoids (fucoxanthin present in $\sim$ 90 and 100 % of the samples at the surface and at the DCM respectively; 19-hexanoyloxyfucoxanthin present in $\sim$ 100 and 95 % of the samples at the surface and at the DCM respectively). Peridinin was only present in a few locations ($\sim$ 42 and 38 % occurrence at the surface and at the DCM respectively). Although zeaxanthin was more sparsely distributed ($\sim$ 67 and 71 % occurrence at the surface and at the DCM respectively) than diadinoxanthin+diatoxanthin (100 % occurrence at both the surface and the DCM). These ratios were higher for both pigments at the surface than at the DCM. $\alpha + \beta$-carotenes were present in minor concentrations in relation to chlorophyll-*a*. Since it is known that all algal groups contain one or the other of these pigments (α- or β-carotene; Larkum and Barrett, 1983; Jeffrey and Vesk, 1997) the less than 100% occurrence might simply reflect concentrations below the detection limit of the method. Finally, a comparison between the ratio of the sum of photosynthetic carotenoids (PS= fu+pe+he+bu) to chlorophyll-*a* and that of the sum of photoprotective carotenoids (PP= ze+(d+t)+(α + β)) to chlorophyll-*a*, showed that PS amounted on average to 50 and 70 % (at the surface and at the DCM respectively) of the chlorophyll-*a* concentration and were more abundant at the DCM than at the surface. The sum of PP represented on average 35 and 20 % (at the surface and at the DCM respectively) of the chlorophyll-*a* concentration and were more abundant at the surface than at the DCM.

Variation with depth was also found in the level of phycoerythrin (relative fluorescence emission at 570 nm) per cyanophyte cell (Figure 2.7). Note that besides the coefficients of determination (r^2), I also calculated the power of the test, that is, the probability of rejecting the null hypothesis when in fact it is false (Sokal and Rholf, 1969), for all regressions. The association between variables was considered

Figure 2.7. Regressions between fluorescence emission by phycoerythrin per liter of sample (emission: 570 nm. excitation: 520 nm) in relative units and the concentration of cyanophyte cells in 21 selected stations (see Figure 2.1). **a)** pooled data from three depths (surface. DCM. and one chosen depth below the DCM); **b)** surface samples; **c)** DCM samples.

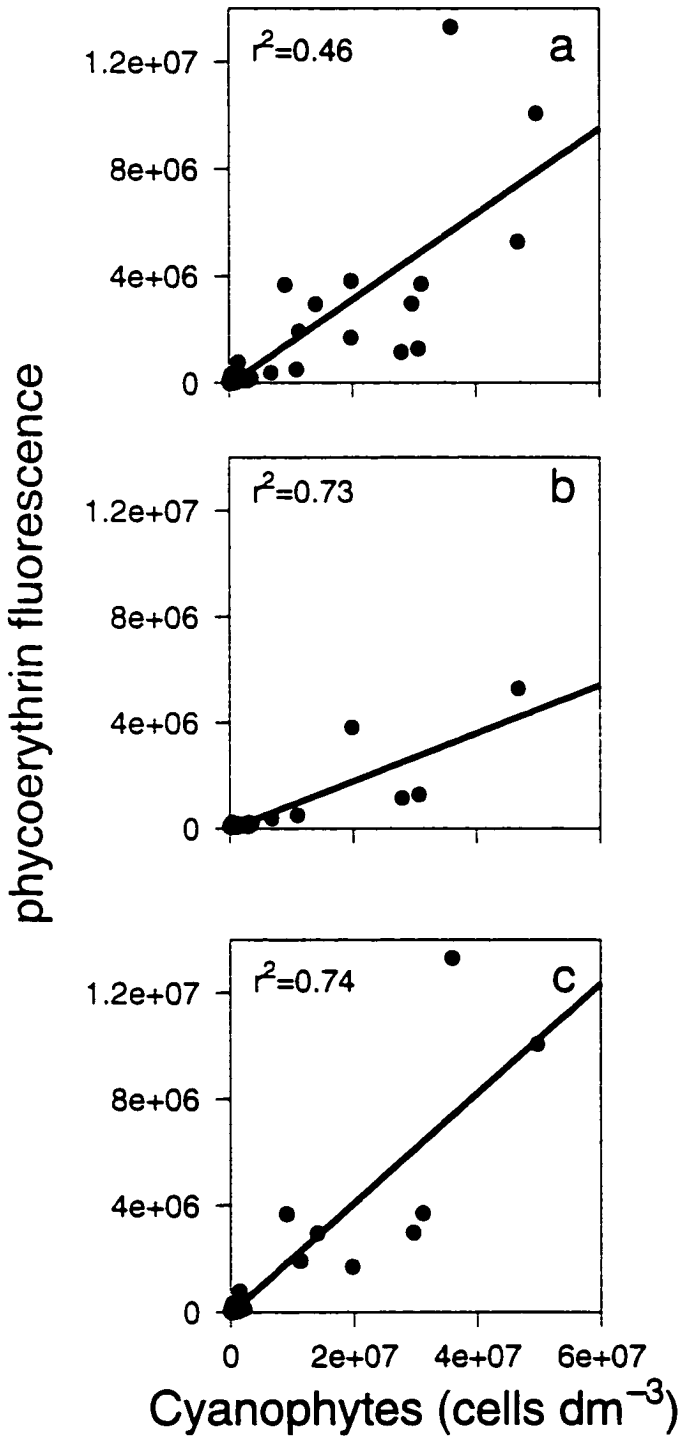
r²=0.46
a
r²=0.73
b
r²=0.74
c
1.2e+07
8e+06
4e+06
0
0
2e+07
4e+07
6e+07
phycoerythrin fluorescence
Cyanophytes (cells dm⁻³)

Figure 2.7

to be strong when, for α=0.05, the power of the test was between 0.8 and 1.0; below 0.8 the associations should be interpreted with caution. For the pooled data from three depths (surface, DCM, and one depth selected from below the DCM) the relationship between relative fluorescence at 570 nm per liter and the number of cyanophytes per liter was strong (power of the test ~1.0) but the coefficient of determination was not very high (r^2 ~0.46). The fit yielded a slope of ~ 0.14. If samples from the surface and from the DCM were analysed separately, the coefficients of determination improved (r^2 ~0.73 for the surface, and r^2 ~0.74 for the DCM) and the slopes were also different (slope ~ 0.09 for the surface and ~ 0.21 for the DCM), indicating a higher concentration of phycoerythrin per cell at the DCM. It should be noted that some of the variability in the fluorescence emission could derive from changes in the relative amounts of phycoerythrobilin and phycourobilin present in the cells (Vernet *et al.*, 1990; Olson *et al.*, 1990; Lantoine and Neveaux, 1997; Wood *et al.*, 1998).

2.3.5 Variability in the shape of the absorption spectra

To investigate the effect of pigment composition (due either to species composition or to photoacclimation) on the absorption spectrum, I analysed the relationships between absorption at different pairs of wavelengths and the ratios of main pigments to chlorophyll-*a*. The ratio $a_{ph}(440)/a_{ph}(676)$ showed a good correlation with the ratio of PP to chlorophyll-*a* concentrations in the samples (r^2 ~0.63, power of the test ~ 1.0; Figure 2.8). Among the PP present in the samples, zeaxanthin seemed to account for most of the variation in $a_{ph}(440)/a_{ph}(676)$. (r^2 for $a_{ph}(440)/a_{ph}(676)$ vs. the ratio of zeaxanthin to chlorophyll-*a* ~0.60, power of the test ~ 1.0; Figure 2.8). The percentage absorption by PP at 440 nm, $\%a_{PP}(440)$, explained up to 62 % of the variance in $a_{ph}(440)/a_{ph}(676)$ (power of the test ~1.0; Figure 2.8).

To evaluate the effect of $a_{PP}(\lambda)$ at different depths, I calculated the average contribution of PP absorption at 440 nm for the pooled data (all depths), as well as for the surface and the DCM samples (Table 2.3). As expected, surface samples

Figure 2.8. Regressions between the ratio of absorption at 440 nm to absorption at 676 nm [$a_{ph}(440)/a_{ph}(676)$] and : **a)** the ratio of the sum of photoprotective pigments to chlorophyll-*a* (PP/ca), **b)** the ratio of zeaxanthin to chlorophyll-*a* (ze/ca), and **c)** the percentage of absorption by photoprotective pigments to total phytoplankton absorption at 440 nm [$\%a_{PP}(440)$].

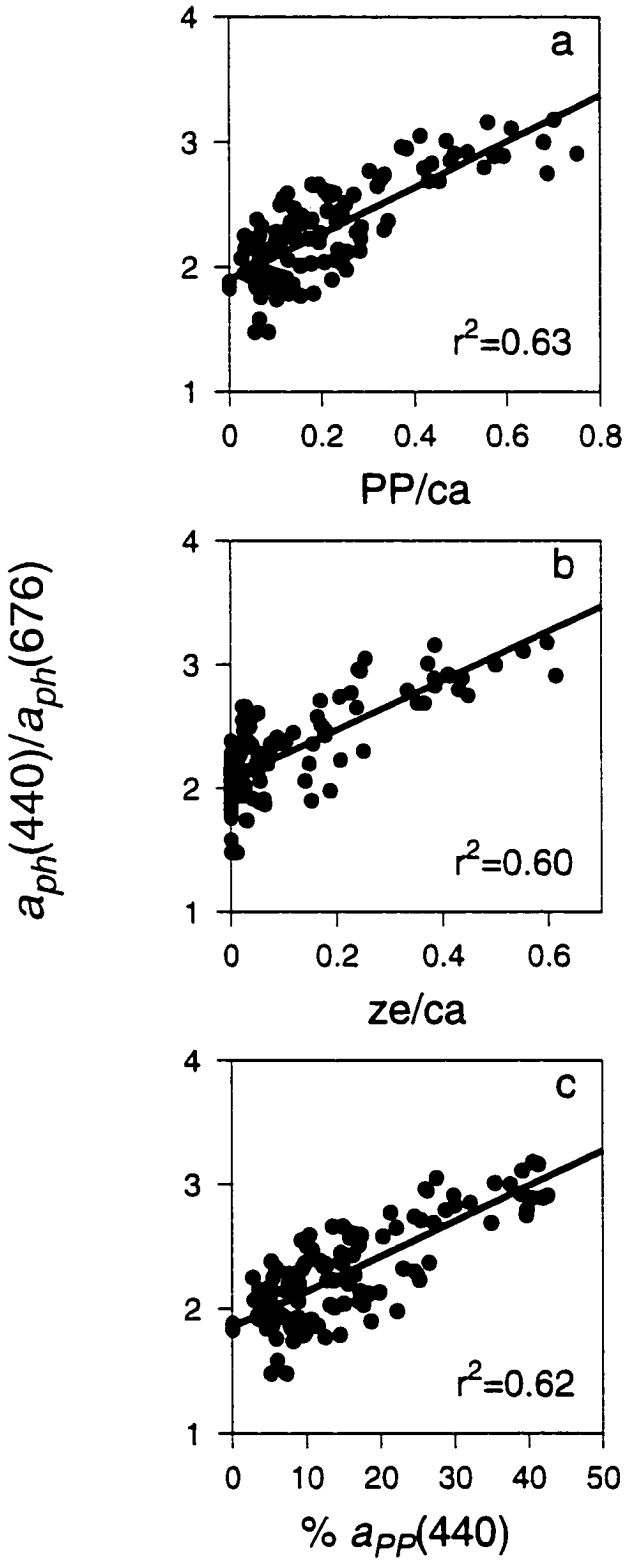

Figure 2.8

Table 2.3 Average (Av.) and coefficient of variation (c.v.) of the percentage contribution of absorption by photoprotective pigments ($a_{PP}(440)$) to total phytoplankton absorption at 440 nm, for samples corresponding to the surface, the depth of the chlorophyll maximum (DCM), and all dephts pooled (Pool).

Depth	Av. $\%a_{PP}(440)$	c.v. $\%a_{PP}(440)$
Surface	23.73	45.70
DCM	13.94	50.96
Pool	15.05	72.77

had the highest $\%a_{PP}(440)$: on average, ~24 % of the absorption at 440 nm at the surface was due to PP. At the DCM, absorption by PP contributed ~14 % of total phytoplankton absorption at 440 nm. On the pooled data, absorption by PP accounted for ~15 % of total phytoplankton absorption at 440 nm.

I also calculated the ratio of absorption at 555 nm, close to the maximum of absorption by phycoerythrin (see Rowan, 1989), to absorption at 623 nm, which corresponds to a peak of absorption by chlorophyll-*a* *in vivo* (Hoepffner and Sathyendranath, 1991). This ratio $a_{ph}(555)/a_{ph}(623)$ showed a low degree of correlation (r^2 ~0.4) though a strong association (power of the test ~ 1.0) with the proportion of cyanophyte cells to total chlorophyll-*a* concentration (Figure 2.9).

2.3.6 Characteristics of phytoplankton in different size-fractions

To assess the contribution of small and large cells to the total chlorophyll-*a*, absorption and fluorescence, I analysed the properties of these variables in two size fractions (<2 μm and >2 μm). Size-fractionation was performed at three depths (surface, DCM, below DCM) on 21 stations (marked with an asterisk on Figure 2.1). However, for this analysis, I used only samples from the DCM, since some of the surface and deep (below the DCM) samples in oligotrophic areas had extremely low phytoplankton concentrations, which resulted in noisy optical measurements, close to, or below, the limit of detection of the instruments.

Contribution of the <2 μm fraction to total biomass and optical properties of phytoplankton: To estimate the contribution of small and large cell sizes to the measured phytoplankton variables in the whole area I calculated the average chlorophyll-*a* concentration, the absorption coefficient at 440 nm, $a_{ph}(440)$, and the fluorescence excitation at 440 nm, $f_c(440)$, for two size-fractions: <2 μm (small), and >2 μm (large), and for the total. The large fraction was estimated by subtracting the value of the small fraction from that of the total. These estimates (Table 2.4) show that the small fraction accounted for ~18 % of the average chlorophyll-*a* concentration, but the contributions to total optical properties, $a_{ph}(440)$ and $f_c(440)$,

Figure 2.9. Regression between the ratio of absorption at 555 nm to absorption at 623 nm $[a_{ph}(555)/a_{ph}(623)]$ and the number of cyanophyte cells per concentration of chlorophyll-a.

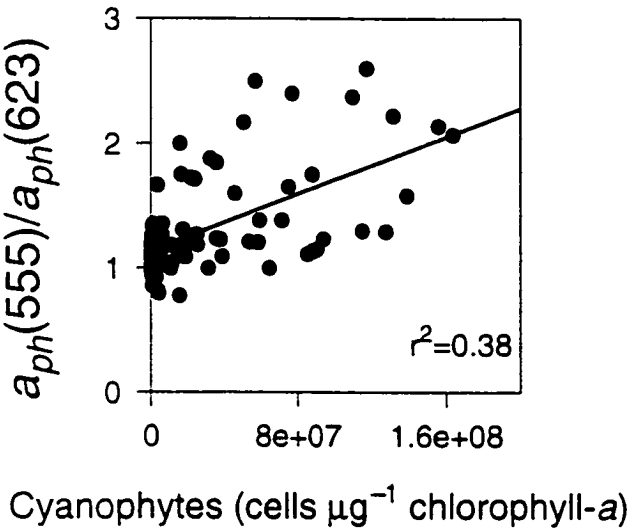
$a_{ph}(555)/a_{ph}(623)$
3
2
1
0
r^2=0.38
0
8e+07
1.6e+08
Cyanophytes (cells µg^{-1} chlorophyll-*a*)

Figure 2.9

Table 2.4 Average (Av.) and coefficient of variation (c.v.) of chlorophyll-a concentration, $a_{ph}(440)$, and $f_c(440)$ for the total (T), >2 μm (G), and <2 μm (L) fractions. The percentage contributions of the <2 μm fraction to the total are also shown.

Variable	Av. T	c.v. T	Av. G	c.v. G	Av. L	c.v. L	% L
chlorophyll-a	1.86	121.5	1.53	145.0	0.33	51.5	17.7
$a_{ph}(440)$	0.078	117.1	0.053	153.8	0.028	56.0	35.3
$f_c(440)$	1108859	117.7	689162	116.9	487270	116.4	43.9

were higher (~35 % and ~44 % respectively). The average specific absorption coefficient at 440 nm for the large fraction was 47 % lower than that for the small fraction (data not shown). The small fraction had a lower degree of variation in the average chlorophyll-*a* concentration and $a_{ph}(440)$ than the large fraction or the total, while variation in $f_c(440)$ was comparable for the two fractions and the total.

At the stations located in oligotrophic subtropical waters of the Canary cruise (e.g., stations: 1, 2, 4, 5, 9, 12, 13, 14, 15), picoplankton contributed between 40 - 100 % of the total chlorophyll-*a* and $a_{ph}(440)$, and this contribution decreased towards eutrophic areas close to Morocco Coast (stations 8, 7) and the Newfoundland Shelf (station 17: Figure 2.10). On the other hand, the contribution of picoplankton to total $f_c(440)$ showed a different pattern (Figure 2.10). At most of the oligotrophic stations (1, 2, 4, 5, 12, 13) the percentage contribution of the small fraction to total $f_c(440)$ was lower than the contribution of the same fraction to total $a_{ph}(440)$. The opposite effect was observed for the remaining stations. In the Labrador Sea, picoplankton contributed between 27 and 42% of the total chlorophyll-*a* concentration and up to 60 % of $a_{ph}(440)$ at the central Labrador Sea (stations 7, 5, 8, and 9). A much lower contribution of the small fraction was observed in stations located in the Labrador Current (stations 3, 10, 11, 13, 14). Except for station 5, where the contribution to fluorescence was lower than that to absorption, picoplankton represented a significant portion of total fluorescence at 440 nm (~ 40 - 80 %).

Variability in the shape of the absorption and fluorescence excitation spectra in different size-fractions: To study the variations in the spectral shapes of the two optical properties measured, I normalised the spectra at 623 nm in the case of absorption, $a^n_{ph}(\lambda)$, and 600 nm in the case of fluorescence, $f^n_c(\lambda)$. I chose 623 nm to normalise the absorption spectra since absorption at that wavelength is less affected than the blue or red peaks by the packaging effect (Stuart *et al.*, 1998). The average $a^n_{ph}(\lambda)$ for the large fraction showed the highest degree of flattening, at both the blue and red peaks, followed by the total, and the small fraction (Figure 2.11). The small fraction showed the highest coefficients of variation (Figure 2.11).

Figure 2.10. Percentage contribution of the <2 μm (small fraction) to the total values of concentration of chlorophyll-*a*, absorption coefficient at 440 nm [$a_{ph}(440)$], and fluorescence at 440 nm [$f_c(440)$], at each station in the two transects.

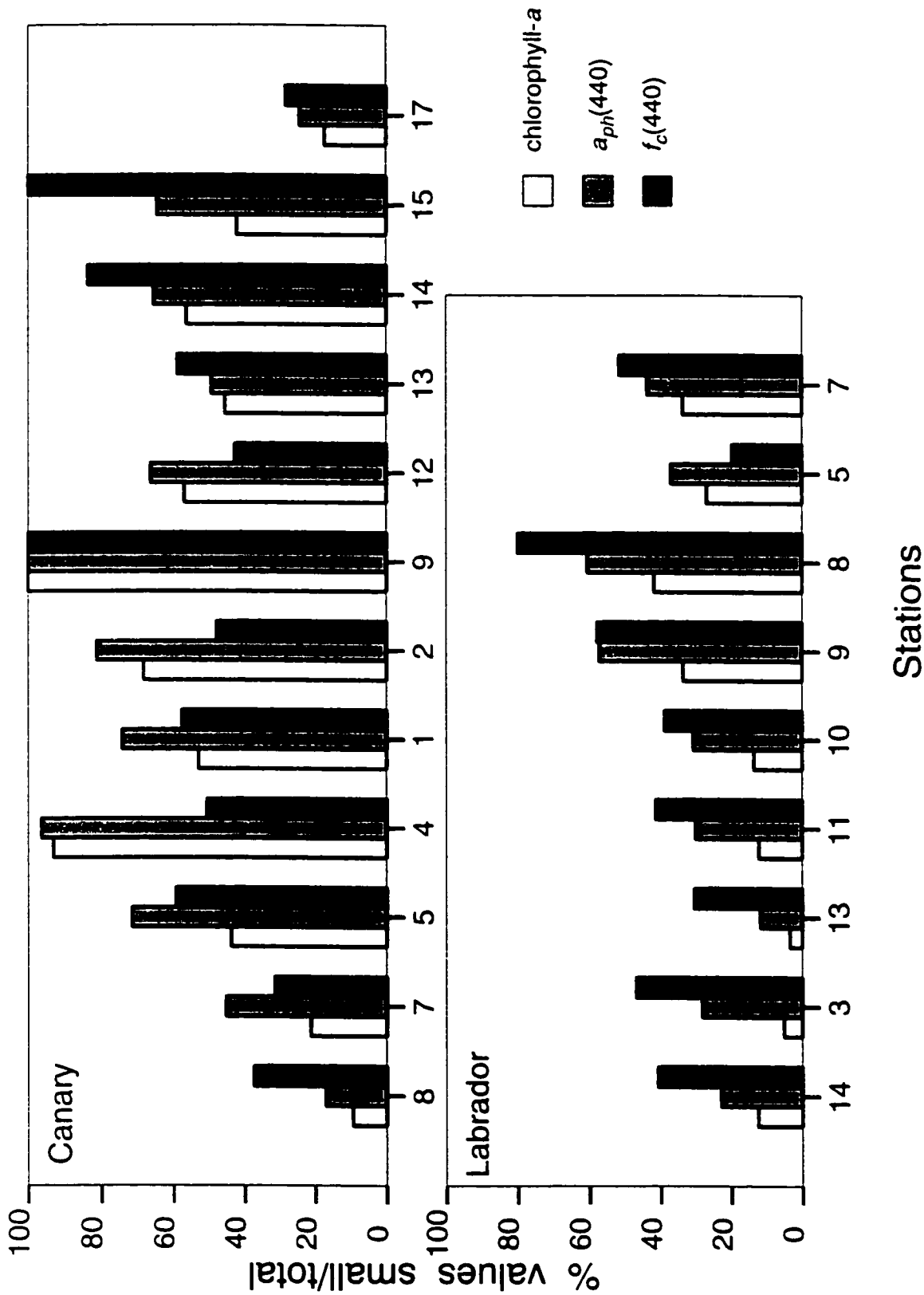
chlorophyll-a
$a_{ph}(440)$
$f_c(440)$
Canary
Labrador
% values small/total
Stations
100
80
60
40
20
0
8
7
5
4
1
2
9
12
13
14
15
17
14
3
13
11
10
9
8
5
7

Figure 2.10

Figure 2.11. Variations in the shape of the absorption and fluorescence excitation spectra for the two size-fractions: >2 μm (large), and <2 μm (small), and for the total. These are averages of spectra from the DCM from 21 selected stations (see Figure 2.1). Absorption spectra are normalised at 623 nm and fluorescence excitation spectra are normalised at 600 nm. The coefficients of variation (c.v.) for each spectrum are shown in the bottom panel.

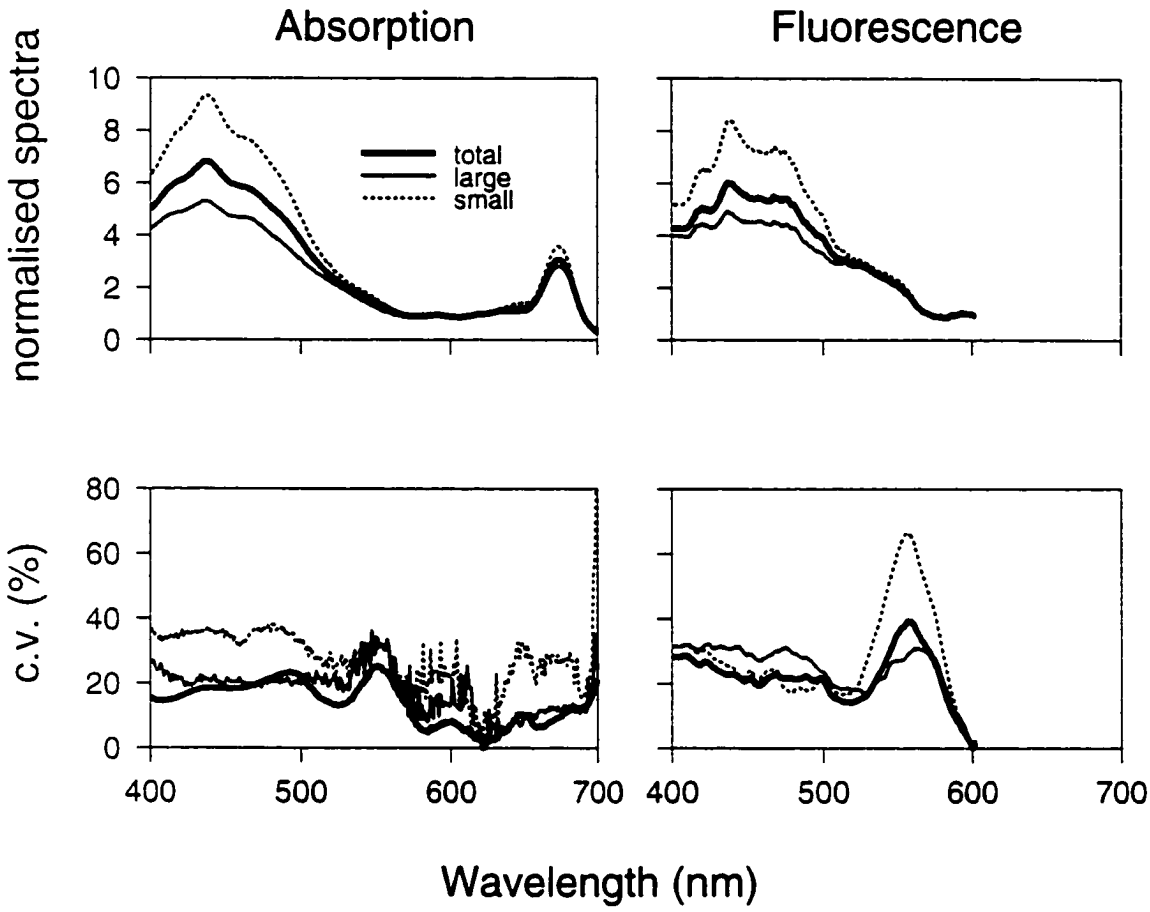
Absorption
Fluorescence
normalised spectra
total
large
small
c.v. (%)
Wavelength (nm)

Figure 2.11

while the c.v. for the large fraction and the total were of similar magnitudes at all wavelengths. This might be attributed in part to larger errors in the determination of absorption for the small fraction, which had the lowest values of absorption, but it might also indicate a real effect, with higher variability in spectral shapes of absorption in picoplankton species than in larger species. Fluorescence excitation spectra were normalised at 600 nm, since this was the longest available wavelength. In this case again the flattening effect at the blue peak affected the large fraction and the total more than the small fraction (Figure 2.11). The coefficients of variation for the different fractions showed a similar pattern, though the large fraction had the highest variation in the blue part of the spectrum ($\sim$ 440 - 490 nm), while the small fraction had the highest variations in the green part of the spectrum ($\sim$ 558 nm: Figure 2.11).

Variability in the difference between absorption and fluorescence for the different size-fractions: To investigate further the variations in the absorption and fluorescence excitation spectra in the different size-fractions I calculated the ratio of absorption to fluorescence at 439 nm, S(439), after normalising the two spectra (absorption and fluorescence) to their values at 545 nm (explained in more detail in Chapter 3). Stations where cyanophytes constituted a substantial part of the phytoplankton community (e.g., Canary cruise stations: 4, 5, 7 and 9) showed the largest values of S(439) (Figure 2.12). At these stations S(439) was higher for the small than for the large fraction or the total, or at least similar to the total in cases where the community was composed entirely of small cells (e.g., station 9). Stations 1, 12 and 13 (Canary) represented intermediate cases where picoplankton made up at least half of the total phytoplankton, and the small fraction seemed to have been a mixture of prochlorophytes, cyanophytes and prymnesiophytes. For the remaining stations (for the two cruises) where the picoplankton community was dominated by prymnesiophytes and chlorophytes, S(439) was lower for the small fraction than for the total, and values for the large fraction were higher than for the total, except for station Labrador-5.

Figure 2.12. Values of the ratio of absorption to fluorescence at 439 nm. where each value was previously normalised at 545 nm. S(439). at each station for each size-fraction. Stations where either the absorption or the fluorescence value for the small fraction was similar to the total do not show a S(439) value for the large fraction.

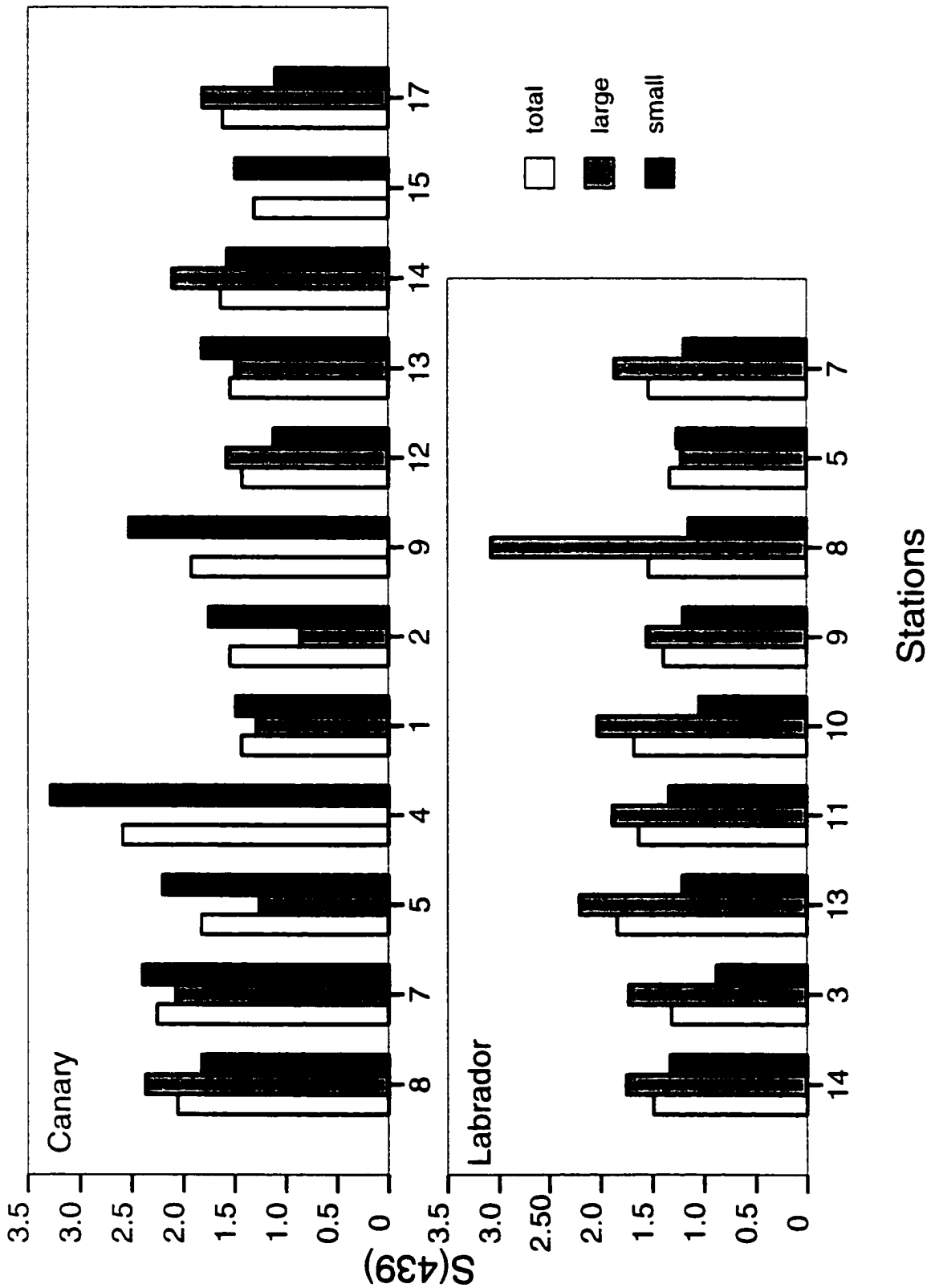
Canary
Labrador
total
large
small
S(439)
Stations
0
0.5
1.0
1.5
2.0
2.50
3.0
3.5
8
7
5
4
1
2
9
12
13
14
15
17
14
3
13
11
10
9
8
5
7

Figure 2.12

2.4 Discussion

2.4.1 Variations in the composition of the phytoplankton population, photoadaptation and photoacclimation

Phytoplankton population: The use of accessory pigments to identify algal groups is limited by the fact that most pigments are not unique to one particular class (see Jeffrey and Vesk, 1997). Nevertheless, the analysis of pigment composition can provide, a simple, preliminary approach to discern qualitatively the most probable composition of the phytoplankton community. Some methods to obtain a quantitative estimation of taxonomic composition of phytoplankton based on pigment composition have been developed (Gieskes *et al.*, 1988; Mackey *et al.*, 1996). These methods are also subject to the limitations imposed by the lack of pigment markers in some groups of phytoplankton and by the variation observed in the pigment ratios within a given taxonomic group. In this study I have used pigment composition only in a qualitative manner, to infer the taxonomic composition of phytoplankton.

It appears that prochlorophytes and cyanophytes were the predominant groups in oligotrophic subtropical waters, diatoms and dinoflagellates in coastal and nutrient-rich waters (Figures 2 and 4, and Table 2.2). Prymnesiophytes were the most ubiquitous group and were present in all types of environment. The averages of pigment ratios (Figure 2.6) suggest that prymnesiophytes and probably diatoms were overall the most abundant groups.

Photoadaptation and depth dependent photoacclimation: Prymnesiophytes contain both 19-hexanoyloxyfucoxanthin (which is also found in a few species of dinoflagellates and diatoms) and fucoxanthin (which is the main carotenoid in diatoms). Different strains of *Phaeocystis* isolated from different environments show large variations in the concentrations of 19-hexanoyloxyfucoxanthin and fucoxanthin (Buma *et al.*, 1991). Furthermore, *Phaeocystis* can apparently change the proportions of these two carotenoids according to conditions of irradiance and iron

concentrations (Van Leeuwe and Stefels, 1998). At low iron concentrations or high irradiances, when the photosystems receive more energy than they can process, this species decreases the amount of fucoxanthin, which Van Leeuwe and Stefels (1998) suggest has a highly efficient energy transfer, and increases the amount of 19-hexanoyloxyfucoxanthin, which they suggest is less efficient at energy transfer. The same pattern has been seen for the relative levels of fucoxanthin and 19-hexanoyloxyfucoxanthin in cultures of the prymnesiophyte *Emiliania huxleyi* grown at different irradiances (Head, unpublished). By contrast, Johnsen *et al.* (1992) suggested that the pigment-complexes of the prymnesiophyte *Chrysochromulina polylepis* are more efficient at transferring energy to the photosystems than the pigment-complexes of the diatom *Skeletonema costatum*. In this case, Johnsen *et al.* (1992) suggested that the higher efficiency in the transfer energy might be due to the presence of 19-hexanoyloxyfucoxanthin (instead of fucoxanthin in the diatom), but also to the presence of chlorophyll-c_3 in the prymnesiophyte.

Diatoms and dinoflagellates prevail in well-mixed (and mainly in middle- to high-latitudes) waters, where average irradiance is lower than in subtropical regions. These types of algae can dramatically increase their levels of photosynthetic carotenoids at low irradiances (Falkowski, 1983; Richardson *et al.*, 1983; Falkowski and La Roche, 1991), and photoacclimate to high irradiances by increasing the levels of PP. The most important PP in both types of algae are diadinoxanthin and diatoxanthin, which are pigments involved in the xanthophyll cycle, which completely avoid the transfer of energy to the photosystems (Demers *et al.*, 1991; Olaizola and Yamamoto, 1994). In diatoms, the concentration of diadinoxanthin+diatoxanthin constitutes $< 10\%$ of the total intracellular concentration of chlorophyll-*a* at low irradiances and can reach $\sim 55\%$ at high irradiances (Sakshaug *et al.*, 1991). Cyanophytes and prochlorophytes, which are predominant in blue subtropical waters, possess high concentrations of the PP zeaxanthin (Figures 5 and 7), which vary between 40 to 200% of the intracellular concentration of chlorophyll-*a* (Kana *et al.*, 1988; Veldhuis and Kraay, 1990). Moreover, it has been shown that

both groups (cyanophytes and prochlorophytes) achieve this modulation not by changing the intracellular concentrations of zeaxanthin with changes in irradiance, but by changing the concentrations of chlorophyll-*a* or divinyl-chlorophyll-*a* (Kana *et al.*, 1988; Veldhuis and Kraay, 1990). At low light, as well as increasing the intracellular concentration of chlorophyll-*a*, cyanophytes also increase the levels of phycobilins (Glover *et al.*, 1985; Li and Wood, 1988; Olson *et al.* 1990; Figure 2.7) and prochlorophytes increase the concentrations of divinyl-chlorophyll-*b* (Veldhuis and Kraay, 1990; Moore *et al.*, 1995; Figure 2.6).

Prymnesiophytes have xanthophyll cycle pigments which can absorb potentially damaging light at high irradiances. Furthermore, they also apparently have the ability to change the composition of their light-harvesting systems from a highly efficient one (containing fucoxanthin) to a less efficient one (containing 19-hexanoyloxyfucoxanthin). This capacity may give them an advantage over diatoms and dinoflagellates, which do not have it, and over those classes which seem to have most of their representatives especially adapted to high light (cyanophytes and prochlorophytes) which have a large proportion of their pigment content dedicated exclusively to photoprotection. This, together with other factors such as their intermediate size-range (Chisholm, 1992), may be why prymnesiophytes are so widely distributed in the world's oceans.

The general changes found in the ratios of various pigments to chlorophyll-*a* (Figure 2.6) and in the phycoerythrin fluorescence per cyanophyte cell (Figure 2.7), are indicative of changes in species composition or photoacclimation within a species, or both. The fact that changes were observable with depth, implies that the rate of mixing in the upper water column was low enough to allow stratification either in the distribution of different species (Moore *et al.*, 1995) or in acclimation of the cells to different irradiances (Falkowski, 1983; Cullen *et al.*, 1988), or both.

2.4.2 Effect of variations in species composition and photoacclimation on the optical characteristics of phytoplankton

Variability in the absorption spectra: The shape of the absorption spectra is influenced by the cell size and the intracellular concentration of pigments through a phenomenon known as the packaging effect, as well as by the pigment composition of the cells (Duysens, 1954; Sathyendranath *et al.*, 1987; Ciotti *et al.*, 1999). I found that, for all samples pooled, the ratio $a_{ph}(440)/a_{ph}(676)$ was correlated with the ratio of PP to chlorophyll-*a* (Figure 2.8). The correlation between zeaxanthin and the ratio $a_{ph}(440)/a_{ph}(676)$ and the ratio of zeaxanthin to chlorophyll-*a* was also high, suggesting that zeaxanthin might be responsible for much of the variation in absorption produced by PP (Figure 2.8). Zeaxanthin is often associated with small cells, mainly picoprokaryotes (cyanophytes and prochlorophytes), which contain high concentrations of this pigment, and picoeukaryotes (e.g., small chlorophytes). Hence, both the small cell size and the enhanced absorption in the blue by zeaxanthin favour the high values of $a_{ph}(440)/a_{ph}(676)$ (Stramski and Morel, 1990; Babin *et al.*, 1996; Bouman *et al.*, in press). Furthermore, the percentage contribution of PP absorption at the blue, % $a_{PP}(440)$, was considerably higher at the surface than at the DCM (Table 2.3).

Contribution of different size-classes to total absorption: At most of the stations the percentage contribution of picoplankton to $a_{ph}(440)$ was greater than their contribution to total chlorophyll-*a*, which is consistent with the fact that small cells have a higher absorption efficiency than large cells (the specific absorption coefficient for the large fraction was 47 % lower than that for the small fraction). The same effect was apparent in the comparison of the normalised (at 623 nm) absorption spectra for the different fractions, which showed higher values of absorption at the blue and red peaks for the small fraction than for the large fraction or for the total (Figure 2.11). The lower variation in the values of chlorophyll-*a* and $a_{ph}(440)$ for the small fraction compared with the large fraction or the total (Table 2.4) would support the idea that there is a background of small cells more-or-less uniformly

distributed in the ocean, on which would be superimposed larger cells in coastal or nutrient-rich areas (Malone, 1980*a*; Yentsch and Spinrad, 1987; Yentsch, 1990). On the other hand, our data do not suggest uniformity in species composition or in the photoacclimation status within the small fraction. For example, when absorption spectra were normalised at 623 nm (Figure 2.11), the highest variation for the small fraction was found at ~ 555 nm, close to the maximum absorption by phycoerythrin.

I examined the relationship between the ratio $a_{ph}(555)/a_{ph}(623)$ and the relative number of cyanophytes in the samples, since a significant difference in this ratio was observed in unialgal cultures: in diatoms the mean $a_{ph}(555)/a_{ph}(623)$ ratio was ~1.00±0.12 (for 7 samples of *Thalassiosira weissflogii* and *Chaetoceros* sp.) whereas the ratio for cyanophytes was ~5.45±0.77 (for 3 samples of *Synechococcus* sp.). Several factors, however, can confound the relationship between the ratio $a_{ph}(555)/a_{ph}(623)$ and the relative number of cyanophytes in natural samples (ratio of cyanophyte cells to total chlorophyll-*a*: Figure 2.9). Part of the variation in this relationship might be due to changes in intracellular concentration of phycoerythrin with irradiance, as previously reported (Glover *et al.*, 1985; Li and Wood, 1988) and observed in this study (Figure 2.7); as well as changes in the proportion of phycoerythrobilin (maximum absorption ~ 550 nm) and phycourobilin (maximum absorption ~ 495 nm; Wood, 1985; Wood *et al.*, 1999) in cyanophytes. The ratio $a_{ph}(555)/a_{ph}(623)$ can also change due to changes in the concentration of photosynthetic carotenoids (which also show some absorption at 555 nm) in eukaryotes induced by photoacclimation. It should be also noted that 623 nm is a minor peak of absorption by chlorophyll-*a*, and hence this region of the spectrum would be more affected by errors in the estimation of absorption.

Variability in fluorescence excitation spectra: The average contribution of picoplankton to total $f_c(440)$ (~ 44%) was larger than their average contribution to total $a_{ph}(440)$ (~ 35%; Table 2.4). This suggests that fluorescence efficiency was, on average, higher than the absorption efficiency in small cells. However, I cannot overrule the possibility that some of this difference might be an artifact caused by

the differences in the filter correction in the absorption spectra (pathlength amplification factor) and in the fluorescence excitation spectra (ratio between fluorescence in suspension and on a filter), and the possible effect of stress on the cells of the small fraction which were pre-filtered through a Nuclepore filter.

There was a larger variation in $f_c(440)$ than in $a_{ph}(440)$, or in total chlorophyll-a concentration, for the small fraction than in the large fraction (Table 2.4). This might have been due to variations in the distribution of phycoerythrin-containing cyanophytes, as the spectral shapes of the fluorescence excitation spectra showed a maximum c.v. at $\sim$ 550 nm (Figure 2.11). Cyanophytes represent an extreme case of imbalance in chlorophyll-a distribution between photosystems (this subject will be explained in more detail in the next two chapters). Their fluorescence excitation spectra show strong fluorescence at the maximum of absorption by phycobilins (associated with PSII), and low fluorescence at the maxima of absorption by chlorophyll-a (mostly located in PSI: Ley and Butler, 1980). This would explain why the contribution of the small fraction to total $f_c(440)$ was lower than its contribution to total $a_{ph}(440)$ at stations where the phytoplankton population was dominated by cyanophytes (Figure 2.10). This was also reflected in the high range of the ratio of absorption to fluorescence, S(439), which was larger for the small fraction at stations dominated by cyanophytes (Figure 2.12).

Lazzara *et al.* (1996) calculated the ratio of fluorescence to absorption (in absolute magnitudes) in different regions (one oligotrophic, one mesotrophic and one eutrophic site) in the North East Atlantic. It is difficult to make a direct comparison between the results of Lazzara *et al.* (1996) and those obtained in this study because of differences in the normalisation of the data. Nevertheless, some similarities in the general trend of changes in the fluorescence to absorption ratio (in the blue region of the spectrum) can be seen. Lazzara *et al.* (1996) found a lower fluorescence-to-absorption ratio in the oligotrophic site than in the mesotrophic site. They attributed this difference mainly to the presence of prokaryotic cells (prochlorophytes and cyanophytes), containing high concentrations of zeaxanthin.

in the oligotrophic site, since zeaxanthin is represented in the absorption and not in the fluorescence spectrum (this subject is discussed in more detail in the next two chapters). Lazzara *et al.* (1996) also mention other possible reasons for this variation in the fluorescence-to-absorption ratio: First, nutrient depletion could possibly decrease the number of active photosystems-II (PSII contributes >95% of fluorescence at ambient temperature) in the oligotrophic area (a correlation between S(439) and nitrate concentrations is discussed in section 3.4.3, Figure 3.4). Secondly, the differential distribution of pigments between photosystem-I and photosystem-II, especially in cyanophytes, could affect this ratio, since both photosystems are represented in the absorption spectrum and only PS-II in the fluorescence spectrum (which is one of the main points discussed in the next two chapters).

For most of the remaining stations, where the picoplankton comprised mainly prochlorophytes, prymnesiophytes, and probably chlorophytes, the contribution of the small fraction to total $f_c(440)$ was larger than its contribution to total $a_{ph}(440)$ and the values of S(439) were lower for the small fraction than for the large fraction or the total. A possible explanation for this low contribution to $f_c(440)$ by the large fraction might be the presence of prymnesiophytes containing 19-hexanoyloxyfucoxanthin, which has been postulated to be less efficient than fucoxanthin at transferring energy to the photosystems. On the other hand, one could also speculate that differences in S(439) between size fractions could be an indication that the fluorescence excitation spectrum was more affected than absorption by the packaging effect. The argument would be that the absorption and fluorescence values for small cells (little affected by packaging) are close at the blue peak, with the differences between them being mainly due to the presence of PP and the arrangement of the photosynthetic apparatus. In addition, a stronger effect of packaging on fluorescence would also contribute to an increase in the ratio S(439) for larger cells.

2.5 Concluding Remarks

These observations confirm that there is a large variation in phytoplankton distribution in these two contrasting regions of the North Atlantic. Variations in the phytoplankton community followed a pattern consistent with photoadaptation to different light environments (i.e., different suites of pigments occurring in different classes). Photoacclimation of cells to different intensities of irradiance, with depth, was also marked. Observations on different size fractions support the hypothesis that there is a background of small cells more-or-less uniformly distributed in the ocean (Malone, 1980*a*; Yentsch and Spinrad, 1987; Yentsch, 1990). Nevertheless, even within the small fraction (< 2 μm) there was considerable variation in the inferred species composition. The differences in species composition (size distribution, pigment composition) and photoacclimation status were clearly reflected in the absorption and fluorescence excitation spectra of the phytoplankton communities.

Models of primary production and new algorithms to retrieve information on phytoplankton from remote sensing should address this variability in the optical characteristics of phytoplankton.

Finally, in this study the analysis was focused on photoadaptation and photoacclimation. There are other factors, not considered here, such as size-dependent variations in the uptake of nutrients (Chisholm, 1992; Graziano *et al.*, 1996), ability to modulate buoyancy (Richardson *et al.*, 1996; Cullen and MacIntyre, 1998), and liability to predation (Kiørboe, 1993) which also influence the distribution of phytoplankton species in the ocean. However, it is difficult to isolate the effect of one factor from that of another, since many act in concert in the ocean. For example, the high-light adaptation of phytoplankton in oligotrophic waters is typically accompanied by adaptation to low nutrients which favours small-size phytoplankton. Without detailed discussion of causality, however, it was observed that the distribution of species and pigments in the two contrasting environments studied here was consistent with strategies for adaptations to different light fields.

In this chapter I presented a general description of the pigment composition, absorption and fluorescence characteristics of phytoplankton in the two study areas of the North Atlantic. In the next chapter I will examine in more detail differences between the shapes of the absorption and fluorescence excitation spectra in field samples from these two cruises, and the possible causes of this variation.

CHAPTER 3

Differences between *In Vivo* Absorption and Fluorescence Excitation Spectra of Natural Samples of Phytoplankton

3.1 Introduction

The action spectrum of phytoplankton represents the photosynthetic rate per unit of incident irradiance as a function of the wavelength. when the photosynthetic rate is measured within the range of linear response of photosynthesis to available irradiance. It can be estimated by incubating the cells under monochromatic light (at low intensity). monitoring a photo-induced reaction and noting the change in the reaction rate with the wavelength of light. The most commonly monitored reactions are oxygen evolution (Engelmann. 1883: Fork. 1963). ^{14}C-labelled CO_2 uptake (Iverson and Curl. 1973: Lewis *et al.*. 1985: Lewis *et al.*. 1988: Schofield *et al.*. 1990: Kyewalyanga *et al.*. 1997). and emission of fluorescence (Neori *et al.*. 1984: Neori *et al.*. 1986: Neori *et al.*. 1988: Culver *et al.*. 1994). The first two of these techniques have been used mainly in laboratory experiments. because they are too cumbersome and time-consuming to be performed during field work (Lewis *et al.*. 1985: Schofield *et al.*. 1990: 1991: Kyewalyanga *et al.*. 1997). On the other hand. determination of the fluorescence excitation spectrum gives a relatively simple and economical way of estimating the action spectrum of photosynthesis.

The shape of the action spectrum of photosynthesis generally resembles the corresponding absorption spectrum of the algae. Therefore. the absorption spectrum of phytoplankton. which is also easy to measure at sea. has been used as a proxy for the shape of the action spectrum (Morel. 1978: 1991: Kyewalyanga *et al.*. 1997). However. some differences may occur between them. depending on the arrangement of the photosynthetic apparatus in different taxonomic groups and on the physiological state of the cells. One source of these differences is that the

absorption spectrum is influenced by all the pigments, including photoprotective pigments which do not contribute to photosynthesis. Fluorescence, on the other hand, represents only the energy effectively transferred to the pigment chlorophyll-*a* of photosystem II, which is potentially usable in photosynthesis (Culver *et al.*, 1994; Sosik and Mitchell 1995; Babin *et al.*, 1995; 1996; Lazzara *et al.*, 1996).

Fluorescence excitation, because of the aforementioned advantages (unaffected by photoprotective pigments and simplicity of measurement), is becoming the method of preference for the estimation of the action spectrum in computations of primary production in the ocean (Sakshaug *et al.*, 1991; Sosik and Mitchell, 1995; Babin *et al.*, 1995). This method is, however, based on the assumption that all the differences between absorption and fluorescence spectra can be ascribed entirely to the presence of photoprotective pigments in natural populations of phytoplankton. This assumption is based more on circumstantial than concrete evidence.

In this study I compare *in vivo* absorption and fluorescence excitation spectra of natural samples of phytoplankton from a wide range of ecological regimes in the North Atlantic, to investigate the variation in the differences between the two types of spectra. I examine the relationship between these differences and the pigment composition of the samples and between them and several environmental variables. In general, these results show that the differences between absorption and fluorescence in diverse oceanographic regimes are variable, and that this variation cannot be entirely explained by the presence of photoprotective pigments. Other possible factors influencing the fluorescence excitation spectrum are discussed. I begin by examining the major factors that potentially could contribute to variations in fluorescence spectra.

3.2 Background

The fluorescence excitation spectrum of phytoplankton represents the re-emission of light (at a fixed wavelength) with variable intensity according to the wavelength of light to which the cell is exposed. The emission of fluorescence is one

of the ways in which the cell releases absorbed excess energy: approximately 18% of the absorbed light is used in photochemical reactions, about 1 to 3% is re-emitted as fluorescence, and the rest is dissipated as heat (Kirk, 1994). An inverse relationship can be expected between the efficiency of emission of fluorescence and the efficiency of photosynthesis (Kautsky and Hirsch, 1931; but see Govindjee, 1995). Nevertheless, the quantitative parameterisation of primary production rates from fluorescence measurements (e.g., pump-and-probe fluorometry: Kolber *et al.*, 1993) is not trivial, due to the complicated pathways of energy flow within the photosynthetic unit (PSU, reviewed in Butler, 1978; Larkum and Barret, 1983; Falkowski, 1992; Kiefer and Reynolds, 1992; Govindjee, 1995). The PSU encompasses the various components of the photosynthetic apparatus. In plants and algae it comprises the two photosystems (PSI and PSII), each of which is composed of a reaction center and a surrounding subantenna of accessory pigments; the electron-carriers of the electron transfer chain (between RCII and RCI); and the light-harvesting-complexes (LHCs), where most of the accessory pigments are located.

All methods of estimating the action spectrum of phytoplankton have the pitfall of being biased towards the action of PSII, instead of the composite action of both photosystems. In the case of fluorescence this bias arises from the fact that at ambient temperature more than 95% of the fluorescence is emitted by PSII, whereas PSI dissipates most of its excess energy as heat (Goedheer, 1969; Kiefer and Reynolds, 1992). If both photosystems work in series during photosynthesis (according to the classic Z-scheme), the process taking place in PSII should be proportional to the total photosynthetic capacity of the cell. However, certain characteristics of the structure and energy distribution inside the PSU can produce significant deviations between the "total" action spectrum and that of PSII alone.

It is sometimes assumed that most of the LHCs transfer the absorbed energy to PSII, while PSI receives energy mainly from its subantenna, composed mostly of chlorophyll-*a* molecules and usually a few carotenoids (reviewed in Larkum and Barret, 1983). In addition, PSII is able to transfer its excess energy to PSI by a

process known as spillover, but the opposite (i.e., transfer of energy from PSI to PSII) does not occur due to differences in energy levels (Butler, 1978; reviewed in Larkum and Barret, 1983; Govindjee, 1995). As a result, when a cell receives light of a wavelength absorbed by PSII, this energy can be "shared", promoting the function of both photosystems in series; but when the cell is irradiated with light of a wavelength that can be absorbed mainly by PSI, this energy cannot be shared. This imbalance in the functioning of the two photosystems becomes important when most of the chlorophyll-*a* of the cell is associated with PSI.

Extreme examples of this type of imbalance can be found in rhodophytes, cryptophytes and cyanophytes, in which more than 70% of the total chlorophyll-*a* can be associated with PSI (Ley and Butler, 1980; Myers *et al.*, 1980). Emerson (1958), working with *Chlorella* cells, noticed a drop in the far-red part of the action spectrum where only chlorophyll-*a* from PSI absorbs light. Emerson also noted that, when the far-red light was supplemented by light of a wavelength absorbed by PSII, there was an enhancement in the photosynthetic rate at the red end of the spectrum due to the synergistic functioning of both photosystems. This effect is usually referred to as the "Emerson enhancement effect". Departures of the fluorescence excitation spectrum from the "total" action spectrum have been noticed in studies with cultures of different species of algae (Neori *et al.*, 1986; 1988). These laboratory experiments showed that, for the species studied at least, most of the light harvested is apparently directed to chlorophyll-*a* of PSII in diatoms and dinoflagellates as opposed to rhodophytes, cryptophytes and cyanophytes. On the other hand, Schofield *et al.* (1990; 1996), through studies of action spectrum by ^{14}C incorporation, have reported that the Emerson enhancement effect can be significant, especially in the blue region of the spectrum, in some species of diatoms, prymnesiophytes and dinoflagellates.

Other differences between the action spectrum and the absorption spectrum of an alga can be caused by diverting energy away from the RCs. This is a mechanism

for protecting the photosystem against photo-damage at high light intensities. Several strategies to adjust the photosynthetic apparatus to fluctuating light conditions have been studied in phytoplankton (reviewed in Richardson *et al.*, 1983; Larkum and Barret 1983; Falkowski and La Roche, 1991). In the case of photoacclimation to low light intensities, the tendency is to increase harvesting of light and its transfer to the RCs, and therefore this type of rearrangement of the photosynthetic apparatus should, in principle, not affect the match between the action and the absorption spectra of algae. It has also been noticed that changes in light quality can produce state transitions, i.e., modify the proportion of energy directed towards PSI and PSII. State-1 transition is caused by exposure to light predominantly absorbed by PSI, and state-2 transition, by exposure to light predominantly absorbed by PSII (Bonaventura and Myers, 1969). Under these state transitions the balance in the operation of the two photosystems tends to be maintained by redirecting the flow of energy. Uncoupling of LHCs from PSII, increase in spillover from PSII to PSI, and increase in cyclic electron flow around PSI, have all been observed under state-2 transition in green algae and dinoflagellates (Kroon *et al.*, 1993; Kroon, 1994) and in cyanophytes (Mullineaux and Allen, 1988). An increase in cyclic electron flow around PSI has also been observed under exposure to high light in experiments with prymnesiophytes, green algae and cyanophytes (Herzig and Dubinsky, 1993). These processes will decrease the amount of light re-emitted as fluorescence. The effects of state transitions on fluorescence (Shubert *et al.*, 1995) and on the quantum yield of photosynthesis (Schofield *et al.*, 1996) have been reported in the literature.

There is also a group of specialised carotenoids which undergoes a fast de-epoxidation when exposed to high light, which also impedes the transfer of energy to the RCs. This process, known as the xanthophyll cycle, first described in green plants (Hager, 1975; Demmig-Adams, 1990) is also present in chromophyte algae. For chromophytes, it involves the conversion of diadinoxanthin into diatoxanthin (Demers *et al.*, 1991; Olaizola and Yamamoto, 1994). Other carotenoids (e.g.,

β-carotene, zeaxanthin, astaxanthin) have also been reported to have photoprotective functions and therefore to increase in concentration at high light intensities (Prézelin, 1981; Dubinsky *et al.*, 1986; Kana *et al.*, 1988; Partensky *et al.*, 1993; Bidigare *et al.*, 1993; Lichtle *et al.*, 1995; Moore *et al.*, 1995).

If photosynthesis is to be estimated by an indirect method, e.g., emission of fluorescence, it is essential to know the distribution of energy among all the competing pathways (photochemical and non-photochemical) inside the PSU (Kiefer and Reynolds, 1992). Different models have been proposed to explain the distribution of energy inside the photosynthetic apparatus (e.g., Butler, 1978; Boardman *et al.*, 1978; Schatz *et al.*, 1988), and experiments have been made to investigate the function of different pigments and LHCs (e.g., Prézelin and Sweeney, 1978; Anderson *et al.*, 1981; Johnsen *et al.*, 1994*b*; Lichtle *et al.*, 1995). Furthermore, recent advances in the knowledge on the fine structure and function of different components of the PSU (e.g., Deisenhofer and Michel, 1988; Krauss *et al.*, 1993; McDermott *et al.*, 1995; Kühlbrandt *et al.*, 1994; Hoffmann *et al.*, 1996), and on genetic regulation of the components of the PSU (e.g., Passaquet and Lichtle, 1995; Bauer and Bird, 1996; Lichtle *et al.*, 1996), are certain to contribute towards improving our insight on the fate of the excitation energy reaching the photosynthetic membranes.

The foregoing summary indicates that the differences between fluorescence and absorption spectra need not be due entirely to the presence of photoprotective pigments. In this chapter, I present data from the North Atlantic on variations in absorption and fluorescence spectra, and examine the factors which contribute to the differences between them. To quantify these differences, I use the ratio S(439), defined as the ratio of absorption to fluorescence at 439 nm, computed after normalising both the spectra to 1 at 545 nm. I then study the relationship between the ratio S(439) and the proportion of photoprotective pigments. I also examine the relationship between S(439) and the proportions of different pigments, to see if changes in specific pigments, representing photoacclimation or changes in species composition, are related to variations in S(439) in natural populations of

phytoplankton. Finally, I investigate the relationship between S(439) and relevant environmental variables (e.g., nitrate concentrations and irradiance) to see if the influence of these factors on the physiology of phytoplankton is reflected in changes in the fluorescence emission.

3.3 Materials and Methods

3.3.1 Sampling

Details of the sampling procedure have been previously described in Chapter 2. An extensive area of the North Atlantic was covered during two cruises in spring 1993 (Canary cruise, Figure 3.1) and spring 1994 (Labrador cruise, Figure 3.1). At each station sea-water samples were collected at seven depths for the determination of pigment and nitrate concentrations, and absorption and fluorescence excitation spectra. Seventeen stations were sampled in the Canary cruise and thirteen in the Labrador cruise. Some of the samples (sometimes corresponding to a whole station and others to some of the depths) had to be discarded because of the extremely low phytoplankton content which resulted in very noisy absorption and fluorescence spectra. Finally, a total of 131 samples was available for this study.

3.3.2 Pigment determination

Samples for pigment determination were preserved in liquid nitrogen until analysis which was within a few months of each cruise. Concentrations of solvent-soluble pigments were measured by high-performance-liquid-chromatography (HPLC) following the technique described by Head and Horne (1993).

Of the main carotenoids found in the samples, 19-butanoyloxyfucoxanthin, fucoxanthin, 19-hexanoyloxyfucoxanthin and peridinin were treated in this work as photosynthetic pigments (PS), and zeaxanthin, α +β-carotene, and diadinoxanthin+diatoxanthin as photoprotective pigments (PP). Since the sample collection and processing time was longer than the period of the xanthophyll-cycle, diadinoxanthin and diatoxanthin were treated as the sum of them, diadinoxanthin+diatoxanthin. Similarly, both α-carotene and β-carotene were found only in

Figure 3.1. Location of the sampling stations.

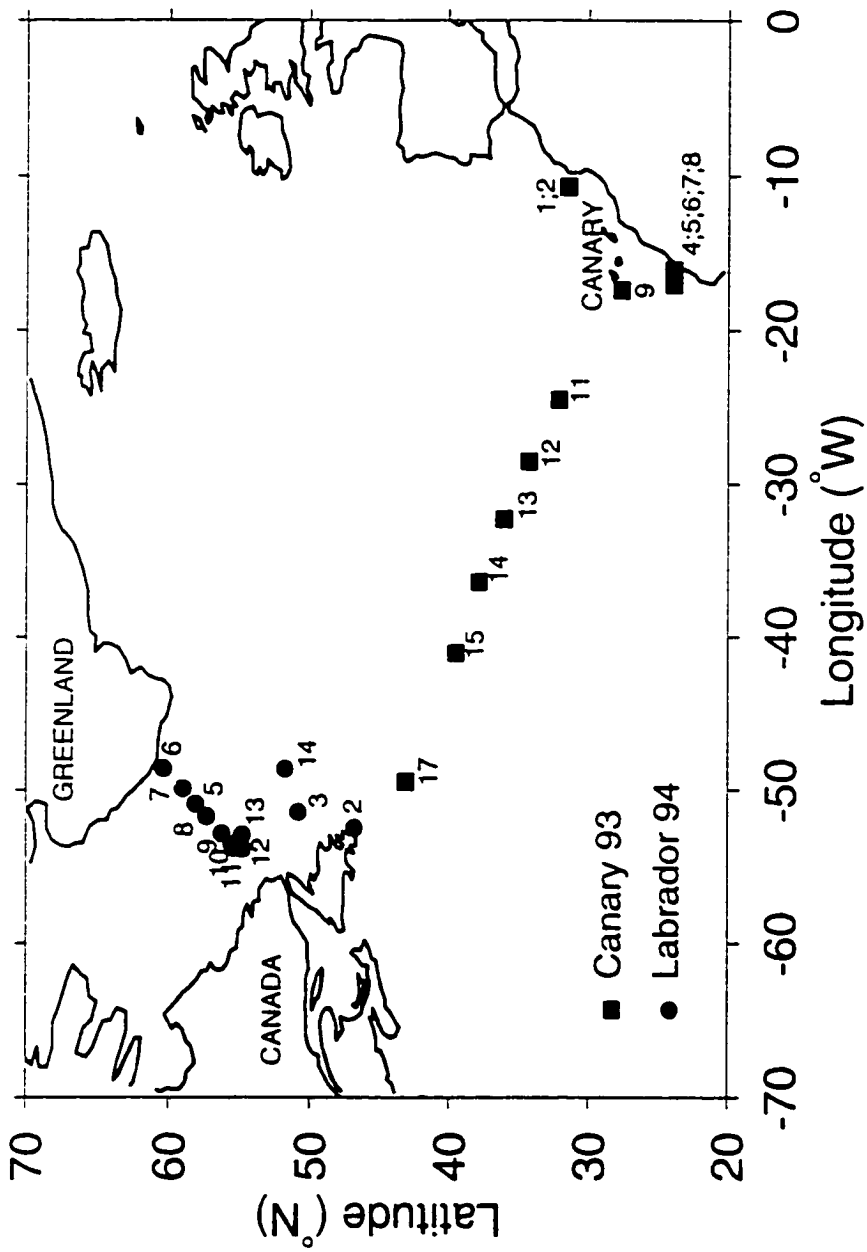
GREENLAND
CANADA
CANARY
Canary 93
Labrador 94
Latitude (°N)
Longitude (°W)
70
60
50
40
30
20
0
-10
-20
-30
-40
-50
-60
-70
1;2
4;5;6;7;8
9
11
12
13
14
15
17
6
7
5
8
9
10
11
12
13
14
3
2

Figure 3.1

minor concentrations, and were treated together in this study. Pigment concentrations for all the samples studied here are shown in Appendix B.

3.3.3 *In vivo* fluorescence excitation

Fluorescence excitation spectra were measured on board immediately after filtering the samples. Spectra were corrected for variation in the response of the excitation lamp by the method of Culver *et al.* (1994), smoothed by taking a running average over 5 nm, and corrected for variations between fluorescence of the cells in suspension and on a filter (see Chapter 2). The corrected fluorescence excitation spectra ($f_c(\lambda)$) were normalised to 1 at 545 nm ($f_c^n(\lambda)$) and the averages of two replicate measurements are reported here. Spectra are shown between 400 and 600 nm, because of calibration problems at the red end of the spectrum. Values of $f_c(\lambda)$ and $f_c^n(\lambda)$, from selected wavelengths, for all the samples analysed here are shown in Appendix B. More details on the process of the fluorescence measurements as well as the values of the correction factors ($C_x(\lambda)$ and $C_s(\lambda)$) are given in Appendix A.

3.3.4 *In vivo* absorption

Optical densities of particulate matter were measured on board on a Beckmann DU-64 spectrophotometer using the filter technique (Yentsch, 1962; Kishino *et al.*, 1985). Absorption spectra of phytoplankton ($a_{ph}(\lambda)$) were obtained from optical densities after correcting for the pathlength amplification factor, transforming decimal to natural logarithms, adjusting for the volume filtered and the area of filtration, and subtracting detrital absorption (see Chapter 2). All absorption spectra were normalised to 1 at 545 nm, a_{ph}^n, and the averages of two replicate measurements are reported here. Values of $a_{ph}(\lambda)$ and $a_{ph}^n(\lambda)$, from selected wavelengths, for all the samples analysed here are shown in Appendix B. More details on the process of the absorption measurements, including all the equations used to convert raw values of optical density to absorption of phytoplankton (e.g., corrections for the pathlength amplification factor and detrital absorption) are given in Appendix A.

3.3.5 Other measurements

During the Canary cruise, on-deck irradiance (PAR: photosynthetically available radiance, 400 - 700 nm) was continuously monitored by a radiometer (LI-COR LI-190 4π-collector). Irradiance profiles (PAR) for the Canary cruise were then calculated from the average daylight irradiance using the model of Sathyendranath and Platt (1988) and the absorption spectra of phytoplankton measured during the cruise. During the Labrador cruise, light penetration underwater (PAR) was measured by a radiometer (LI-COR LI-192 4π-collector) attached to the submersible pumping system. Relative profiles of irradiance (PAR) were computed for the two cruises, taking the value of irradiance at zero meters as 100%.

Nitrate concentration was measured using an Alpkem RFA autoanalyzer. Picoplankton composition was determined on board by flow cytometer analysis following the method described in Li (1994). Different groups of picoplankton (i.e., *Prochlorococcus* spp., *Synechococcus* spp., and picoeukaryotes) were identified by interactive analysis of multiple bivariate scatterplots as described by Olson *et al.* (1993). PAR and CTD data were kindly provided by E. Horne, nutrients by J. Anning, and flow cytometric data by W.K.W. Li. Values of σ_t, temperature, and nitrate concentrations for all the samples analysed here are shown in Appendix B. Data on picoplankton composition and irradiance at different depths in station Canary 5 are shown in Appendix B.

3.4 Results

3.4.1 Variability in absorption and fluorescence excitation spectra

The variation in the shape of the spectra (normalised to 1 at 545 nm) was high, both for the *in vivo* absorption (Figure 3.2a) and for the fluorescence excitation (Figure 3.2b) for all the samples pooled together (n=131). Considered on a percentage basis the degree of variation is similar for absorption (standard deviation

Figure 3.2. Top panel: absorption spectra (gray dotted lines) of all the samples used in this study (n=131); the average spectrum and the average $\pm$ the standard deviation are also shown (solid black lines). Bottom panel: idem but for the fluorescence excitation spectra.

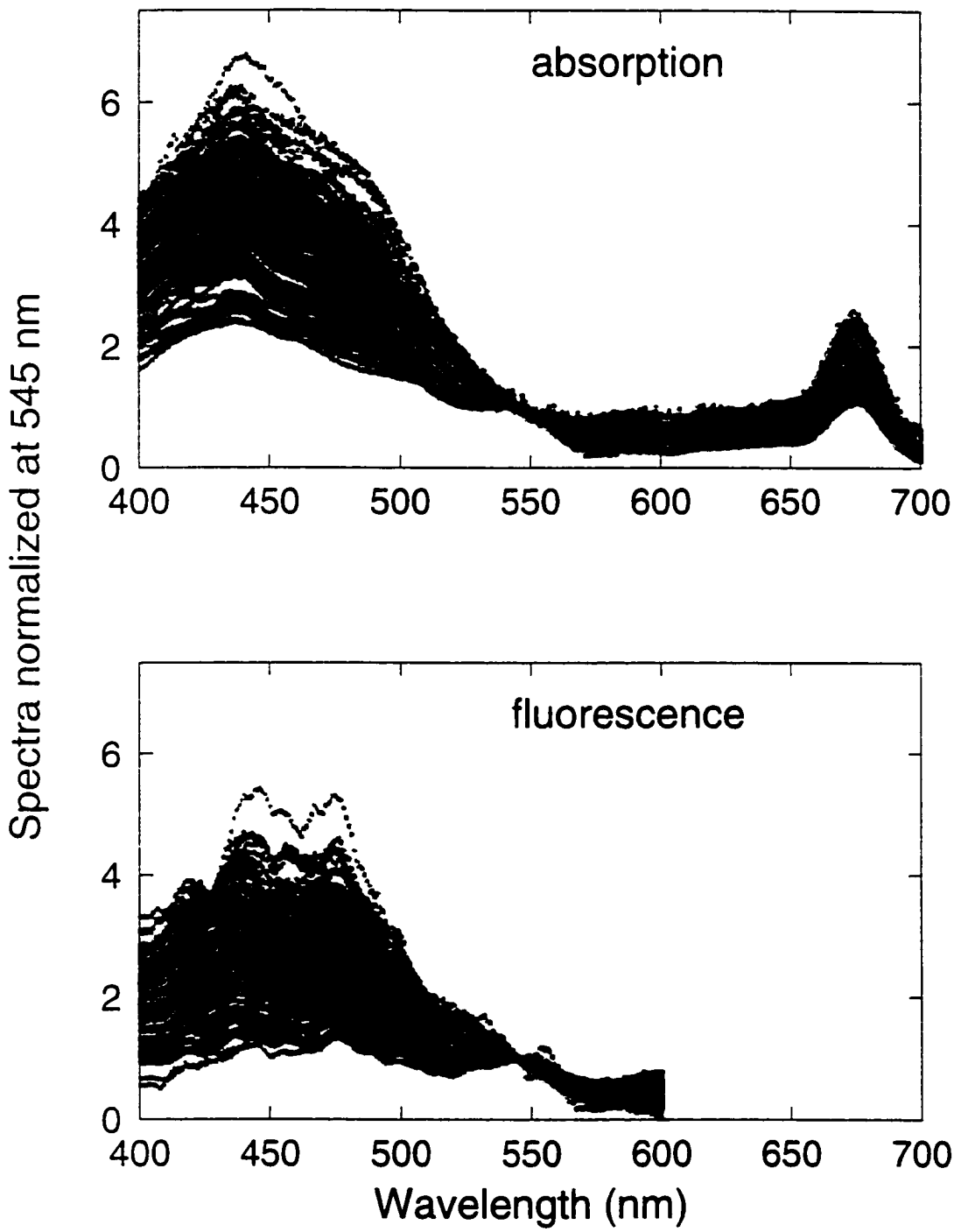
absorption
fluorescence
Spectra normalized at 545 nm
Wavelength (nm)
0
2
4
6
400
450
500
550
600
650
700

Figure 3.2

from the mean at 439 nm, $\sim$ 20%) and for the fluorescence (standard deviation from the mean at 439 nm, $\sim$ 27%).

3.4.2 Variability in the ratio of absorption to fluorescence at 439 nm

The ratio of the normalised absorption ($a^n_{ph}(\lambda)$) to the normalised fluorescence excitation ($f^n_c(\lambda)$) at the blue peak (439 nm), denoted S(439), was calculated for all the samples. To assess the variation in S(439) the samples were grouped into 5 categories according to a rank in the ratio S(439) (see Figure 3.3). The values of S(439) ranged from 1.0 (no difference between absorption and fluorescence) to 3.0 (absorption 3 times higher than fluorescence). Overall, more than 70% of the samples had large ratios of absorption to fluorescence, S(439) >1.2 (i.e., absorption higher than fluorescence by 20% or more). Closer inspection showed that $\sim$ 11% of the samples fell into group 1 characterised by a small S(439) (< 1.1), another $\sim$12% into group 2, with values of S(439) between 1.1 and 1.2, $\sim$35% belonged to group 3, with values of S(439) between 1.2 and 1.5, $\sim$24% to group 4, with S(439) values between 1.5 and 1.75, and finally $\sim$18% remained in group 5, with values of S(439) >1.75 (Figure 3.3).

This separation into ranks of S(439), though arbitrary, was aimed at pinpointing the variables which were significantly associated with variations in S(439). I investigated the distribution of environmental variables and pigment ratios in these five categories of samples. Results of the analysis of variance on the characteristic variables and *a posteriori* comparisons among groups are shown in Table 3.1. Except for the ratio of photosynthetic pigments to chlorophyll-*a*, the values of the variables did not follow a normal distribution. Therefore, except for the ratio of photosynthetic pigments to chlorophyll-*a*, a Kruskal-Wallis one way analysis of variance on ranks was performed. *A posteriori* pairwise multiple comparison among groups was carried out using Dunn's method for the non-normally distributed variables and Student-Newmann-Keuls method for the normal one: pairs of groups were considered significantly different in the distribution of a given variable when $P<0.05$.

Figure 3.3. Percentage of samples, from the pooled data, in each category of S(439).

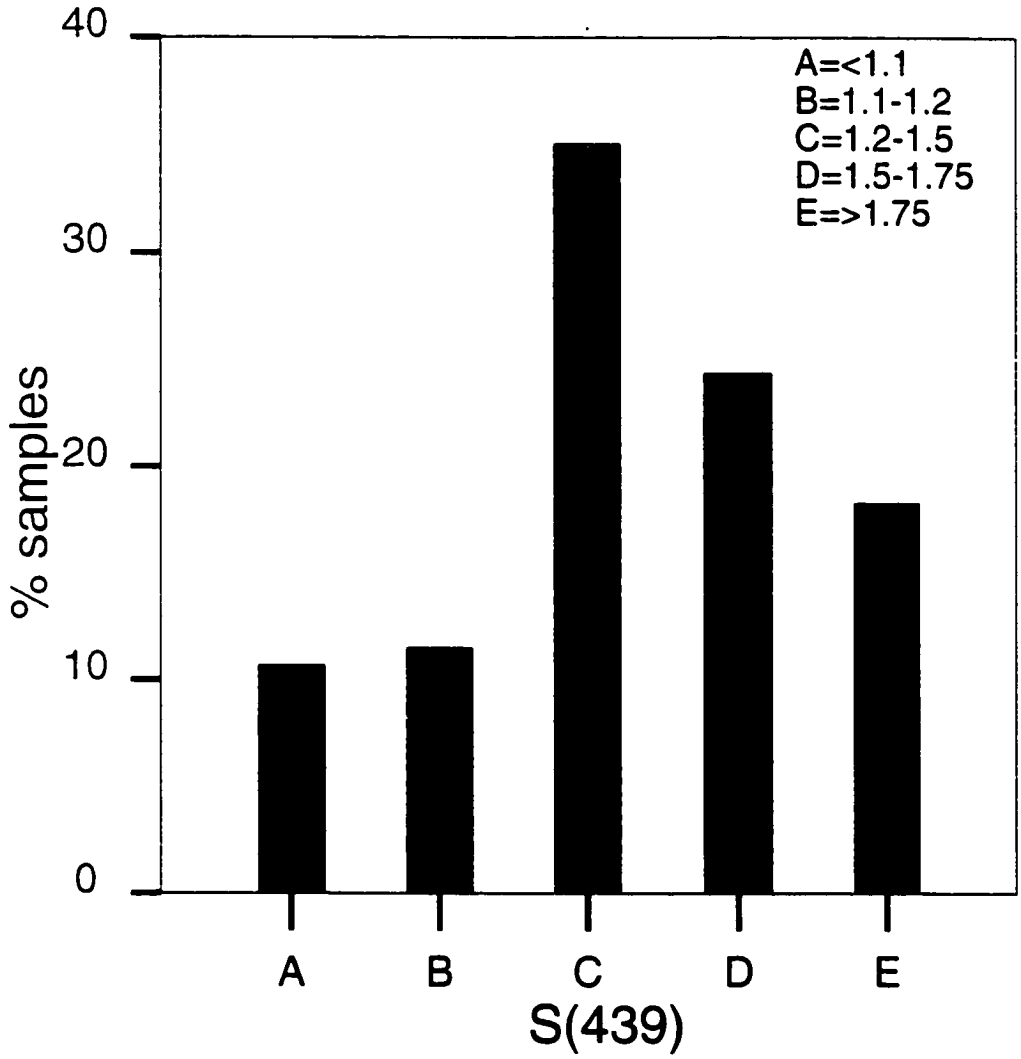

Figure 3.3

From the 16 variables analysed (Table 3.1) only 12 proved to have significantly different distributions between at least 2 groups; for the other 4 variables (the ratios of diadinoxanthin+diatoxanthin, chlorophyll-*b* and peridinin to chlorophyll-*a*; and the sum of phaeopigments) there were no significant differences among groups.

Neighbouring pairs of groups were not significantly different from each other for any of the variables considered (except for groups 4 and 5 with respect to the ratios of the sum of photosynthetic pigments (PS), and chlorophyll-c_3 to chlorophyll-*a*, which even if significantly different, represented only a minor proportion of the total pigments). This is not unexpected due to the arbitrary way in which the groups were separated. But a comparison between distant groups showed some significant differences. Group 1, with the lowest values of S(439) (<1.1) is significantly different from the group with the largest values of S(439) (> 1.75) for 8 out of 12 of the significant variables. Group 1 (S(439) <1.1) belongs to high NO_3^- and high σ_t environments with low concentrations of chlorophyll-*a*, low values of total photoprotective pigments (PP) relative to chlorophyll-*a*, (ratios of zeaxanthin and α+β-carotene to chlorophyll-*a* close to 0.0) and high ratios of PS to chlorophyll-*a* (high ratios of fucoxanthin to chlorophyll-*a*). Group 1 is also significantly different from group 4 (S(439) between 1.5 - 1.75) for 6 of the variables: NO_3^- concentration, chlorophyll-*a* concentration, and the ratios of PP, α+β-carotene, 19-butanoyloxyfucoxanthin and 19-hexanoyloxyfucoxanthin to chlorophyll-*a*. Group 2 (S(439) between 1.1 - 1.2) is significantly different from group 5 (S(439) > 1.75) in 4 of the variables; group 2 has low ratios of PP to chlorophyll-*a* (low ratios of zeaxanthin and α+β-carotene to chlorophyll-*a*) and high ratios of PS to chlorophyll-*a*. Groups 3 and 5 are also distinguished by significant differences in 8 variables; that is, samples from group 3 correspond to high σ_t environments, with a small ratio of PP to chlorophyll-*a* (low ratios of zeaxanthin and α+β-carotene to chlorophyll-*a*), and high ratios of PS (high ratio of fucoxanthin to chlorophyll-*a*), chlorophyll-c_1+c_2 and chlorophyll-c_3 to chlorophyll-*a*.

Table 3.1 Variables significantly different (Y) or not (N) in the analyses of variance among the 5 groups of samples categorised by their S(439) value (group 1: <1.1; group 2: 1.1 - 1.2; group 3: 1.2 - 1.5; group 4: 1.5 - 1.75; group 5: >1.75).

Groups	VARIABLE											
compared	NO_3^-	σ_t	ca	PP/ca	PS/ca	ze/ca	$\alpha+\beta$/ca	c_1+c_2/ca	c_3/ca	bu/ca	fu/ca	he/ca
1 vs. 5	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	N
1 vs. 4	Y	N	Y	Y	N	N	Y	N	N	Y	N	Y
1 vs. 3	Y	N	N	Y	N	N	N	N	N	N	N	Y
1 vs. 2	N	N	N	N	N	N	N	N	N	N	N	N
2 vs. 5	N	N	N	Y	Y	Y	Y	N	N	N	N	N
2 vs. 4	N	N	N	N	N	N	Y	N	N	N	N	N
2 vs. 3	N	N	N	N	N	N	N	N	N	N	N	N
3 vs. 5	N	Y	N	Y	Y	Y	Y	Y	Y	N	Y	N
3 vs. 4	N	N	N	N	N	N	N	N	N	N	N	N
4 vs. 5	N	N	N	N	Y	N	N	N	Y	N	N	N

ca : chlorophyll-*a*
PP : sum of photoprotective pigments
PS : sum of photosynthetic pigments
ze : zeaxanthin
$\alpha+\beta$: $\alpha+\beta$-carotene
c_1+c_2 : chlorophyll-c_1 + c_2
c_3 : chlorophyll-c_3
bu : 19-butanoyloxyfucoxanthin
fu : fucoxanthin
he : 19-hexanoyloxyfucoxanthin

Individual columns of Table 3.1 provide information on the relative contribution of each variable to the distinction between groups. Some variables were useful to distinguish groups only in 1 or 2 pairs. On the other hand, NO_3^- concentration, the ratios of PP, PS, zeaxanthin, and α+β-carotene to chlorophyll-a, were able to distinguish between groups in at least 3 cases. Based on this, I focused the analysis on the relationship between S(439) and these variables, which were selected for their ability to explain some of the variance in S(439), for a large change in this quantity. The other variables which do not differ significantly among groups might still explain a part of the variance in S(439) for small changes in S(439) (e.g., within a group), but this issue is not examined further in this study.

3.4.3 Relationship between S(439) and selected variables

Having singled out variables that are significantly different among the five S(439) groups, I proceeded to investigate the relationships between these selected variables and S(439). Linear regression analysis was performed. The association between variables was considered to be strong when, for an α=0.05, the power of the test fell between 0.8 and 1.0; below 0.8 the associations should be interpreted with caution. For the pooled data the highest correlations between S(439) and the selected variables were found for the ratios of : α+β-carotene ($r^2 \sim 0.51$), PP ($r^2 \sim 0.34$), and zeaxanthin ($r^2 \sim 0.27$) to chlorophyll-a (Figure 3.4). There is a tendency for S(439) to increase with an increase in the ratio of PP to chlorophyll-a and particularly with increase in the ratios of zeaxanthin and α+β-carotene to chlorophyll-a. The relationship between S(439) and NO_3^- concentration, and the ratio of PS to chlorophyll-a, have low and negative correlations (r^2 ~0.18, and ~0.11 respectively). All these correlations have low coefficients of determination, although their association between variables (power of the test >0.80) is strong. These low r^2 values indicate that no single variable is sufficient to explain the variance in S(439) for the pooled data. Thus, it is difficult to draw general conclusions from the pooled data. If we narrow down to individual stations (choosing only those stations with 4 or more available data points) the relationship between S(439) and

Figure 3.4. Linear regressions between S(439) and some selected variables. Pooled data. ca: chlorophyll-*a*; PP: sum of photoprotective pigments; ze: zeaxanthin; $\alpha + \beta$: $\alpha + \beta$-carotene; PS: sum of photosynthetic pigments; NO_3^-: nitrate concentration in μM.

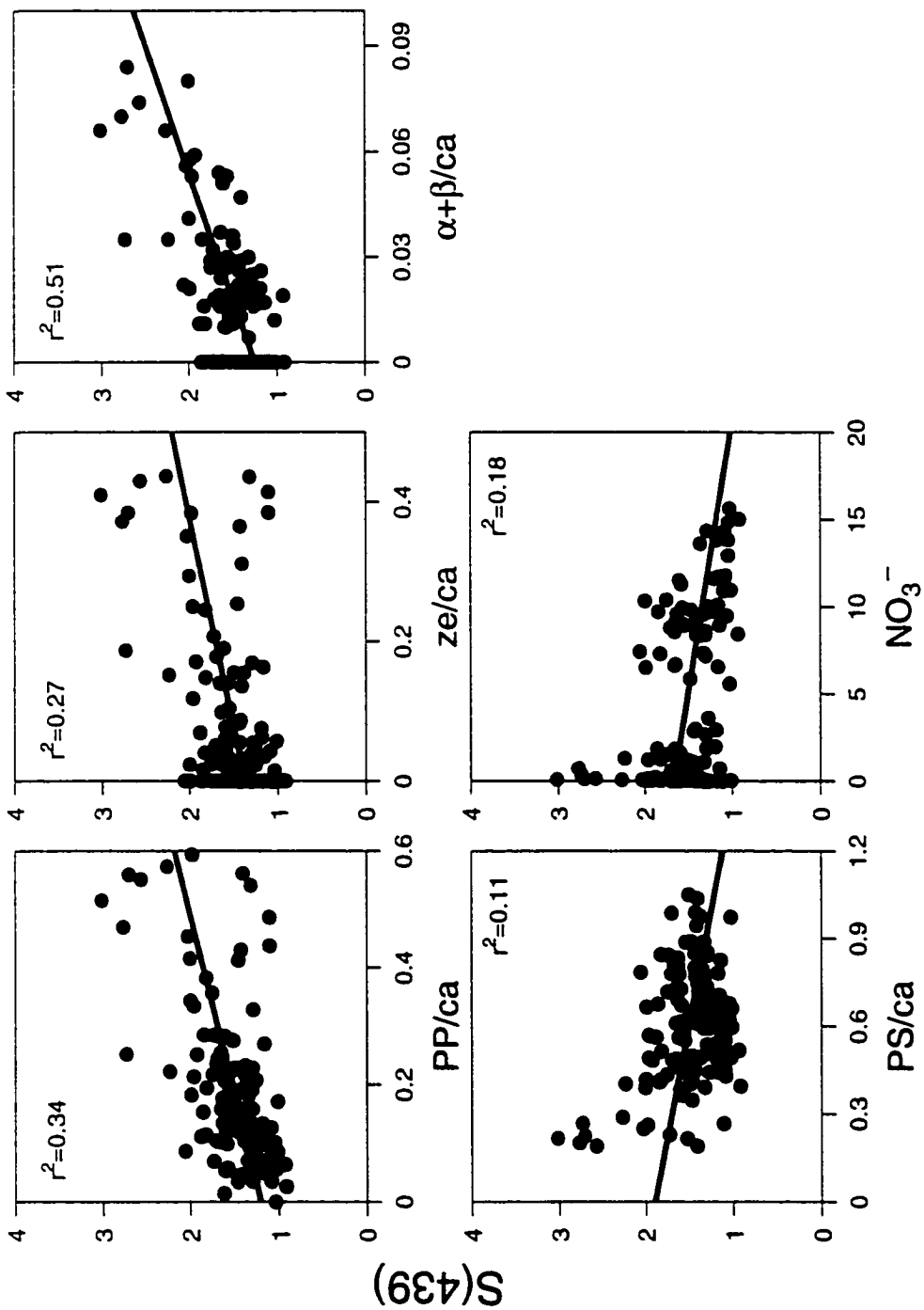

Figure 3.4

any of the selected variables shows a large range in the values of the coefficient of determination. For example, the relationship between S(439) and the ratio of PP to chlorophyll-*a* at individual stations ranged between r^2=0 and r^2=0.95.

I chose two stations from very different environments (station Canary 5 and station Labrador 9. Figure 3.1) for a more detailed analysis of the relationship among S(439), pigment composition, phytoplankton composition (when available), and some environmental variables (NO_3^- concentration and irradiance). Changes in the shape of the spectra as well as changes in the magnitude of S(439) are readily seen in the absorption and fluorescence excitation spectra (both normalised to 1 at 545 nm) from different depths for the two selected stations (Figure 3.5). These two stations are discussed in detail below.

3.4.4 Station Canary 5

Profiles of main variables: Station Canary 5 was situated close to the Canary Islands (Figure 3.1). The σ_t profile shows stratification, with an upper mixed layer separated by a pycnocline at $\sim$ 20 m from an intermediate layer which was in turn separated by a second pycnocline at $\sim$ 40 m from a region of gradual increase in σ_t with depth (Figure 3.6a). Nitrate concentrations were almost nil in the surface layer; there was a nitracline in the intermediate layer, after which NO_3^- concentrations gradually increased with depth (Figure 3.6a). The chlorophyll-*a* maximum ($\sim$2.0 μg/l) was located at the bottom of the intermediate layer ($\sim$ 40 m), immediately below the nitracline ($\sim$ 30 m) (Figure 3.6a). All of the sampled depths were located above the 1% surface irradiance (irradiance I at depth z, I_z=1.6% I_0 at 75 m) (Figure 3.6a). The profiles of the ratios of PS and PP to chlorophyll-*a* were relatively featureless throughout the upper mixed layer after which the ratio of PS to chlorophyll-*a* increased and that of PP decreased with depth (Figure 3.6a). The S(439) profile had a maximum at $\sim$ 20 m after which it steadily decreased with depth (Figure 3.6a). Figure 3.6c depicts the profiles of the ratio of the main photosynthetic and photoprotective carotenoids to chlorophyll-*a*. From these profiles the predominance of zeaxanthin among the photoprotective carotenoids becomes

Figure 3.5. Absorption and fluorescence excitation spectra from different depths at stations Canary 5 and Labrador 9. The two types of spectra are normalised to 1 at 545 nm.

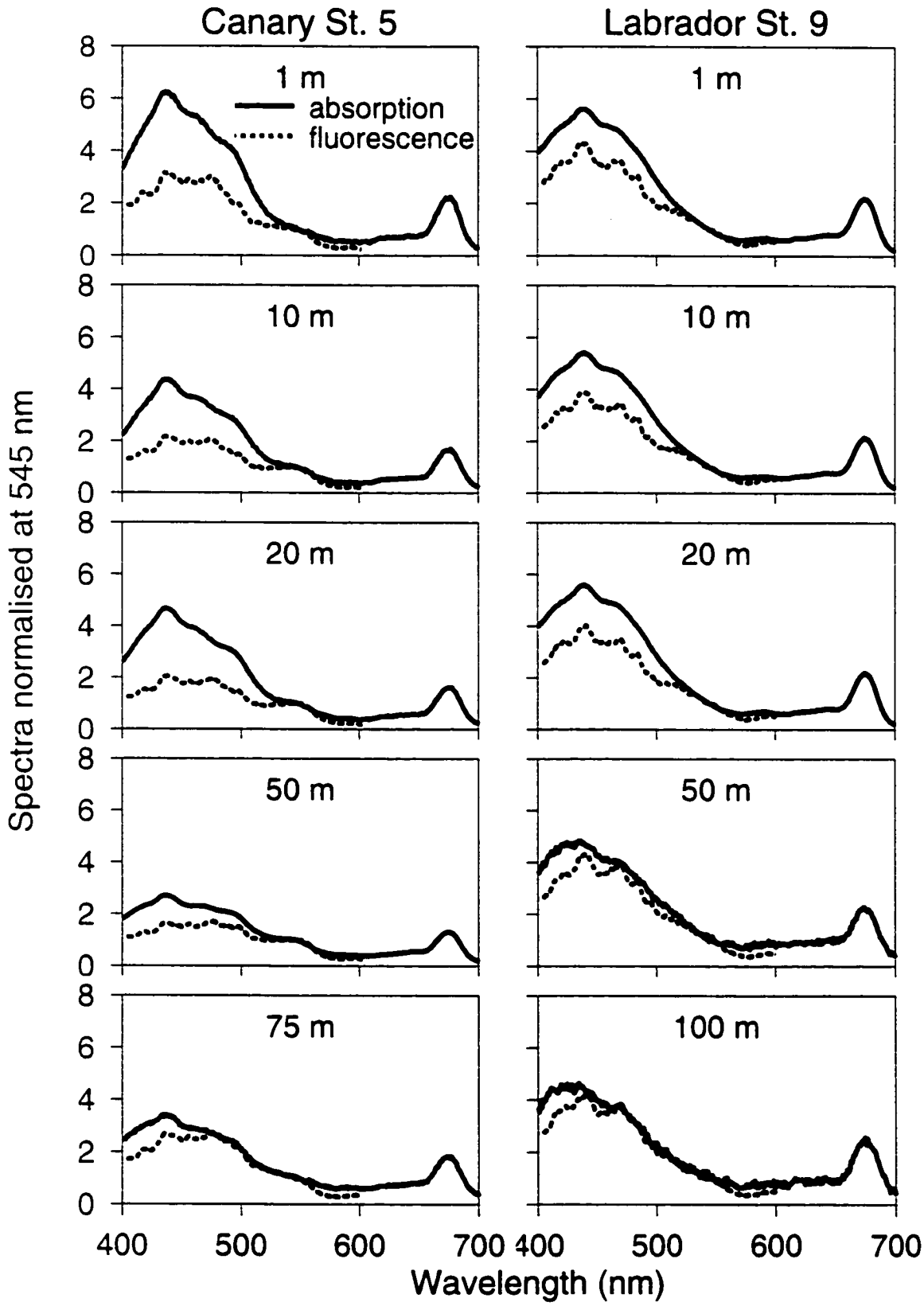
Canary St. 5
Labrador St. 9
1 m
absorption
fluorescence
10 m
20 m
50 m
75 m
100 m
Spectra normalised at 545 nm
Wavelength (nm)
0
2
4
6
8
400
500
600
700

Figure 3.5

Figure 3.6. Profiles of main variables in two selected stations: Canary 5 and Labrador 9. σ_t: density: I_z: irradiance at depth z (% surface irradiance): NO_3^-: nitrate concentration (μM): ca: chlorophyll-*a* (mg m^{-3}): S(439): absorption over fluorescence at 439 nm: PS: sum of photosynthetic pigments: PP: sum of photoprotective pigments: ze: zeaxanthin: he: 19-hexanoyloxyfucoxanthin: va: divinyl-chlorophyll-*a*: d+t: diadinoxanthin+diatoxanthin: fu: fucoxanthin.

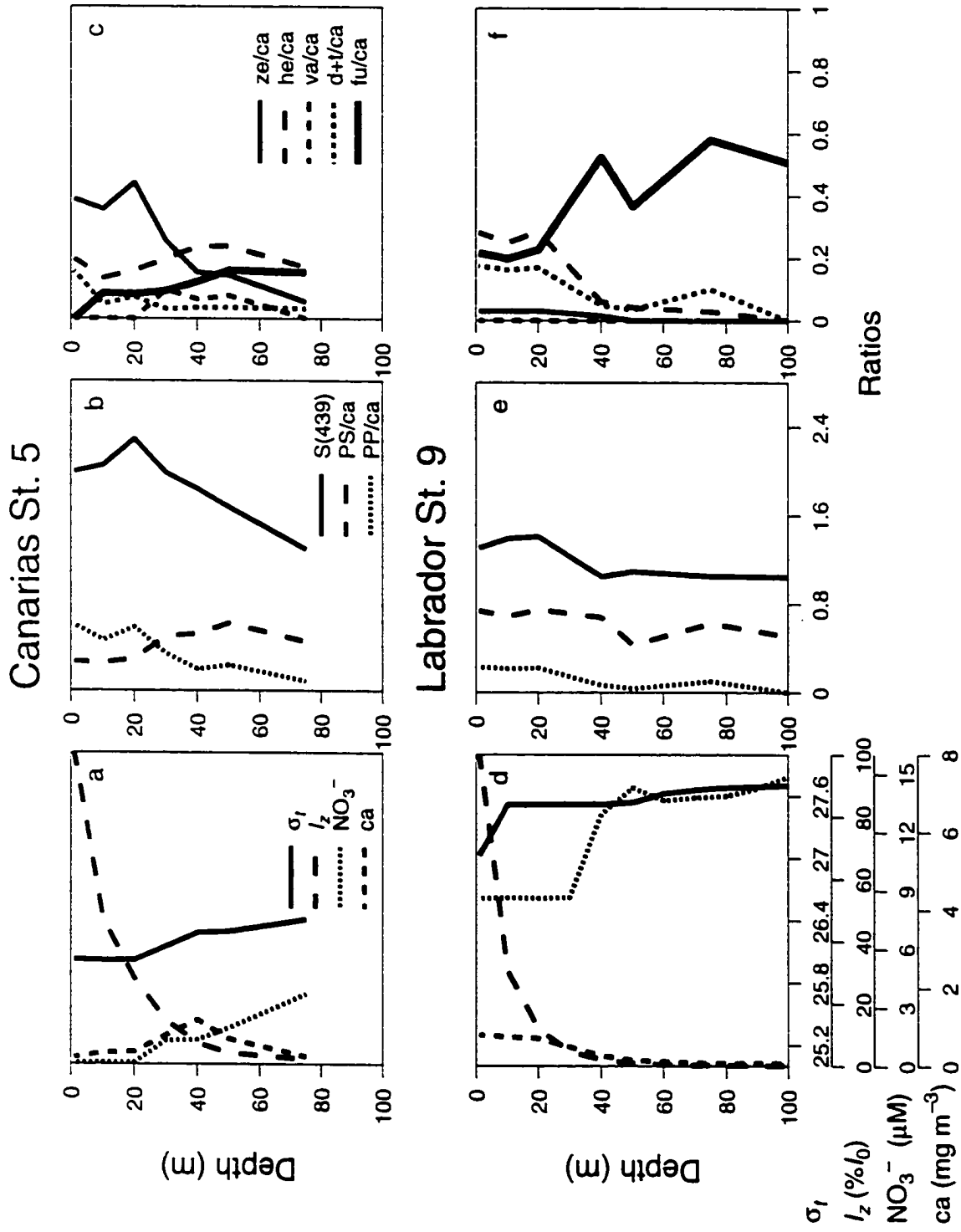
Canarias St. 5
Labrador St. 9
Depth (m)
Ratios
a
b
c
d
e
f
σt
Iz
NO3-
ca
S(439)
PS/ca
PP/ca
ze/ca
he/ca
va/ca
d+t/ca
fu/ca
σt
Iz (%I0)
NO3- (μM)
ca (mg m-3)

Figure 3.6

evident, with the maximum ratio of zeaxanthin to chlorophyll-*a* at ~ 20 m. Low values of the ratio of diadinoxanthin+diatoxanthin to chlorophyll-*a* had a maximum at the surface.

Among the photosynthetic carotenoids, 19-hexanoyloxyfucoxanthin and fucoxanthin were predominant, and their ratios to chlorophyll-*a* increased with depth. Finally, to represent the presence of prochlorophytes, the ratio divinyl-chlorophyll-*a* to chlorophyll-*a* is shown, indicating a minor contribution of divinyl-chlorophyll-*a* to the total chlorophyll-*a* between 20 and 75 m.

Relationship among variables: At this station (Canary 5) there was good correlation between S(439) and the ratio PP to chlorophyll-*a* (r^2 ~0.74), and between S(439) and the ratio of zeaxanthin to chlorophyll-*a* (r^2 ~0.84) (Table 3.2). There were also correlations between the ratio of PP to chlorophyll-*a* and irradiance at depth (I_z) (r^2 ~0.63), and the ratio of zeaxanthin to chlorophyll-*a* and I_z (r^2 ~0.50) (Table 3.2). These correlations (except for that between S(439) and the ratio of zeaxanthin to chlorophyll-*a*) should be interpreted with caution because the powers of these tests are below the desired level (<0.80; see Table 3.2). On the other hand, the highest correlations were found between the ratio of zeaxanthin to chlorophyll-*a* and the percentage of cyanophytes in the sample (r^2 ~0.92) and between S(439) and the percentage of cyanophytes in the sample (r^2 ~0.87), and both of these relationships show a strong association between the variables (power of the test >0.80) (Table 3.2). There was also a positive correlation between S(439) and the ratio of $\alpha + \beta$-carotene to chlorophyll-*a* (r^2 ~0.61) although it was not very strong (power of the test ~0.56). There were also low degrees (r^2 <0.42) of negative correlation between S(439) and the proportion of photosynthetic carotenoids (i.e., their sum PS or individual pigments such as fucoxanthin or 19-hexanoyloxyfucoxanthin relative to chlorophyll-*a*), which were also not strong (power of the test <0.80). Finally there was a close (r^2 ~0.89) negative correlation between S(439) and the concentration of NO_3^- which was strong (power of the test ~0.94).

Table 3.2 Correlations between selected variables in the station Canary 5. Number of observations = 7.
* desired power of test: 0.80

Variables	r^2	*Power of test
S(439) vs. PP/ca	0.74	0.74
PP/ca vs. I_z	0.63	0.58
S(439) vs. ze/ca	0.84	0.87
ze/ca vs. I_z	0.50	0.42
ze/ca vs. %cyan.	0.92	0.97
S(439) vs. %cyan.	0.87	0.92
S(439) vs. $\alpha+\beta$/ca	0.61	0.56
S(439) vs. PS/ca	0.31	0.24
S(439) vs. fu/ca	0.42	0.34
S(439) vs. NO_3^-	0.89	0.94

PP : sum of photoprotective pigments
PS : sum of photosynthetic pigments
ze : zeaxanthin
%cyan. : % of cyanophytes
$\alpha+\beta$: $\alpha+\beta$-carotene
ca : chlorophyll-a
fu : fucoxanthin
I_z : irradiance at depth z

3.4.5 Station Labrador 9

Profile of main variables: This station located in the middle of the Labrador Sea (Figure 3.1) showed a subsurface pycnocline at $\sim$ 10 m (mainly associated with changes in salinity, data not shown) after which the density profile was uniform. Nitrate concentrations were relatively high throughout the water column, though a marked nitracline at $\sim$ 30 m separated the upper mixed layer from a zone of high NO_3^- concentrations at depth. The chlorophyll-*a* concentration was uniform ($\sim$ 1.0 μg/l) up to $\sim$ 20 m after which it decreased with depth. The bottom of the euphotic zone (I_z=1% I_0), was located at $\sim$ 50 m (Figure 3.6d). The profile of the ratio PS to chlorophyll-*a* was relatively uniform with depth, while that of PP to chlorophyll-*a* and S(439) showed the highest values in the upper layer up to $\sim$ 20 m (Figure 3.6e). The main photosynthetic carotenoids were 19-hexanoyloxyfucoxanthin in the upper layer (up to $\sim$ 20 m) and fucoxanthin at depth (increasing below 20 m). Zeaxanthin was a minor component and the ratio of diadinoxanthin+diatoxanthin to chlorophyll-*a* showed a relatively uniform profile with two small maxima at $\sim$ 20 m and 75 m (Figure 3.6f); divinyl-chlorophyll-*a* was non-detectable.

Relationship among variables: This station showed a strong positive correlation between S(439) and the ratio of PP to chlorophyll-*a* (r^2 <0.84, power of the test 0.88), and specifically between S(439) and the ratio of diadinoxanthin+diatoxanthin to chlorophyll-*a* (Table 3.3). However, the correlations between the ratio of PP to chlorophyll-*a* and I_z and diadinoxanthin+diatoxanthin to chlorophyll-*a* and I_z were low and not strong (see Table 3.3). On the other hand, the correlations between S(439) and the main photosynthetic carotenoids (ratios of fucoxanthin and 19-hexanoyloxyfucoxanthin to chlorophyll-*a*) were high and strong (Table 3.3). Contrary to expectations, however, the relationship between S(439) and 19-hexanoyloxyfucoxanthin to chlorophyll-*a* was positive, which lead to a low correlation between S(439) and the ratio of the total PS to chlorophyll-*a* (Table 3.3).

Table 3.3 Correlations between selected variables in the station Labrador 9. Number of observations = 7.
* desired power of test: 0.80

Variables	r^2	*Power of test
S(439) vs. PP/ca	0.84	0.88
PR/ca vs. I_z	0.45	0.36
S(439) vs. d+t/ca	0.78	0.79
d+t/ca vs. I_z	0.42	0.33
S(439) vs. PS/ca	0.44	0.36
S(439) vs. fu/a	0.87	0.91
S(439) vs. he/ca	0.93	0.98
S(439) vs. NO_3^-	0.91	0.96

PP : sum of photoprotective pigments
PS : sum of photosynthetic pigments
d+t : diadinoxanthin+diatoxanthin
he : 19-hexanoyloxyfucoxanthin
ca : chlorophyll-*a*
I_z : irradiance at depth z
fu : fucoxanthin

The correlation between S(439) and NO_3^- concentration is again high, strong and negative (r^2 <0.91, power of the test 0.96) in this station.

3.5 Discussion

3.5.1 Normalisation of the fluorescence and absorption spectra

The first issue to solve before comparing the shape of the *in vivo* absorption and fluorescence excitation spectra of phytoplankton, is how to normalise the spectra. According to the theory of energy transfer, the shape of the normalised fluorescence excitation spectrum should be at the most equal (i.e., assuming that the action of PSII exactly represents the total action spectrum, and that there are no photoprotective pigments or other non-photochemical processes of quenching of energy working in the cell) or lower (i.e., if any of the previous assumptions is not valid) than the absorption spectrum.

Elsewhere absorption and fluorescence excitation spectra of phytoplankton have been used to calculate ratios of absorption, and of fluorescence, at pairs of wavelengths (Neori *et al.*, 1984; SooHoo *et al.*, 1986; Mitchell and Kiefer, 1988*b*). However, most of these early works did not make the detritus correction to obtain "clean" phytoplankton absorption. Despite this, conspicuous features in these reports were the larger changes in the [green: blue] ratio for the fluorescence excitation spectra than for the absorption spectra. These were attributed to a higher efficiency of energy transfer to chlorophyll-*a* at the wavelength of absorption corresponding to the main light-harvesting pigments. Therefore, if both (absorption and fluorescence) spectra were normalised to 1 at a peak of chlorophyll-*a* absorption, the fluorescence spectrum would overshoot that of absorption in the green region. Indeed, such an effect has been shown by Johnsen and Sakshaug (1993) working with corrected spectra of a dinoflagellate normalised to 1 at 676 nm. These authors also attributed this disparity between absorption and fluorescence to a differential efficiency in the transfer of energy by the different pigments at different wavelengths. They concluded, based on existing literature, that the transfer of energy to PSII

chlorophyll-*a* at 676 nm should have been ~ 85% of the absorption. Johnsen and Sakshaug (1993) also mentioned that a part of the difference between the absorption and fluorescence spectra could be caused by an imbalance in the energy distribution between the two photosystems.

If we compare the fluorescence excitation spectra of rhodophytes, cryptophytes, or cyanophytes reported in the literature (Neori *et al.*, 1986; 1988; Johnsen and Sakshaug, 1996) with their corresponding absorption spectra, both normalised to 1 at a maximum of chlorophyll-*a* absorption, it is obvious that a serious overshooting of the fluorescence over the absorption spectra would be apparent in the region of absorption of phycobilins. This is because chlorophyll-*a* associated with PSI is "invisible" to the fluorescence excitation spectrum. Recently, Johnsen and Sakshaug (1996), reviewing the question of scaling, have reinforced the criterion of normalising the fluorescence spectra to the red peak of absorption according to the percentage of chlorophyll-*a* in PSII. This procedure would be useful for work with cultures, provided the distribution of chlorophyll-*a* between photosystems is known, but the problem would still remain for field samples of mixed (and sometimes unknown) species composition. These authors have also pointed out that normalising the absorption and fluorescence spectra to the region of absorption by fucoxanthin (520 - 535 nm) would avoid the overshooting of fluorescence over absorption.

In this study I decided to normalise both fluorescence excitation and absorption spectra to 1 at 545 nm to ensure that no overshooting of fluorescence over absorption would occur, and to be able to compare the two types of spectra quantitatively at the blue peak using the ratio S(439). This wavelength (545 nm) corresponds to one of the peaks of absorption of phycoerythrin, which was an important light-harvesting pigment in these samples (according to emission fluorescence spectra, see Chapter 2). In addition, absorption spectra of pigment-protein complexes show that absorption at 545 nm is dominated by complexes containing photosynthetic carotenoids (Anderson *et al.*, 1981; Bidigare *et al.*, 1990*b*; Nelson and Prézelin, 1990; Johnsen *et al.*, 1994*b*). This normalisation carries the implicit assumption that all the light

absorbed at 545 is harvested by photosynthetic pigments and that they transfer the energy captured with 100% efficiency exclusively to chlorophyll-*a* from PSII. If part of the light absorbed at 545 nm is not transferred to chlorophyll-*a* of PSII, normalisation will underestimate the ratio between absorption and fluorescence. It is recognised, however, that the use of a single wavelength for the normalisation (545 nm) which for some spectra may correspond to a low value of absorption, plus the errors associated with the corrections of both absorption and fluorescence spectra, might introduce errors in the estimation of S(439). Nevertheless, after testing different normalisations, this one seemed to be the most appropriate for these data, especially because of the presence of phycoerythrin in many of the samples. More laboratory research, using species with different arrangements of the photosynthetic apparatus and pigment composition, may help to test further the efficacy of different normalisation procedures.

3.5.2 General variability in S(439)

The first observation emerging from this study is that there is a large variation in the values of S(439) (Figure 3.3), which was due as much to variations in the *in vivo* absorption spectra as to variations in the fluorescence excitation spectra (Figure 3.2). This has implications in the use of fluorescence for the estimation of photosynthesis, i.e., the caveats on the use of a fixed shape for the absorption spectrum of phytoplankton in the spectral computations of primary production, would also apply to the use of a fixed shape for the fluorescence excitation spectrum. It should be mentioned, though, that part of the variability in the values of S(439) might be caused by differences in the factors used to estimate absorption and fluorescence in suspension from the respective values on a filter (see methods).

According to the analysis of variance, the group corresponding to the lower values of S(439) is characterised by conditions mostly encountered at depth in the ocean (i.e., high σ_t, low chlorophyll-*a* concentrations, low values of photoprotective pigments (PP) relative to chlorophyll-*a*, high values of photosynthetic pigments (PS) relative to chlorophyll-*a*, high NO_3^- concentrations), compared with the conditions

characterising the group of samples with the highest values of S(439). Thus, there is a general agreement with the basic concept of photoacclimation (Falkowski *et al.*, 1994). On the other hand, the attempts made to classify groups by depth or by geographical region did not yield statistically significant differences. Nevertheless, some general trends were evident: the highest values of S(439) were found close to the west coast of Africa (Canary Islands) while the lowest values of S(439) were found close to the east coast of North America (The Grand Banks of Newfoundland) and in the middle of the Labrador Sea. These results contrast somewhat with the distribution of fluorescence efficiency across the North Atlantic reported by Olaizola *et al.* (1996) for the same cruise (Canary 1993), although differences in the method of measuring fluorescence and in the normalisation of the data do not allow a direct comparison.

3.5.3 Relationship between photoprotective pigments and S(439)

The function of photoprotective pigments in plants and algae has been well studied (Prézelin, 1981; Dubinsky *et al.* 1986; Siefermann-Harms, 1987; Demmig-Adams, 1990; Demers *et al.*, 1991; Bidigare *et al.*, 1993; Olaizola and Yamamoto, 1994). The absorption of light by these pigments has been proposed to be the main cause of difference between the absorption and fluorescence excitation spectra (e.g., Culver *et al.*, 1994; Sosik and Mitchell, 1995; Lazzara *et al.*, 1996) and for low quantum yields of photosynthesis (e.g., Babin *et al.*, 1995; 1996). In this study, for all the samples pooled together, the proportion of photoprotective pigments (PP-to-chlorophyll-*a* ratio) could explain only ~34% of the variance in S(439) (Figure 3.4). This indicates that differences between fluorescence emission and absorption are probably determined by more than one factor. On the other hand, pigments tend to covary with each other, and a certain degree of covariance can be expected amongst environmental variables (e.g., nutrient concentrations, irradiance, σ_t, etc.) as well. Therefore, as a first approach, I analysed individual relationships between S(439) and the variables considered, rather than attempting multiple regressions.

Of all the pigments, those able to explain a large part of the variance in S(439) were: zeaxanthin, whose function as a photoprotective pigment has been widely reported (e.g., Kana *et al.*, 1988; Moore *et al.*, 1995; Uhrmacher *et al.*, 1995) and $\alpha + \beta$-carotene (note that α-carotene was only present in a few samples where prochlorophytes were present), whose photoprotective function in the core of the RCs (Prézelin, 1981; Larkum and Barret, 1983) and in intrathylakoid globules (Ben-Amotz *et al.*, 1982; 1989; Sánchez-Saavedra *et al.*, 1996) has been repeatedly mentioned (Table 3.1, Figure 3.4).

3.5.4 Relationship between some selected variables and S(439)

The decrease in S(439) associated with the increase in the proportion of photosynthetic pigments (ratio of PS to chlorophyll-*a*), can be expected to be concomitant with a decrease in the ratio of PP to chlorophyll-*a* with a decrease in light. A relationship between variations in fluorescence emission and nitrate concentration has been noticed in physiological studies on cultures (Geider *et al.*, 1993; Kolber *et al.*, 1988; Sosik and Mitchell, 1991) where it was shown that nutrient stress can produce changes in the PSUs, and consequently in the transfer of energy, similar to those induced by photoacclimation to high light levels. Furthermore, some authors have related variations in the fluorescence excitation spectra (Sosik and Mitchell, 1995; Lazzara *et al.*, 1996) or fluorescence efficiency (Babin *et al.*, 1996; Olaizola *et al.*, 1996; Graziano *et al.*, 1996) to the nitrate environment (distance to the nitracline, or NO_3^- concentration) in the ocean.

3.5.5 Possible causes of variations in S(439) in two particular environments

The linear relationship between S(439) and each of the main variables for individual stations showed a wide range in the values of the coefficients of determination (e.g., r^2 from 0 to 0.95 for PP/ca vs. S(439)). This again implies that differences between fluorescence and absorption spectra cannot be explained by a single factor (e.g., ratio of PP to chlorophyll-*a*). Rather, they seem to be related, directly or indirectly, to different variables, which can be broadly attributed to variations in

species composition and the physiological state of the cells. Nevertheless I cannot rule out that a part of the reason for the low correlation between S(439) and the selected variables at certain stations might be due to errors associated with the correction of the spectra and the normalisation procedure. In particular, there are some cases where some clear relationships can be drawn between S(439) and certain variables.

Two oceanic stations were selected for detailed analyses where characteristic relationships between fluorescence and selected variables were very apparent. The most striking feature in the results was the high degree of covariance between S(439) and the PP-to-chlorophyll-*a* ratio, and more specifically the zeaxanthin-to-chlorophyll-*a* ratio at the Canary 5 station, which may be a perfect example of the effect of non-photochemical quenching of energy by photoprotective pigments. On the other hand, the proportion of cyanophytes in the phytoplankton assemblage, which is related to the presence of zeaxanthin in this station, had an even higher degree of covariance with S(439). Hence, this station also represents an example of the imbalance in chlorophyll-*a* content between photosystems in cyanophytes. This effect has been noticed already in the ocean (Lewis *et al.*, 1988). Besides, at station Canary 5 the variations in irradiance explain only half of the variance in the ratio of zeaxanthin to chlorophyll-*a* and with a low level of strength, while the proportion of cyanophytes in the sample explains >90% of the variance in the ratio of zeaxanthin to chlorophyll-*a* (Table 3.2). Therefore, the ratio zeaxanthin to chlorophyll-*a* might be more an indication of the number of cyanophytes than of a change in the photoacclimation of the cells. Thus, the effect of differences in energy distribution between photosystems, represented here by the presence of a group with an extreme case of imbalance, can be at least as important as the effect of photoprotective pigments in producing differences between absorption and fluorescence at the blue peak.

At the Labrador 9 station, although direct information on species composition was not available, the pigment composition indicated the presence of mainly

diatoms (chlorophyll-c_1 + c_2, fucoxanthin) and prymnesiophytes (chlorophyll-c_3, 19-hexanoyloxyfucoxanthin, 19-butanoyloxyfucoxanthin). There was a high degree of covariance between S(439) and the ratio PP to chlorophyll-*a* at this station, but the most interesting feature was the positive covariance between S(439) and 19-hexanoyloxyfucoxanthin to chlorophyll-*a*, which was also evident at other stations (data not shown). This is contrary to what might be expected, since 19-hexanoyloxyfucoxanthin is a light-harvesting pigment. It is possible that this positive covariance between S(439) and the ratio of 19-hexanoyloxyfucoxanthin to chlorophyll-*a* might reflect the low efficiency of energy transfer by 19-hexanoyloxyfucoxanthin (Van Leeuwe and Stefels, 1998).

The knowledge on the structure of the photosynthetic apparatus and on the energy distribution inside it, comes mainly from studies of terrestrial plants, of photosynthetic bacteria, and to a lesser extent, of algae. According to the available information the regulation of the transfer of energy inside the photosynthetic membranes is a very dynamic process, and different groups of organisms have evolved to handle it in different ways. Hence, even if there are some species in which most of the chlorophyll-*a* is associated with PSII, there is no reason to believe that this should be the general case. In addition, energy distribution might also be subject to state-transitions.

3.6 Concluding Remarks

In this study I found that differences between absorption and fluorescence in natural samples of phytoplankton in the North Atlantic were highly variable, and that this variation could not be explained solely by the presence of photoprotective pigments. Furthermore, I found evidence that differences in energy distribution between PSI and PSII can be also a contributing factor to differences between absorption and fluorescence spectra in the ocean.

Finally, these results support the idea that the correct use of fluorescence measurements (fluorescence excitation spectra or pump-and-probe, pulse amplitude

modulation, etc.) for the estimation of primary production should take into account all the possible causes of fluctuations in fluorescence (e.g., Kiefer and Reynolds, 1992; Govindjee, 1995; Cunningham, 1996). Fluorescence techniques provide a powerful tool for the study of photophysiology of phytoplankton and they will help to improve our understanding of the physiology of photosynthesis in the ocean. The natural environment is a complex system, and interpretation of observations from different oceanic regions is not straightforward. There is certainly a need for more laboratory experiments under controlled conditions to investigate further the process of energy transfer in the photosystems in different species of phytoplankton.

This objective will be addressed in the next chapter, where I investigate the causes of variations in the shapes of the absorption and fluorescence excitation spectra in cultures of two species of diatom and one species of cyanophyte grown at different irradiances.

CHAPTER 4

Changes in the *In Vivo* Absorption and Fluorescence Excitation Spectra with Growth Irradiance in Three Species of Phytoplankton

4.1 Introduction

In the visible part of the spectrum, which is important for photosynthesis, the optical characteristics of phytoplankton are determined by their pigment composition, as well as the manner in which they are packaged into the cell. Pigments do not occur free in solution inside cells, but are bound to specific proteins, forming pigment-protein-complexes or chromoproteins (Prézelin, 1981; Larkum and Barrett, 1983; Porra *et al.*, 1987). These are the main component of the photosynthetic apparatus, which is located in the thylakoid membranes. One definition of the photosynthetic unit (PSU) is the smallest unit able to carry out photosynthesis (Prézelin, 1981). Chromoproteins are arranged in a highly specific way forming different components within the PSU. These main components are (see Figure 4.1): the light-harvesting-complexes (LHCs), the photosystems (PSI and PSII), which in turn are composed of a reaction center (RCI and RCII) and a subantenna, or intrinsic antenna, (reviewed in Prézelin, 1981; Larkum and Barrett, 1983; Prézelin and Boczar, 1986).

The absorption spectrum of a particular species of phytoplankton represents the contributions of the individual absorptions of all of the pigment-protein-complexes present in the cell, as well as other absorbing materials. Light-harvesting-complexes make the bulk of the pigment content of the cell and, therefore, they are the main agents responsible for absorption. The fact that pigments are enclosed within a cell forming intricate structures also affects their optical characteristics. This is known as the "packaging effect" (Duysens, 1956) and it means that cell size and intracellular concentration of pigments also influence phytoplankton absorption (Morel

Figure 4.1. Models to describe the arrangement of the photosynthetic apparatus and energy flow inside it (as indicated by the arrows). **a:** "lake" model. **b:** "puddle" model. **c:** special case of "puddle" model corresponding to cyanophytes. Modified from Knox (1975) and Larkum and Barrett (1983).

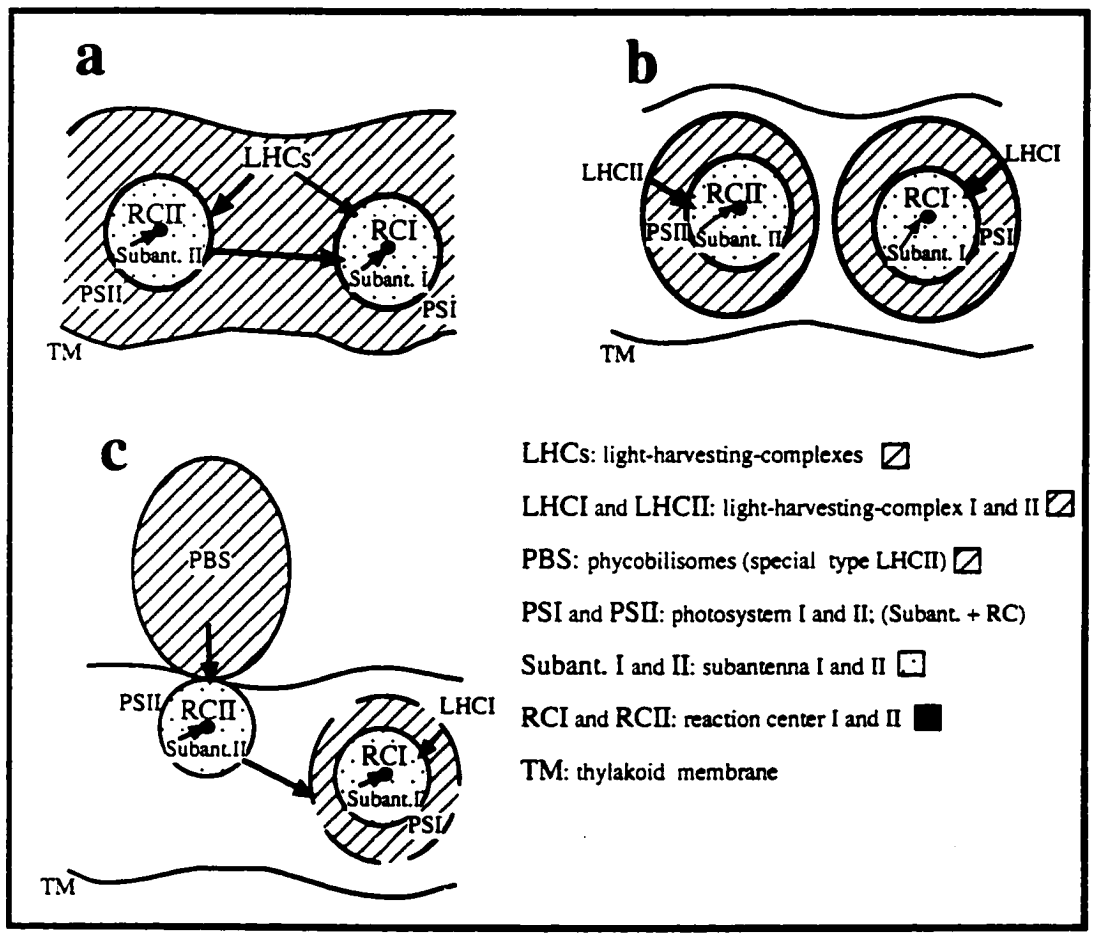
a
LHCs
RCII
Subant. II
RCI
Subant. I
PSII
PSI
TM
b
LHCII
RCII
PSII
Subant. II
RCI
LHCI
Subant. I
PSI
TM
c
PBS
PSII
RCII
Subant.II
LHCI
RCI
Subant.I
PSI
TM
LHCs: light-harvesting-complexes
LHCI and LHCII: light-harvesting-complex I and II
PBS: phycobilisomes (special type LHCII)
PSI and PSII: photosystem I and II; (Subant. + RC)
Subant. I and II: subantenna I and II
RCI and RCII: reaction center I and II
TM: thylakoid membrane

Figure 4.1

and Bricaud, 1981; Kirk, 1994; Sathyendranath *et al.*, 1987; Larkum and Barrett, 1983).

The fluorescence excitation spectrum of an alga at ambient temperature, by contrast, results from the re-emission of light reaching chlorophyll-*a* of PSII (Goedheer, 1969; Kiefer and Reynolds, 1992; Govindjee, 1995). The fluoresced light has a longer wavelength than the absorbed light, and has a peak in intensity at ~680 nm (Clayton, 1980; Govindjee, 1995). For a fixed wavelength of emission, the intensity of fluorescence varies with the wavelength of incident light. This wavelength dependence is caused by the differential absorption of light by the pigment-protein-complexes (mainly LHCs), which funnel the captured energy to the subantenna of PSII, which is the main source of fluorescence (Govindjee, 1995). In order to understand variations in fluorescence emission, and thus in the shape of fluorescence excitation spectra, it is critical to understand the structures of the PSU in different species and how the flow of energy can be altered under different physiological conditions. The general flow of energy inside the PSU may be described as follows: light energy is captured by the LHCs and transferred to the subantennae of the photosystems, which in turn funnel it to the RCs. Finally, the electron transfer chain between RCII and RCI produces the initial products of photosynthesis (ATP and NADPH). The transfer of energy to be finally used in photochemistry is not 100% perfect and part of the energy absorbed is dissipated as heat and fluorescence. Fluorescence emission will increase when the RCs are closed (the electron transfer chain is reduced; reviewed in Govindjee, 1995; Falkowski and Raven, 1997).

The subantennae surrounding the RCs contain mainly chlorophyll-*a*, but they also contain small amounts of chlorophyll-*b* or chlorophyll-*c* (depending on the species) and carotenoids (Larkum and Barrett, 1983; Prézelin and Boczar, 1986). Hence, they should also contribute to the differences between fluorescence and absorption spectra. In addition, there are pigments which absorb light but do not transmit the energy to any of the photosystems, called non-photosynthetic or photoprotective pigments, PP (Prézelin, 1981; Siefermann-Harms, 1987). They may

be located in different parts of the cell: surrounding the RCs (probably as a part of the pigment-protein complexes in the subantannae); forming a part of certain LHCs (Johnsen *et al.*, 1997); or even outside the thylakoids (Ben-Amotz *et al.*, 1982: Omata and Murata, 1983; Murata *et al.*, 1981). Regardless of their location, they will also contribute to differences between fluorescence and absorption spectra.

It is also well known that the structure of the PSU is not static, and that the composition of the photosynthetic apparatus and the flow of energy through it undergo changes in response to changes in environmental conditions, e.g., changes in light quantity and quality, nutrient concentration and temperature (reviewed in Larkum and Barrett, 1983; Prézelin and Boczar, 1986; Falkowski and Raven, 1997). Some of these responses, designed to re-direct the flow of energy within the PSU, for example state-transitions (Bonaventura and Myers, 1969; Mullineaux and Allen, 1988; Kroon, 1994; Shubert *et al.*, 1995), and the xanthophyll cycle (Demmig-Adams, 1990; Olaizola and Yamamoto, 1994) are known to be extremely rapid (response time of seconds to minutes). Some other responses, on the other hand, involve long-term acclimation with changes in the composition of the PSU. This latter type of response includes increases in levels of PP at high irradiance (Bidigare *et al.*, 1993; Moore *et al.*, 1995), and increase in levels of LHCs at low irradiance (Richardson *et al.*, 1983; Falkowski and La Roche, 1991).

Based on the assumption of an equal distribution of energy between photosystems, it has been proposed that the fluorescence excitation spectrum should represent the "photosynthetic-absorption-spectrum" (i.e., phytoplankton absorption without the contribution of PP), and as such it should serve as a proxy for the action spectrum of photosynthesis in studies of primary production in the ocean (Sakshaug *et al.*, 1991; Babin *et al.*, 1995; Sosik and Mitchell, 1995). On the other hand, it is known that phycobilin-containing algae (rhodophytes, cryptophytes and cyanophytes) show an extreme imbalance in energy distribution between photosystems (Haxo and Blinks, 1950; Neori *et al.*, 1986; Warnock, 1990). Phycobilins, which

are the main LHCs in these algae, are water-soluble pigment-protein complexes arranged in elaborate structures called phycobilisomes, located in the cytoplasm and connected to PSII (reviewed in Larkum and Barrett, 1983; Rowan, 1989). These phycobilisomes transfer the energy collected to PSII, while PSI receives energy from pigment-protein complexes containing most of the chlorophyll-*a* of the cell (Goedheer, 1969; Myers *et al.*, 1980; Bidigare *et al.*, 1989). Therefore, cyanophytes show a striking difference between fluorescence and absorption spectra (Johnsen and Sakshaug, 1996). Other algae (e.g., dinoflagellates, diatoms) have also shown small imbalances in energy distribution between photosystems (Schofield *et al.*, 1990; 1996; Johnsen *et al.*, 1994*b*; 1997). Lewis *et al.* (1988) reported such a type of PSII biased action spectrum during a bloom of *Trichodesmium* (ex *Oscillatoria*) in the Sargasso Sea. Furthermore, in a study conducted in the North Atlantic, Chapter 3, it was found that differences between the shapes of the absorption and fluorescence excitation spectra were not exclusively related to levels of PP in the samples, especially in areas where cyanophytes dominated the phytoplankton community.

A few studies have dealt with the efficiency of energy transfer to photosystems in phytoplankton (Goedheer, 1969; Johnsen *et al.*, 1994*b*; 1997), but we still do not understand very well how the LHCs are arranged and how energy transfer works in all species. In order to explain energy transfer inside the PSU I chose two simple models (Figure 4.1), which are modifications of the "lake" and "puddle" models proposed by Knox (1975). Originally (Knox, 1975) the models described energy transfer between photosystems of the same type. I have extended this concept to explain energy transfer between the two different photosystems (PSI and PSII). Basically, the lake model the LHCs form a matrix in which the photosystems are embedded. In this model most of the energy captured is directed to PSII and a minor part to PSI; PSII can then re-direct excess energy to PSI by spillover (Butler, 1978). Energy absorbed by all the LHCs is shared by both photosystems, and thus the shape of the fluorescence excitation spectrum (representing energy reaching PSII) should resemble that of the absorption spectrum (representing both

PSI and PSII), once the effects of PP have been removed. In the puddle model, each photosystem would be served by a particular LHC (LHCI or LHCII). In this case, if LHCI and LHCII have different pigment compositions, then the fluorescence excitation spectrum will miss absorption of pigments exclusive to LHCI. This will lead to a difference in the shapes of the fluorescence and absorption spectra.

In this study I conducted a series of laboratory experiments to examine the shapes of absorption and fluorescence excitation spectra of three different species of phytoplankton. I used the diatoms *Thalassiosira weissflogii* (which is frequently used in physiological experiments) and *Chaetoceros* sp. (which was previously found to be abundant in the Labrador Sea and was isolated from that source, see Chapters 2 and 3) and a cyanophyte *Synechococcus* sp. (cyanophytes were previously found to be abundant close to Canary Islands, see Chapters 2 and 3). I chose these species because they are commonly occurring species in natural assemblages and because they were expected to have different types of LHCs and patterns of energy distribution within their photosynthetic apparatuses. A simplified description of the distribution of pigments in different types of pigment-protein-complexes in diatoms and cyanophytes is shown in Table 4.1. As a first approach I assumed that the pathway of energy inside the PSUs in diatoms would correspond to a lake model and in cyanophytes to an extreme case of a puddle model (Figure 4.1). In this chapter, I examined the variations in the shape of absorption and fluorescence spectra and their potential relationship to changes in the structure of the PSU, as reflected in the pigment composition of the cells. I also evaluated the contributions of PP (Table 4.2) to the difference between absorption and fluorescence spectra, by growing the cultures at different irradiances.

Table 4.1 Pigment composition of the main pigment-protein-complexes in diatoms (Prézelin and Boczar 1986, Boczar and Prézelin 1989) and cyanophytes (Larkum and Barrett 1983).

Diatoms	
Complex	Components
LHCs:	• chlorophyll-*a*-chlorophyll-*c*-fucoxanthin-protein • chlorophyll-*a*-chlorophyll-*c*-protein • chlorophyll-*a*-fucoxanthin-protein
PSII:	• Subantenna II: chlorophyll-*a*-(*)-protein • RCII: chlorophyll-*a*-protein (P680)
PSI:	• Subantenna I: chlorophyll-*a*-(*)-protein • RCI: chlorophyll-*a*-protein (P700)
Cyanophytes	
Complex	Components
LHCs:	• phycobilins • chlorophyll-*a*-protein
PSII:	• Subantenna II: chlorophyll-*a*-(*)-protein • RCII: chlorophyll-*a*-protein (P680)
PSI:	• Subantenna I: chlorophyll-*a*-(*)-protein • RCI: chlorophyll-*a*-protein (P700)

LHCs : Light-harvesting-complexes
PSI and PSII: photosystem I and II
RCI and RCII: reaction center I and II
* : probably minor amounts of other pigments

Table 4.2 Separation of the main carotenoids present in diatoms and cyanophytes into photosynthetic (PS) and photoprotective (PP).

	PS	PP
diatoms	fucoxanthin	diadinoxanthin diatoxanthin β-carotene
cyanophytes		zeaxanthin β-carotene

4.2 Materials and Methods

4.2.1 Phytoplankton Cultures

Cultures of *Chaetoceros* sp. (a strain isolated from the Labrador Sea by D.V. Subba Rao), *Thalassiosira weissflogii* (clone CCMP 1336, Provasoli-Guillard National Center for Culture of Marine Phytoplankton) and *Synechococcus* sp. (clone CCMP 837, Provasoli-Guillard National Center for Culture of Marine Phytoplankton) were grown in f/2 medium under continuous illumination provided by a rack of cool-white fluorescent tubes. *Chaetoceros* sp. and *Thalassiosira weissflogii* were grown in semicontinuous cultures, maintained at exponential growth by frequent addition of fresh medium, at 10 °C and 20 °C. *Synechococcus* sp. was grown in batch cultures at 20 °C. *Chaetoceros* sp. was grown at two irradiances (PAR: photosynthetically available radiation, 400 - 700 nm): 30 and 100 μmol quanta $m^{-2} s^{-1}$; *Thalassiosira weissflogii* was grown at five irradiances (PAR): 20, 50, 100, 250, and 450 μmol quanta $m^{-2} s^{-1}$; and *Synechococcus* sp., at three irradiances (PAR): 20, 50, and 100 μmol quanta $m^{-2} s^{-1}$. All cultures were initiated from small inocula and grown for at least 10 days at the designated irradiance before performing the measurements, to ensure complete acclimation to the growth conditions. Note that the highest irradiances at which *Chaetoceros* sp. was grown was lower than the highest irradiance for *Thalassiosira weissflogii*. *Chaetoceros* sp. was isolated from the cold waters of the Labrador Sea and grown at 10 °C; thus, the cells were probably predisposed to a photosynthetic apparatus acclimated to low temperatures, which is somewhat equivalent to acclimation to high light (Maxwell *et al.*, 1995) which reduces their ability to grow at higher irradiances. In the case of *Synechococcus* sp. I moved cells growing at very low light (<20 μmol quanta $m^{-2} s^{-1}$) directly to the higher irradiances without allowing them to gradually adjust to increasing irradiances as suggested by Kana and Glibert (1987). Consequently, the maximum irradiance at which the cells were able to grow was 100 μmol quanta $m^{-2} s^{-1}$.

Cultures were acclimated to dim light, < 10 μmol quanta $m^{-2} s^{-1}$, for at least 30 min. before they were filtered, to eliminate fast changes in the energy flow within the PSU, such as the xanthophyll cycle or state transitions. Samples of 10 ml of culture were filtered through GF/F glass-fibre filters for pigment determination and kept in liquid nitrogen until analysis. Another aliquot of 10 ml was inoculated with DCMU [3-(3.4-dichlorophenyl) 1-1 dimethylurea] to a final concentration of 18 μM (to block the electron transfer and enhance the fluorescence emission by PSII), filtered through GF/F filter after $\sim$ 10 min., and the filters were then immediately used to measure the fluorescence and absorption spectra. All measurements were done in triplicate for *Chaetoceros* sp. and *Thalassiosira weissflogii*, and in duplicate for *Synechococcus* sp. Results shown are the averages of these replicates.

In the case of *Chaetoceros* sp. and *Thalassiosira weissflogii*, cells were counted on the day of each experiment, using a haemocytometer. Cell size was measured using a calibrated ocular eyepiece in the microscope. Two dimensions were measured for the diatoms and the volumes, considering a cylindrical shape, were calculated; from these estimates the cell radius was calculated for a spherical equivalent volume. For *Synechococcus* sp., samples were fixed with 2% glutaraldehyde and stored in cryovials at -70 °C until analysis by flow cytometry. Cell numbers were counted using a flow cytometer following the method described by Li *et al.* (1994). Flow cytometric measurements of forward scattered light were used to estimate cell size (Olson *et al.*, 1989) by transforming them into equivalent-spherical-diameter using a coefficient obtained for calibrated plastic beads. This estimation of cell volume is affected by differences in the shape and in the refractive indexes of the plastic beads, used in the calibration, and these of the cells measured. Hence, cell volumes calculated by flow cytometry should not expected to be the same as those estimated by microscopy. Flow cytometric data were kindly supplied by W.K.W. Li.

4.2.2 Pigment determination

The efficiency of pigment extraction by different solvents from *Chaetoceros* sp. was examined. A modification of the solvent mixture proposed by Wright

et al. (1991) and Kraay *et al.* (1992), using 98% ethanol:2% 0.5M ammonium acetate (instead of 98% methanol), proved to be the best for this species. It is known that the extraction of pigments from cyanophytes is more difficult than from diatoms, but because of technical problems it was impossible to check the efficiency of extraction of different solvents from *Synechococcus* sp.. Nevertheless, according to Wright *et al.* (1997), the commonly-used method of soaking the samples in 90% acetone can cause a severe underestimation of pigments, particularly in cyanophytes. Therefore, all filters were extracted in a mixture of 98% ethanol:2% 0.5M ammonium acetate by grinding using an electrical homogeneizer. Filters were taken from the liquid nitrogen and immediately ground keeping the temperature low ($\sim$ 5 °C) by maintaining the grinding-container in an ice bath, to minimise the conversion of chlorophyll-*a* into chlorophyllide-*a* by the action of chlorophyllase (Barrett and Jeffrey, 1964; Owens and Falkowski, 1982). Samples were then analysed by HPLC according to the method of Head and Horne (1993).

4.2.3 *In vivo* fluorescence

In vivo fluorescence excitation spectra were measured using a Photon Technology International QM-1 spectrofluorometer equipped with a red-sensitive photomultiplier and a reference photodetector with a photodiode array for the correction of the excitation spectrum. To avoid any interference by stray light, two interference filters were used: a cut-off filter located immediately after the excitation monochromator to eliminate light of wavelengths above 700 nm, and another cut-off filter located immediately before the emission monochromator to eliminate light of wavelengths below 720 nm. The efficacy of the correction procedure was tested by comparing the shapes of the fluorescence excitation and absorption spectra of a 90% acetone solution of pure chlorophyll-*a*, which gave a good match. Fluorescence excitation spectra were recorded between 380 and 700 nm at an emission wavelength of 730 nm. The excitation slits were set at a bandpass width of 2 nm and the emission slits at 4 nm. The blank was obtained by filtering the filtrate of the corresponding sample through a GF/F filter. Filters were wetted with filtered seawater

before reading. The shapes of the spectra were also corrected for differences between measurements in suspension and on filter as described in Chapter 2. The ratio of the fluorescence excitation spectrum on a filter to that in suspension was calculated from triplicate measurements for each species (data not shown). All measurements of fluorescence excitation spectrum were then divided by their correspondent ratio ($C_s(\lambda)$=filter/suspension). Spectra were then smoothed using a running average of 5 nm and normalised to 1 at 545 nm, $f_c^n(\lambda)$, as discussed in Chapter 3, and the average from replicate samples was calculated in each case. The mean coefficient of variation, from the coefficients of variation calculated from the replicate values of $f_c^n(440)$ in each case, from all experiments (n=10) was 3.0 %±2.3. More details on the process of the fluorescence measurements as well as the values of the correction factors ($C_s(\lambda)$) are given in Appendix A.

4.2.4 *In vivo* absorption

In vivo absorption spectra were measured using the filter technique (Yentsch, 1962; Mitchell and Kiefer, 1984), with a Shimadzu UV-2101 PC double-beam spectrophotometer equipped with an integrating sphere. Sample and blank filters were wetted in filtered seawater and placed in the spectrophotometer immediately after the fluorescence measurements. The optical density at 750 nm was subtracted from the spectrum (Sathyendranath *et al.*, 1987; Bricaud and Stramski, 1990). Optical densities ($D(\lambda)$) were transformed into absorption ($a_p(\lambda)$) by converting decimal to natural logarithm (factor=2.3) and taking into account the volume filtered and the area of the filter (X= volume filtered divided by the area of the filter). Spectra were corrected for the pathlength amplification factor using a quadratic equation (Mitchell, 1990; Hoepffner and Sathyendranath, 1992):

$$a_p(\lambda) = \frac{2.3 * (A * [D(\lambda)] + B * [D(\lambda)]^2)}{X}.$$

where the coefficients A and B were determined individually for each species (for *Chaetoceros* sp. A=0.47 and B=0.19, for *Thalassiosira weissflogii* A=0.35 and

B=0.20, for *Synechococcus* sp. A=0.41 and B=0.32). Absorption by detrital material was corrected for, to obtain $a_{ph}(\lambda)$, using the method proposed by Kishino *et al.* (1985) as modified by Hoepffner and Sathyendranath (1992) for *Chaetoceros* sp. and *Thalassiosira weissflogii* and the theoretical approach of Hoepffner and Sathyendranath (1993) for *Synechococcus* sp.. Finally, spectra were normalised to 1 at 545 nm, the normalised spectra are denoted here as $a^n_{ph}(\lambda)$, and the average from replicate samples was calculated in each case. The mean coefficient of variation, from the coefficients of variation calculated from the replicate values of $a^n_{ph}(440)$ in each case, from all experiments (n=10) was 3.6 %±2.6. More details on the process of the absorption measurements, including all the equations used to convert raw values of optical density to absorption of phytoplankton (e.g., corrections for the pathlength amplification factor and detrital absorption) are given in Appendix A.

4.2.5 Estimation of the absorption by photoprotective pigments

The contribution of PP to the normalised absorption spectra was estimated using the method proposed by Bidigare *et al.* (1990*b*) as modified by Babin *et al.* (1996). Briefly, the absorption spectrum was reconstructed ($a_r(\lambda)$) using pigment concentrations (p_i) and the corresponding specific *in vivo* absorption coefficients ($a^*_i(\lambda)$) given by Bidigare *et al.* (1990*b*):

$$a_r(\lambda) = \sum_{i=1}^{n} p_i a^*_i(\lambda).$$

An initial estimate of absorption by PP ($a_{pp}(\lambda)$) was also computed. Since there were some discrepancies between the shapes of the reconstructed absorption spectra and the measured absorption spectra, I used a procedure similar to that described in Babin *et al.* (1996) to obtain a corrected estimate of the normalised absorption by PP. This method uses the shapes of absorption by pure pigments *in vitro* (slightly shifted, see Bidigare *et al.*, 1990*b*), which are not the best representation of the absorption spectra of pigments forming pigment-protein-complexes *in vivo* (see Johnsen *et al.*, 1994). The bias produced by estimating *in vivo* absorption spectra of individual pigments from *in vitro* ones, could only be assessed by measuring

absorption from isolated pigment-protein-complexes from different species of phytoplankton. Nevertheless, admitting that this method (Babin *et al.*, 1996) is not perfect, it represents a first approach to estimate in a simple way an approximation of the contribution of absorption by photoprotective pigments to total phytoplankton absorption. The normalised absorption by PP ($a^n_{pp}(\lambda)$) was estimated as follows:

$$a^n_{pp}(\lambda) = \frac{a^n_{ph}(\lambda) * a_{pp}(\lambda)}{a_r(\lambda)}.$$

In other words, the normalised phytoplankton absorption spectrum was multiplied by the ratio of the initial estimate of absorption by PP to the total reconstructed absorption at the same wavelength. More details on the estimation of $a_{pp}(\lambda)$ are given in Appendix A.

4.3 Results

4.3.1 Changes in pigment composition with irradiance

I investigated variations in the pigment ratios in the three species when exposed to the two extreme growth irradiances, hereafter referred to as the lowest light (LL) and the highest light (HL). For *Thalassiosira weissflogii* LL=20 μmol quanta $m^{-2} s^{-1}$ and HL=450 μmol quanta $m^{-2} s^{-1}$, for *Chaetoceros* sp. LL=30 μmol quanta $m^{-2} s^{-1}$ and HL=100 μmol quanta $m^{-2} s^{-1}$, and for *Synechococcus* sp. LL=20 μmol quanta $m^{-2} s^{-1}$ and HL=100 μmol quanta $m^{-2} s^{-1}$. These pigment ratios at different irradiances are later used to infer changes in the distribution of pigments in different parts of the PSU. I especially wanted to study variations in the proportion of photosynthetic carotenoids, PS, and the proportion of photoprotective pigments, PP (Table 4.2) in relation to chlorophyll-*a*. I also estimated cell size in the three species at LL and HL. Because cell size changed with irradiance, I preferred to look at intracellular concentration (per unit cell-volume) of pigments

rather than the amount of pigments per cell. Cell volumes in diatoms might be affected by the presence of vacuoles, since this effect was not considered in this study intracellular concentrations of pigments might be underestimated.

In *Thalassiosira weissflogii*, the ratios of chlorophyll-*c* to chlorophyll-*a* (cc/ca) and PS/ca were higher at LL than at HL, while the ratio PP/ca was higher at HL (Table 4.3). This species seemed to have compensated a general increase in pigments content per cell at LL by increasing its volume by ~64%, compared with that at HL. The concentration of chlorophyll-*a* increased in proportion to cell size, therefore the intracellular concentration remained almost the same at the two irradiances. The intracellular concentration of chlorophyll-*c* was ~48% higher at LL. Intracellular concentrations of photosynthetic carotenoids were also slightly higher at LL (~12% more than at HL), while intracellular concentrations of PP were >70% higher at HL than at LL (Table 4.3). These results are consistent with changes in cell volume and intracellular concentrations of chlorophyll-*a* in the same species found by Sosik *et al.* (1989), but they are in contrast to the findings by Falkowski *et al.* (1985), who found that cell volume decreased with a decrease in irradiance. These discrepancies are probably due to the wide variation observed in cell sizes of *Thalassiosira weissflogii*, from 500 μm^3 (Berges *et al.*, 1996) to 2800 μm^3 (Costello and Chisholm, 1981). These variations in cell size can be explained by the large genetical variation observed in isolates of *Thalassiosira weissflogii* (Armbrust and Chisholm, 1992), which can undergo different cycles of auxosporation.

In *Chaetoceros* sp., in contrast to the situation in *Thalassiosira weissflogii*, the ratios cc/ca and PS/ca were higher at HL than at LL, similar to the direction of changes in PP/ca (Table 4.3). In *Chaetoceros* sp., which is smaller than *Thalassiosira weissflogii*, cell volume actually increased with irradiance (Table 4.3). Consequently, the total intracellular concentration of chlorophyll-*a* was ~30% higher at LL than at HL. The intracellular concentrations of chlorophyll-*c* and PS were also higher at LL than at HL (~10%), while intracellular concentrations of PP were ~36% higher at HL (Table 4.3).

Table 4.3 Cellular volume (μm^3), intracellular concentration of pigments (mg m^{-3} 10^{-5}), and ratios among them in the three species studied. The standard deviations for cell size determination (n~50 to 100 for the diatoms *T. weissflogii*, *T.w.*, and *Chaetoceros* sp., *Chaet.*, and n>5000 for *Synechococcus* sp., *Syne.*) are indicated. Triplicate measurements of pigment concentration showed a coefficient of variation of <5% (except in some cases for β-carotene which was found in minor concentrations). The percentage difference of the values at low light (LL) from those at high light (HL) are also shown.

sp.	Irr.	cell vol.	ca	cc	PS	PP	cc/ca	PS/ca	PP/ca
T.w.	LL	2304±346	41.98	4.07	18.95	3.48	0.13	0.45	0.08
	HL	1407±367	43.28	2.74	16.88	12.30	0.09	0.39	0.28
	% dif.	64.0	-3.0	48.7	12.3	-71.7	46.7	15.6	-70.8
Chaet.	LL	49±16	139.56	32.04	95.20	11.02	0.23	0.68	0.08
	HL	65±30	105.56	29.97	82.90	17.37	0.28	0.79	0.17
	% dif.	-24.6	32.2	6.9	14.8	-36.5	-19.0	-13.1	-52.1
Syne.	LL	0.30±0.04	171.99	–	–	106.52	–	–	0.62
	HL	0.45±0.05	87.70	–	–	88.08	–	–	1.00
	% dif.	-34.0	96.1	–	–	20.9	–	–	-38.0

ca : chlorophyll-*a*
cc : chlorophyll-*c*
PS : sum of photosynthetic pigments
PP : sum of photoprotective pigments

In *Synechococcus* sp., as in the other species, the ratios of PP/ca also increased at HL (Table 4.3). Since I was not able to measure the concentration of phycobilins, the main LHCs in this species, I do not know how their concentrations changed with irradiance. As in *Chaetoceros* sp. cell volume increased with irradiance in *Synechococcus* sp.. These results are consistent with what was found by Stramski *et al.* (1995), but contrasting with those reported by Kana and Glibert (1987), who found that cell volume increased with a decrease in irradiance. This difference might be due to differences in cell sizes and physiological responses to growth conditions by different strains of *Synechococcus*. The intracellular concentration of chlorophyll-*a* was >90% higher at LL than at HL, while the concentration of PP was only ~20% higher at HL (Table 4.3). Hence, the increase in PP/ca at HL was mainly due to a decrease in the amount of chlorophyll-*a* rather than to the increase in the concentration of PP (see Kana *et al.* 1988). Values of intracellular concentrations of all pigments in the three species studied are given in Appendix C.

4.3.2 Variation in the absorption and fluorescence excitation spectra at low and high light

Variation in the normalised absorption and fluorescence excitation spectra at LL and HL was examined. If each species is considered separately, differences between absorption spectra at LL and HL were always larger than the corresponding differences in the fluorescence excitation spectra (Figure 4.2). The coefficient of variation between the normalised (545 nm) absorption spectra at the blue peak (439 nm), between LL and HL was: 34.6% for *Chaetoceros* sp., 41.6% for *Thalassiosira weissflogii*, and 35.8% for *Synechococcus* sp.. The c.v. between the normalised (545 nm) fluorescence spectra at the blue peak was: 26.3% for *Chaetoceros* sp., 24.9% for *Thalassiosira weissflogii*, and 22.7% for *Synechococcus* sp.. On the other hand, if results from all the experiments on the three species were considered together, it is obvious that variations in the shape of fluorescence spectra would be larger than those of the absorption spectra.

Figure 4.2. Absorption and fluorescence excitation spectra, both normalised to 1 at 545 nm, at low light (LL) and high light (HL) for the three species studied.

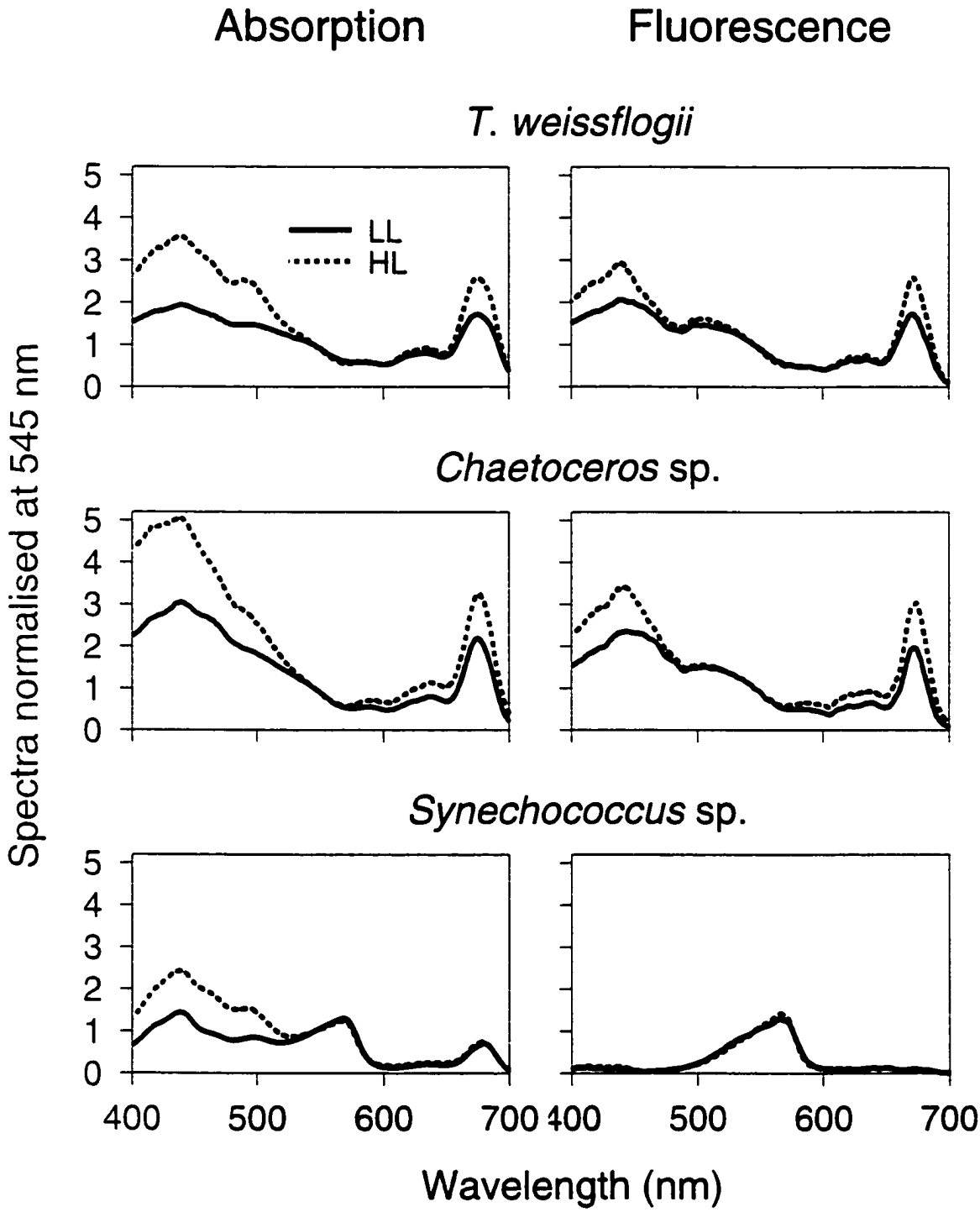
Absorption
Fluorescence
T. weissflogii
Chaetoceros sp.
Synechococcus sp.
LL
HL
Spectra normalised at 545 nm
0
1
2
3
4
5
400
500
600
700
Wavelength (nm)

Figure 4.2

4.3.3 Accounting for differences in the spectra at low and high light

Possible causes of differences between the absorption, and between fluorescence, spectra at LL and HL were investigated. For each species, I first subtracted the normalised (at 545 nm) LL spectrum from the normalised HL spectrum to obtain Δa^n_{ph} for each case. In the same way, I calculated Δf^n_c as the difference between the normalised fluorescence spectra at HL and LL. In addition, I calculated Δa^n_{pp} by subtracting the normalised PP absorption spectrum at LL from that at HL, and finally I calculated the difference between Δa^n_{ph} and Δa^n_{pp} ($\Delta a^n_{ph} - \Delta a^n_{pp}$). A comparison of all these difference spectra is shown in Figure 4.3, which shows that *Chaetoceros* sp. gave the highest values of Δa^n_{ph}, both in the red and blue regions of the spectrum, followed by *Thalassiosira weissflogii*, and then *Synechococcus* sp. which gave the lowest values of Δa^n_{ph} and showed differences only in the blue part of the spectrum.

For all three species, Δf^n_c followed the shape of Δa^n_{ph} in the red region (Figure 4.3). On the other hand, marked differences were observed in the spectral changes in the blue part of the spectrum. In *Chaetoceros* sp., Δa^n_{pp} covered only a small part of Δa^n_{ph}, and the $\Delta a^n_{ph} - \Delta a^n_{pp}$ spectrum was larger than Δf^n_c. In *Thalassiosira weissflogii*, Δa^n_{pp} accounted for a large part of Δa^n_{ph}, but there a $\Delta a^n_{ph} - \Delta a^n_{pp}$ spectrum was still discernible and in this case, the fluorescence difference spectrum Δf^n_c seemed to match the $\Delta a^n_{ph} - \Delta a^n_{pp}$ spectrum. In *Synechococcus* sp. by contrast it appears that almost all the difference spectrum Δa^n_{ph} was accounted for by an increase in the proportion of PP, relative to chlorophyll-*a* (PP/ca), and there was hardly any difference between the fluorescence spectrum at LL and that at HL (Δf^n_c, Figure 4.3). Thus, different species showed different responses in their optical characteristics with varying irradiance, and it is evident, as I shall discuss later, that the effect of PP was not the only factor affecting variations in the absorption and fluorescence spectra with light level.

Figure 4.3. Difference spectra (HL-LL), all normalised to 1 at 545 nm, for the three species studied: total phytoplankton absorption at HL minus total phytoplankton absorption at LL (Δa^n_{ph}); PP absorption at HL minus PP absorption at LL (Δa^n_{pp}); difference total phytoplankton absorption minus difference PP absorption ($\Delta a^n_{ph} - \Delta a^n_{pp}$); fluorescence at HL minus fluorescence at LL (Δf^n_c).

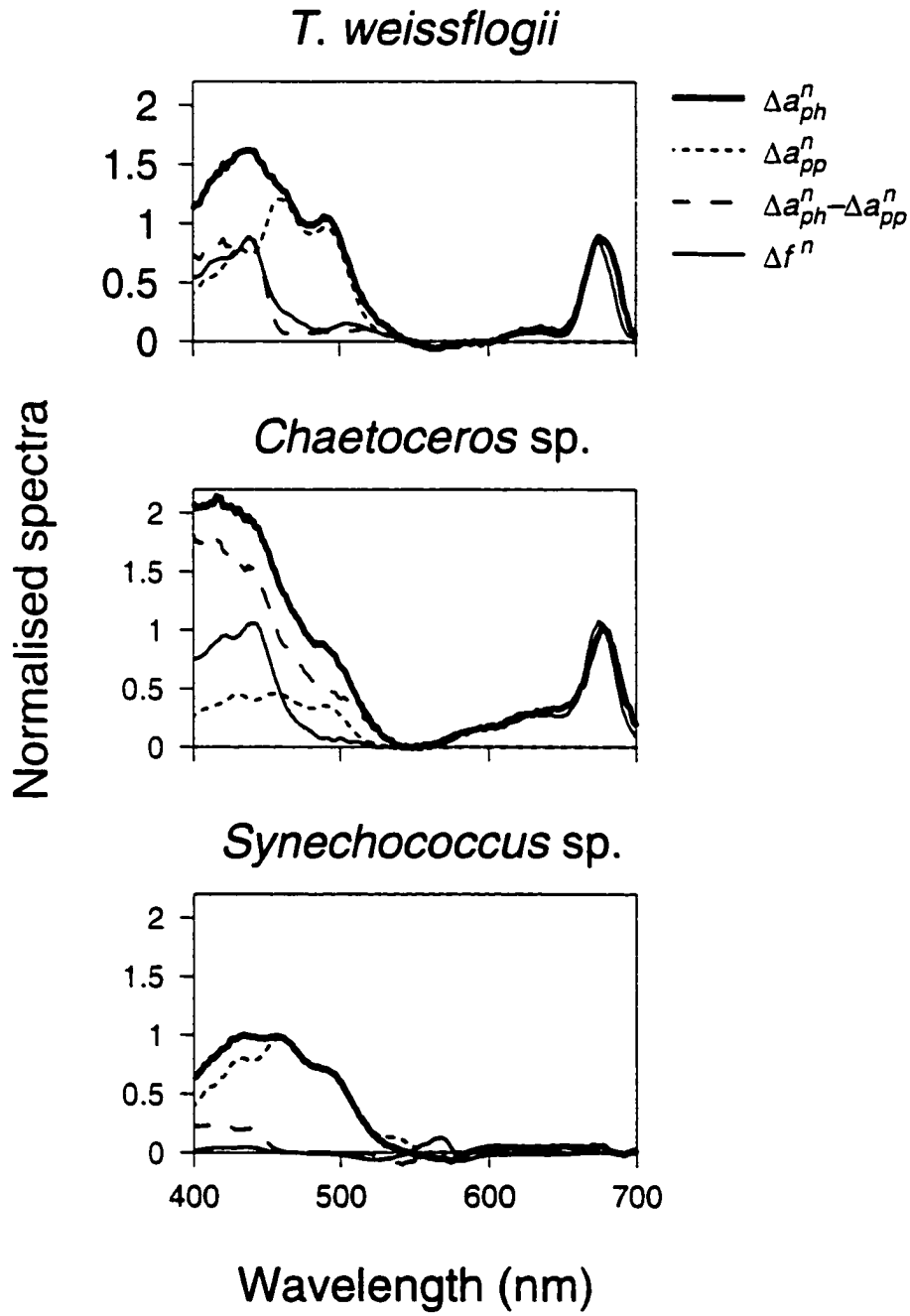
T. weissflogii
Chaetoceros sp.
Synechococcus sp.
Normalised spectra
Wavelength (nm)
Δa_{ph}^n
Δa_{pp}^n
$\Delta a_{ph}^n - \Delta a_{pp}^n$
Δf^n
0
0.5
1
1.5
2
400
500
600
700

Figure 4.3

4.3.4 Comparison between absorption and fluorescence spectra at each irradiance

I also examined the shapes of the normalised absorption and fluorescence spectra at each irradiance, and the corresponding difference spectrum (absorption - fluorescence, $a^n_{ph} - f^n_c$) in relation to the absorption by PP. For *Thalassiosira weissflogii*, the shape of the fluorescence excitation spectrum was similar to that of the absorption spectrum at LL, when the contribution of PP to absorption was very low (Figure 4.4). At HL, the normalised fluorescence spectrum was lower than the absorption spectrum in the blue region, and $a^n_{ph} - f^n_c$ matched the shape of the PP absorption in that region. In *Chaetoceros* sp., the spectra for LL showed the same trend as in *Thalassiosira weissflogii*, though $a^n_{ph} - f^n_c$ was slightly larger than the absorption by PP. At HL, the difference spectrum was much larger than absorption by PP. In the two diatoms, both at LL and HL, the difference spectrum showed a conspicuous peak at the far red region ($\sim$ 690 nm). In the case of *Synechococcus* sp., for both LL and HL, the shape of the fluorescence spectrum resembled the shape of phycoerythrin absorption (maximum at $\sim$ 567 nm) and almost completely missed the peaks at the blue and red regions of the spectrum, where chlorophyll-*a* absorbs. Therefore, the difference spectrum ($a^n_{ph} - f^n_c$) showed absorption by chlorophyll-*a* and PP.

It should be noticed that the representation of the *in vivo* absorption spectrum of PP, as derived from the methods of Bidigare *et al.* (1990*b*) and Babin *et al.* (1996), was not perfect, as was evident from its tendency to extend towards wavelengths corresponding to phycobilins absorption (Figure 4.4). Nevertheless, it provides a reasonable approximation of the spectral contribution of PP to absorption.

4.3.5 Relationship of differences between absorption and fluorescence spectra and the proportion of PP

Differences between the normalised (545 nm) absorption and fluorescence spectra were quantified as the ratios of absorption to fluorescence at 439 nm, S(439), and

Figure 4.4. Comparison between absorption and fluorescence excitation spectra, both normalised to 1 at 545 nm, for the three species studied at low (LL) and high light (HL). The correspondent absorption by PP, the difference total phytoplankton absorption minus PP absorption ($a^n_{ph} - a^n_{pp}$), and the difference (total phytoplankton absorption-fluorescence: $a^n_{ph} - f^n_c$) spectra are also shown.

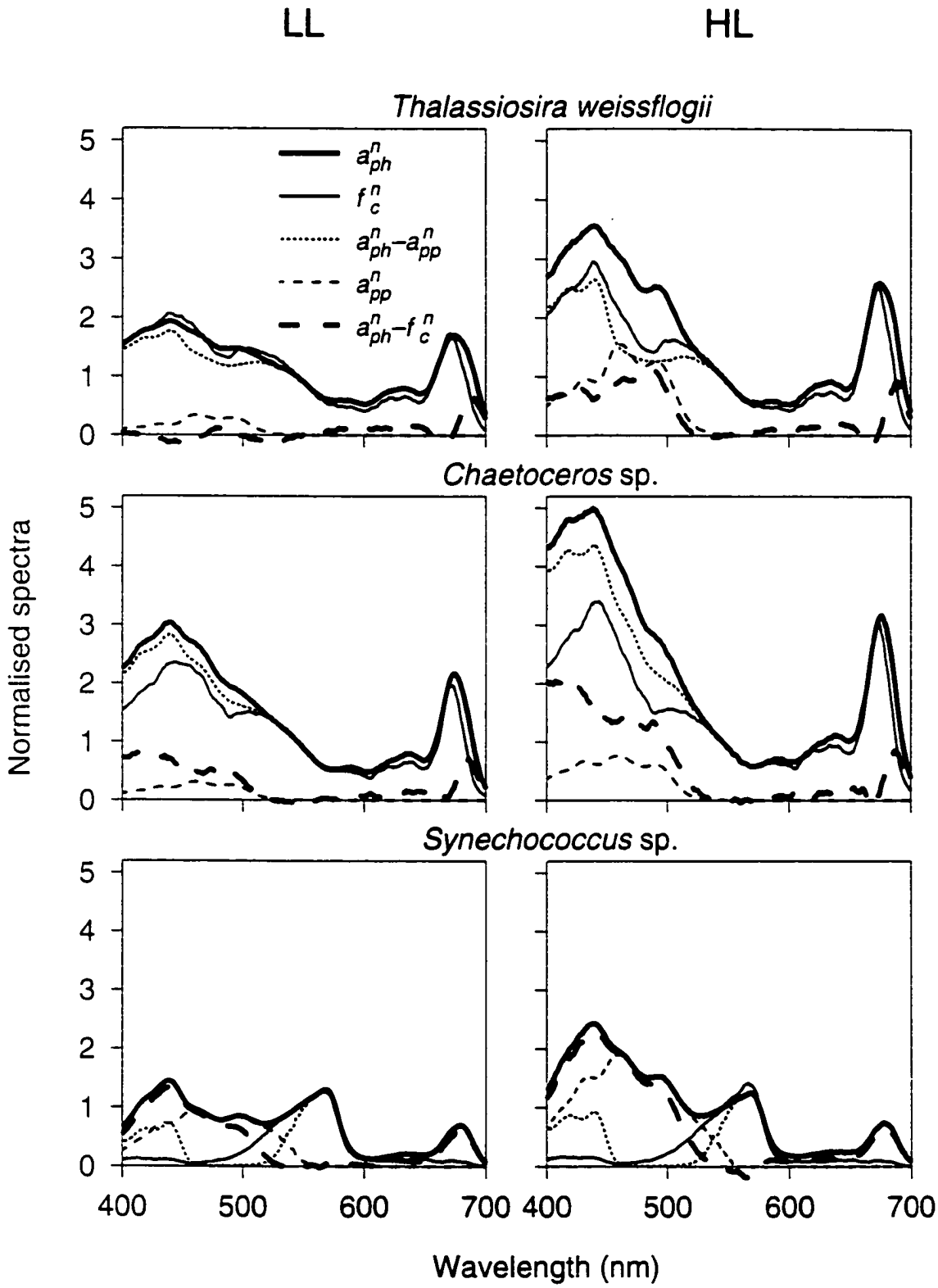
LL
HL
Thalassiosira weissflogii
Chaetoceros sp.
Synechococcus sp.
a^n_{ph}
f^n_c
$a^n_{ph}-a^n_{pp}$
a^n_{pp}
$a^n_{ph}-f^n_c$
Normalised spectra
0
1
2
3
4
5
400
500
600
700
Wavelength (nm)

Figure 4.4

at 676 nm. S(676) for all the growth irradiances. The relationship between S(439) and the ratio PP to chlorophyll-*a* (PP/ca) for *Thalassiosira weissflogii* showed a good correlation (r^2 ~0.95; Figure 4.5a). If the values for *Chaetoceros* sp. and for *Synechococcus* sp. were plotted in the same graph, points for the former species lay close to the regression line for *Thalassiosira weissflogii*, but those for *Synechococcus* sp. lay well above the regression line. Since PP absorb only in the blue-green region of the spectrum, while chlorophyll-*a* absorbs in both blue and red regions of the spectrum, a better way to single out the effect of PP should be to examine the ratio S(439)/S(676), in which the effect of chlorophyll-*a* of PSI is cancelled out. Consistent with this argument, the relationship between S(439)/S(676) and PP/ca showed a good correlation for *Thalassiosira weissflogii* (r^2 ~0.99) and the values for *Synechococcus* sp. were closer to this regression line (Figure 4.5b).

4.4 Discussion

4.4.1 Changes in the PSU

Studies at high resolution of the molecular structure of LHCs (e.g., peridinin-chorophyll-protein complex in dinoflagellates, Hofmann *et al.*, 1996; chlorophyll-*a*-chlorophyll-*b*-protein complex in terrestrial plants, Kühlbrandt *et al.*, 1994) have shown that these are very organised and elaborate structures. Hence, it seems likely that the structure of any individual LHC should be conservative (i.e., it should maintain the same ratios of its constituent molecules), whereas the cell may change the concentrations of different types of LHCs according to physiological growth conditions (Johnsen *et al.*, 1997). I believe that looking at changes in the components of the PSU (i.e., concentrations of different LHCs and ratio of PSI:PSII) in different species, or under different physiological conditions, should provide a better way of understanding changes in the fluorescence excitation spectra, than looking at changes, for example, in the total concentrations of isolated pigments. Fluorescence emission originates mainly from the subantenna of PSII (Govindjee, 1995), i.e., from a small fraction of very specialised pigment-protein complexes. Hence, knowing how

Figure 4.5. Relationships between absorption to fluorescence ratios (S) and the ratios of photoprotective pigments to chlorophyll-*a* (PP/ca), for the three species studied. **a:** absorption/fluorescence at 439 nm [S(439)] versus PP/ca. Inset shows the values for *T. w.* and *Chaet.* in an expanded scale. **b:** [S(439)/S(676)] versus PP/ca. In both cases the regression line is only for *Thalassiosira weissflogii.*

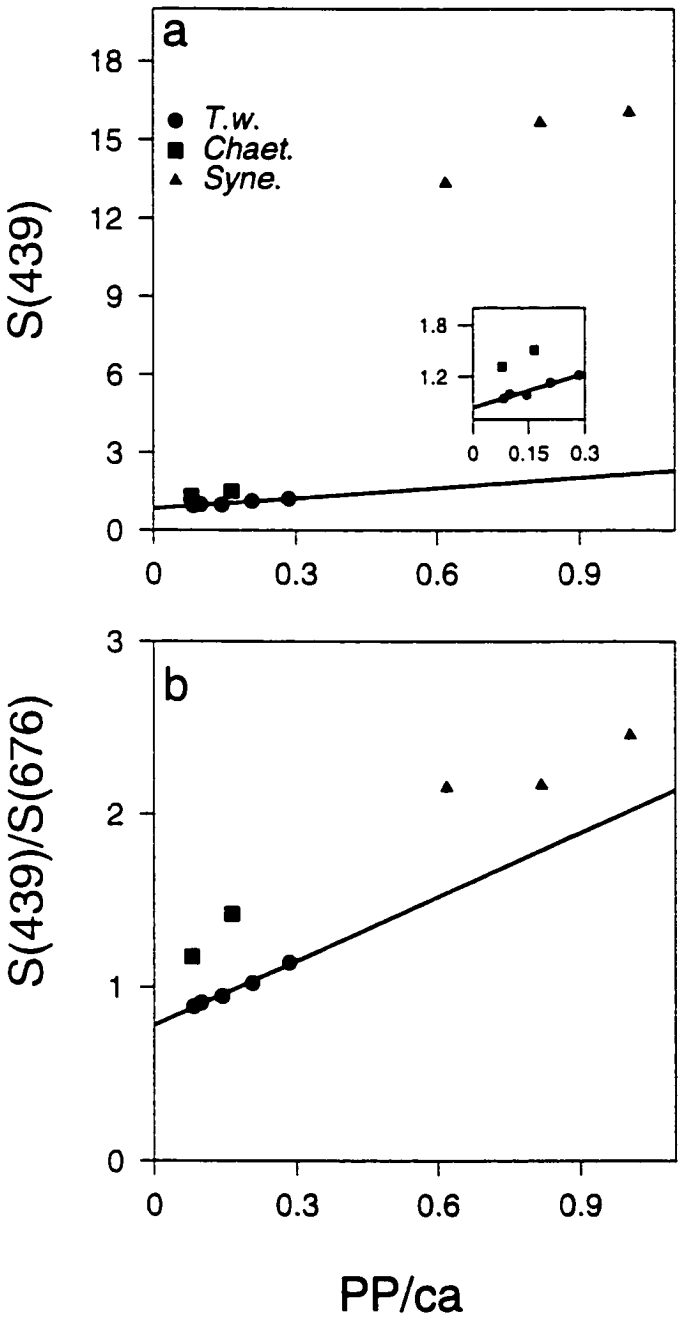
a
T.w.
Chaet.
Syne.
S(439)
18
15
12
9
6
3
0
1.8
1.2
0
0.15
0.3
0
0.3
0.6
0.9
b
S(439)/S(676)
3
2
1
0
0
0.3
0.6
0.9
PP/ca

Figure 4.5

the total cellular amount of chlorophyll-*a* changes under different conditions does not tell us much about expected variations in the fluorescence excitation spectrum. unless we know how the proportion of those particular subantenna II complexes changed within the PSU, as well as how the proportion of LHCs serving subantenna II changes (i.e. how energy is distributed between the photosystems). To my knowledge, the structure of LHCs in diatoms has not been described in sufficient detail to unequivocally identify the composition of different types of LHCs (see Prézelin and Boczar, 1986; Boczar and Prézelin, 1989). Thus, I could not give any detailed quantitative assignment of pigments to the different complexes.

Despite the absence of quantification, however, changes in the ratios amongst the pigments should provide some information as to how the different components of the PSU change with irradiance, if we take into account the basic pigment distribution within the PSU (Table 4.1). In *Thalassiosira weissflogii*, both cc/ca and PS/ca ratios were higher at LL, while the intracellular concentration of chlorophyll-*a* did not change (Table 4.3). We can infer from this that this species had more chlorophyll-*a* in subantennae complexes at HL. Thus, at HL, there were fewer LHCs per photosystem, as it is expected in photoacclimation to high light (Richardson *et al.*, 1983; Falkowski and LaRoche, 1991). *Chaetoceros* sp., on the other hand, seemed to have more chlorophyll-*a* in subantennae complexes at LL, since both the cc/ca and PS/ca ratios were lower at LL, concomitant with a higher intracellular concentration of chlorophyll-*a* (Table 4.3). This is consistent with an increase in the proportion of PSIIs at LL, as it has been demonstrated by Falkowski *et al.* (1981) for *Skeletonema costatum* (PSI:PSII ratio changed from ~1.0 at HL to ~0.5 at LL). This might also influence the way in which the packaging will affect the fluorescence spectrum (coming only from PSII) and the absorption spectrum (representing all parts of the PSU).

In the case of *Synechococcus* sp., I could not quantify the concentrations of phycobilins (the main LHC for PSII), although there are reports that they increase at LL (Kana and Glibert., 1987). Besides, the ratio of the normalised (at 545 nm)

absorption value at 567 to that at 676 nm (Figure 4.2) suggests that the amount of phycoerythrin (the main phycobilin in the clone used here) increased in proportion to the amount of chlorophyll-*a*, an observation which is consistent with the constant ratio chlorophyll-*a* to phycoerythrin observed by Bidigare *et al.* (1989) for *Synechococcus* sp. grown under different light conditions. The increased intracellular concentration of chlorophyll-*a* at LL was likely located in the subantannae of the photosystems (mainly in PSI) and perhaps forming more specialized LHCIs, depicted by the dashed line in Figure 4.1c. On the other hand, the two PP in cyanophytes, β-carotene and zeaxanthin, behaved differently (results not shown). The intracellular concentration of β-carotene decreased with irradiance (~3 fold higher at LL than at HL), the opposite of what was found by Kana *et al.* (1988). Nevertheless these results might agree with the idea of β-carotene serving a mainly light-harvesting function proposed by Goedheer (1969). The intracellular concentration of zeaxanthin, which is the most abundant PP, remained almost constant (~1% difference between LL and HL), consistent with the constant concentration of zeaxanthin per cell found by Kana *et al.* (1988). It has been shown that in the cyanophyte *Anacystis nidulans* most of the zeaxanthin is located in the cytoplasmic membrane and the cell envelope (Omata and Murata, 1983; Murata *et al.*, 1981) which certainly is a strategic location for a photoprotective pigment.

4.4.2 Variations in the absorption and fluorescence spectra in relation to the organisation of the PSU

It should be noted that when differences in the spectra at LL and HL were examined, normalised spectra were used. Hence, changes in absorption, or fluorescence spectra, are all relative to their value at 545 nm, which is close to the maximum of absorption of phycoerythrin and on the tail of absorption spectra by photosynthetic carotenoids. In general, for all the species, the increase in absorption at HL at the red peak (~676 nm) may be due largely to a change in the packaging effect at different irradiances, but it may also reflect changes in absorption by different pigments, since absorption at this wavelength is relative to 545 nm. In the

blue region of the spectrum the change in absorption from HL to LL, Δa^n_{ph}, can be attributed to a combination of the packaging effect, a relative increase in the concentrations of PP, and a relative decrease in the levels of photosynthetic pigments at HL. For *Thalassiosira weissflogii*, the change in fluorescence at HL and LL, Δf^n_c, was similar to the difference $\Delta a^n_{ph} - \Delta a^n_{pp}$ (Figure 4.3). This change can therefore be attributed to the same causes as changes in absorption, once the effect of PP has been eliminated. In *Chaetoceros* sp. the remaining spectrum $\Delta a^n_{ph} - \Delta a^n_{pp}$ was even larger than Δf^n_c (Figure 4.3). This could be due to part of the chlorophyll-*a* being in PSI. It should be noted, however, that at least part of this Δa^n_{ph} in *Chaetoceros* sp. may have been caused by an increase in the concentrations of degradation products of chlorophyll-*a* (since this species proved to have a high chlorophyllase activity) or by errors in the corrections made for the presence of detritus.

In order to explain variations in the absorption and fluorescence spectra for the different species and under different light conditions, I also examined the actual difference spectrum absorption - fluorescence, $a^n_{ph} - f^n_c$.(Figure 4.4). This, as expected, shows some of the trends already deduced from Figure 4.3. For example, in *Chaetoceros* sp. at HL, $a^n_{ph} - f^n_c$ was much larger than the absorption spectrum of PP. This might, again, indicate that there was some difference in the chlorophyll-*a* distribution between the photosystems or that the packaging effect affects absorption and fluorescence in different ways. In *Synechococcus* sp. it was evident that the fluorescence excitation spectrum, both at LL and HL, shows an extremely low contribution of chlorophyll-*a* (Figure 4.4), so that $a^n_{ph} - f^n_c$ cannot be entirely accounted for by PP absorption, even though the proportion of PP to chlorophyll-*a* increased with irradiance (see pigments section). In cyanophytes, as mentioned above, the main LHCs are phycobilins which transfer the energy to PSII, while most of the chlorophyll-*a*-protein complexes are associated with PSI which contributes only $<$ 5% of the fluorescence at ambient temperature. Thus, significant differences between absorption and fluorescence spectra are expected. One interesting feature seen in the difference spectra ($a^n_{ph} - f^n_c$) of the diatoms was a conspicuous peak at

the red-end of the spectrum. This shift of the red peak between absorption and fluorescence might be an indication of absorption by subantenna-chlorophyll-*a* from PSI which does not fluoresce, and which is known to absorb in the far red (at ~ 700 nm; Prézelin, 1981; Johnsen *et al.*, 1997).

In order to distinguish between the effect of PP and that of the differential distribution of chlorophyll-*a* between photosystems, on the $(a_{ph}^n - f_c^n)$ difference, I looked at the ratios of absorption to fluorescence at the red [S(676)] and the blue [S(439)] peaks. The ratio PP/ca increased with growth irradiance in the three species (Table 4.3). Because PP/ca was much higher in *Synechococcus* sp. than in the two diatoms, we can expect S(439) to be greater for the former than for the latter, as is seen in Figure 4.5a. The magnitude of increase in S(439) for *Synechococcus* sp., however, was far higher than one would expect from extrapolating from the data for *Thalassiosira weissflogii*. If we were to pool the data about variations in S(439) with changes in PP/ca for *Synechococcus* sp. with that for the diatoms shown in Figure 4.5a, we would get a high coefficient of determination (r^2=0.90). It would be a mistake, however, to conclude from this that all the changes in S(439) were due to changes in PP. The relationship between [S(439)/S(676)] and PP/ca (Figure 4.5b) provided a better way to discern the effect of PP on the difference $(a_{ph}^n - f_c^n)$. On the other hand, providing there is no bias due to the normalisation at 545 nm, the ratio S(676) should represent a measure of the effect of unequal distribution of chlorophyll-*a* between PSII and PSI.

4.4.3 Comparison between field and laboratory results

These experimental results confirm previous field results from a study in the North Atlantic (Chapter 3). For example, it is obvious that for the pooled data for the three individual species, the variation in the shape of the fluorescence excitation spectra would be higher than the variation in the shape of the absorption spectra (Figure 4.2), as was the case in the field (Chapter 3). In the laboratory, *Synechococcus* sp. showed the largest difference between absorption and fluorescence spectra, which was due to the particular distribution of chlorophyll-*a* inside the PSU. This

implies that in cyanophytes energy distribution inside the PSU corresponds to an extreme case of the "puddle model" (Figure 4.1c), and these results were consistent with the highest values of S(439) being found in nature in areas dominated by cyanophytes (Chapters 2 and 3). The two species of diatoms studied showed some variations in the difference between absorption and fluorescence spectra ($a^n_{ph} - f^n_c$). In *Thalassiosira weissflogii* this could be attributed mainly to absorption by PP implying that in this diatom the energy is distributed according to the "lake model" (Figure 4.1a), where both photosystems receive energy from all the LHCs in the PSU. In the case of *Chaetoceros* sp., however, part of the difference ($a^n_{ph} - f^n_c$) was not due to absorption by PP. This unexplained portion of $a^n_{ph} - f^n_c$ could have been caused in part by errors in the methods, but it seems also reasonable to suggest that in this species perhaps some of the LHCs were associated exclusively with PSI (puddle model), so that they did not contribute to the fluorescence spectrum. This is also consistent with field results in areas dominated by diatoms or prymnesiophytes where only some of the differences in the ratio S(439) could be explained by the presence of PP (Chapter 3). Recently, Culver *et al.* (1999) have suggested also that differences between absorption and fluorescence spectra (normalised at the red peak) of diatom-dominated field samples from Puget Sound (Washington, USA), could have been due to differential distribution of chlorophyll-*a* between PSII and PSI. As mentioned above, we still lack information on how all the different species of phytoplankton manage the energy flow inside the PSU, but it is possible that, besides the extremes of lake model (all the energy shared by PSII and PSI: e.g., *Thalassiosira weissflogii*) and puddle model (each photosystem has its own LHC: e.g., cyanophytes), there could be intermediate cases where only some of the LHCs are unique to PSII or PSI.

More detailed studies of the type performed by Johnsen *et al.* (1994*b*; 1997) separating different pigment-protein complexes or those carried out by Goedheer (1969) and Subramaniam *et al.* (1999) investigating changes in the fluorescence spectra at ambient temperature (caused mainly by PSII) and at low temperature

(-196 °C: showing also the contribution of PSI), should help us to understand how different LHCs distribute energy to PSI and PSII in different species of phytoplankton and under different physiological conditions.

4.5 Concluding Remarks

It has been shown through laboratory experiments with different species of phytoplankton that differences between absorption and fluorescence excitation spectra are not exclusively due to the presence of photoprotective pigments, confirming earlier field results (Chapter 3). Differences in the arrangement of the photosynthetic apparatus also contribute to differences between absorption and fluorescence spectra. Cyanophytes, which are extensively distributed and abundant in the open ocean (Johnson and Sieburth, 1979; Li *et al.*, 1983; Lewis *et al.*, 1988; Capone *et al.*, 1997; 1998), represent an extreme case in which the fluorescence spectrum differs significantly from the absorption spectrum. Other species, including diatoms (this study) and dinoflagellates (Johnsen *et al.*, 1994*b*; 1997) also show some degree of difference between absorption and fluorescence due to unequal distribution of chlorophyll-*a* between photosystems. Given this apparent influence of the arrangement of the photosynthetic apparatus on the fluorescence spectrum of phytoplankton, it would be advisable to evaluate the consequences of using the fluorescence spectrum as a proxy of the action spectrum of photosynthesis by phytoplankton.

CHAPTER 5

General Discussion and Conclusions

This chapter is divided into four sections. The first section provides a summary of the results obtained in the three main chapters of the thesis. The second gives a brief perspective of the most abundant phytoplankton groups and the factors that determine their distribution in the ocean. This brief review will put the observations of phytoplankton composition and their optical characteristics during the two cruises analysed in Chapter 2 into an ecological context. The third section focuses on differences in the arrangement of pigments within the photosynthetic apparatus in different groups of phytoplankton and the consequences for the estimation of primary production in the ocean. This section will explain the physiological relevance of the field and laboratory results on the fluorescence excitation spectra of phytoplankton reported in Chapters 3 and 4. Finally, in the last section, general conclusions are drawn from the main findings in this thesis.

5.1 Summary

In **Chapter 2** it was shown that, in the areas of the North Atlantic studied here, different groups of phytoplankton were distributed in a manner that is consistent with expectations based on previous work, from the point of view of photoadaptation. For example, in clear oligotrophic waters phytoplankton groups, such as cyanophytes and prochlorophytes, containing high levels of photoprotective pigments were predominant, whereas in shelf and high-latitude areas, where irradiance is on average low, groups containing high levels of blue-green-absorbing photosynthetic pigments were more abundant. Apart from these expected trends, it was noted that prymnesiophytes were one of the most abundant (possibly together with diatoms) and ubiquitously distributed phytoplankton groups. This can, to a great extent, be attributed to the "flexible" ensemble of pigments in their photosynthetic apparatus, which can contain varying proportions of different types of

photosynthetic pigments (with varying capacities for energy transfer) and an adequate photoprotective system.

The biomass and maximum values of absorption and fluorescence of the small-size fraction of the phytoplankton community (<2 μm) were, on average, less variable than those of the total phytoplankton population. This supports the hypothesis that there is a background of small cells which is more-or-less uniformly distributed in the ocean. By contrast, however, variations in spectral characteristics (absorption and fluorescence excitation) were as high for the small fraction as for the large fraction of phytoplankton, suggesting large variations in species composition or in the capacity for photoacclimation.

Variations in optical characteristics were caused by changes in the pigment composition of phytoplankton. For example, the ratio of absorption at 440 nm to that at 676 nm was positively correlated with the ratio of photoprotective pigments (especially zeaxanthin) to chlorophyll-*a*. High concentrations of photoprotective pigments are usually found in small cells, which by virtue of their small size are relatively unaffected by packaging. Hence, the presence of photoprotective pigments might be an indication of low packaging effect, nevertheless, this was not the sole cause of increase in the ratio of absorption at 440 nm to that at 676 nm. It was shown that absorption by photoprotective pigments was responsible for a substantial part of total absorption at 440 nm, which also enhanced the ratio of absorption at 440 nm to that at 676 nm.

Marked differences in the fluorescence excitation at 440 nm and in the ratio of the normalised (at 545 nm) absorption to fluorescence at 439 nm, S(439), were found to vary with phytoplankton composition and differences in size-class. Thus, the contribution of the small fraction to the total fluorescence at 440 nm was generally larger than the contribution of the small fraction to total absorption at 440 nm, so that the ratio S(439) was generally lower for the small fraction than for the large fraction or the total. One possible interpretation is that large cells have a higher packaging effect on the fluorescence than on the absorption spectrum.

Another interesting aspect of the variation in the fluorescence excitation spectra relates to what it tells us about the arrangement of pigments within the photosynthetic apparatus in different species. At stations where cyanophytes were abundant, the fluorescence excitation spectrum had very low values of fluorescence at 440 nm, which rendered a high ratio S(439). This can be related to two particular features in cyanophytes: they contain a high concentration of zeaxanthin (which does not transfer energy to PSII), and they have most of the chlorophyll-*a* (absorbing in the blue) associated with PSI which does not fluoresce at ambient temperature.

In **Chapter 3** it was shown that the shape of the fluorescence excitation spectrum was as variable as the shape of the absorption spectrum for phytoplankton communities throughout the study area. Hence, the fluorescence excitation spectrum cannot be considered a more conservative optical property of phytoplankton than the absorption spectrum, as has been suggested previously (Sosik and Mitchell, 1995). In fact the ratio between normalised (545 nm) absorption to fluorescence at 439 nm, S(439), varied considerably, which could be related in part to differences in phytoplankton composition and environmental conditions. Notably, the ratio of photoprotective pigments to chlorophyll-*a* explained, on average, only 34% of the variance in S(439), so that the difference between absorption and fluorescence excitation spectra was not only due to the presence of photoprotective pigments, as has often been assumed.

At one station in the subtropical North Atlantic there was a higher correlation between the ratio of zeaxanthin to chlorophyll-*a* and the number of cyanophytes, than between S(439) and the ratio of photoprotective pigments to chlorophyll-*a*. Hence, the difference between absorption and fluorescence excitation spectra must have also been due to the different distribution of pigments in the photosynthetic apparatus of cyanophytes. At one station in the Labrador Sea, where diatoms and prymnesiophytes were abundant, there was a high correlation between the ratio of photoprotective pigments to chlorophyll-*a* and the ratio S(439). Also, at this station there was a high positive correlation between S(439) and the ratio of

19-hexanoyloxyfucoxanthin to chlorophyll-*a*, which could be an indication that 19-hexanoyloxyfucoxanthin has a lower capacity to transfer energy to PSII than other photosynthetic pigments such as fucoxanthin. Thus, in Chapter 3 it was shown that differences between the absorption and the fluorescence excitation spectra are due not only to the presence of photoprotective pigments, but also to the arrangement of different pigments within the photosynthetic apparatuses of different species.

In **Chapter 4**, the results of laboratory experiments with cultures of different species of phytoplankton, representative of species found in the field (two diatoms: *Thalassiosira weissflogii* and *Chaetoceros* sp.; and a cyanophyte: *Synechococcus* sp.) are described. It was shown that the manner in which pigments are organised within the different parts of the photosynthetic apparatus has a profound impact on the shape of the fluorescence excitation spectrum, and hence, on the difference between the absorption and fluorescence excitation spectra. The photosynthetic apparatuses of diatoms and cyanophytes correspond to "lake" and "puddle" models respectively. Thus, in some diatoms, in this case in *Thalassiosira weissflogii*, which has a lake structure for its photosynthetic apparatus, it can be assumed that both photosystems (PSI and PSII) share all types of light-harvesting-complexes (LHCs). Thus, except for the absorption by photoprotective pigments (at high irradiance), the shapes of the absorption spectrum (representing all parts of the photosynthetic apparatus) and the fluorescence excitation spectrum (representing only LHCs serving PSII) are similar. By contrast, cyanophytes, which have a puddle structure for the photosynthetic apparatus, have two types of LHCs: one serving PSI (LHCI) and one serving PSII (LHCII). Hence, pigments found in LHCI (mainly chlorophyll-*a*) are not represented in the fluorescence excitation spectrum. This produces a large difference between the shapes of the absorption and fluorescence excitation spectra, as had been found in natural samples presented in Chapters 2 and 3. Another diatom, *Chaetoceros* sp., did show some differences in shape between its absorption and fluorescence excitation spectra, which could not be attributed to absorption by photoprotective pigments. In this case, some of the difference in S(439), might have

been due to an unequal distribution of pigments between LHCs serving PSI and PSII, an intermediate case between lake and puddle models.

Even though there is no direct evidence to support the idea, it seems possible that packaging does not have the same effect on fluorescence as it does on absorption, because of the differences in the organisation of pigments within PSI and PSII. If such a differential packaging effect exists, then one might speculate that some differences in S(439) might be due to changes in the packaging effect which accompany unequal increases in LHCs serving PSII and PSI. This idea deserves future study.

5.2 Distribution of Phytoplankton Species in the Ocean

Early observations of phytoplankton distribution in the ocean focused on large cells, or netplankton (>20 μm), such as diatoms and dinoflagellates. This bias in the size of cells studied was due to the limitations imposed by the method of sampling and preservation and by the optical instrumentation used to analyse the samples (Mills, 1989). Thus, early studies led to the belief that this size class of algae, and among them diatoms, was the major contributors to primary production in the ocean (Werner, 1977; Guillard and Kilham, 1977). The highest concentrations of these groups were found in areas characterised by the input of high concentrations of nutrients (e.g., coastal, frontal, and upwelling areas). On the other hand, the large central basins of the ocean, depleted in nutrients, were considered to be permanent deserts.

In the present study, if we would look only at the >2 μm component of phytoplankton biomass (Figures 2.3, 2.5 and 2.10), then we would reach similar conclusions. That is, it would have appeared that the phytoplankton community was dominated by large cells, mainly diatoms, in coastal and nutrient rich areas (Figures 2.3, 2.5, and Table 2.2). The observations were further biased by the fact that the two cruises analysed in this thesis took place in spring, when maximum abundance of large diatoms in shelf areas is expected (see Margalef, 1978). Under optimal nutrient supply, diatoms have high growth rates (e.g., up to 3 doublings

per day in *Thalassiosira weissflogii*, Costello and Chisholm, 1981). On the other hand, their large volume and their lack of motility together with their high nitrate requirement, can explain why diatoms tend to dominate in coastal or frontal areas, where a compromise between mixing (maintaining them in the euphotic zone) and high nutrient supply is reached (Margalef, 1978).

The pigment composition of diatoms is such that they absorb mainly blue-green light (main harvesting pigments chlorophyll-*a*, chlorophyll-*c*, and fucoxanthin). The main photoacclimation mechanism in diatoms is to maximise light absorption at low irradiance by increasing the amount of photosynthetic pigments, which is consistent with the type of environment to which they are best adapted: areas of high mixing and coastal waters which tend to be turbid at times. They also possess a mechanism of photoacclimation to high irradiance involving diadinoxanthin and diatoxanthin in the so-called xanthophyll cycle (Demmig-Adams, 1990; Demers *et al.*, 1991), which provides a fast (order of minutes) photoprotection to the RCs. This also represents an advantageous feature for dealing with the sudden exposure to high irradiances during mixing.

The other main group of large cells, dinoflagellates, is known to produce extensive blooms in coastal areas occasionally (Lam and Ho, 1989; Carreto *et al.*, 1985; Negri *et al.*, 1992). Some of these are dominated by toxin-producing species, and some species produce intense discolouration of the surface waters, hence their common denomination as "red tides". Because of these particular features and their social-economical implications, the study of dinoflagellate blooms is receiving increasing attention (see Hallegraeff, 1993). Aside from extensive blooms, dinoflagellates are an important part of the phytoplankton community in shelf waters, and especially in temperate stratified waters in summer, after the diatom spring bloom (Margalef, 1978). One of the reasons why dinoflagellates grow under relatively low nutrient concentrations is their motility, which allows them to photosynthesize during the daylight close to the surface and take up nutrients at night close to the nutricline (Margalef, 1978; Santos and Carreto, 1992; MacIntyre *et al.*, 1997; Cullen and

MacIntyre, 1998). Dinoflagellates also possess pigments able to absorb blue-green light: Their main LHCs contain peridinin, chlorophyll-*c* and chlorophyll-*a* (Prézelin and Sweeney, 1978; Hoffman *et al.*, 1996), and they can increase the amount of these photosynthetic pigments substantially under low light conditions. In addition, they also possess xanthophyll-cycle pigments for photoprotection under high irradiance. In this study dinoflagellates were more abundant on the continental-shelf area south of Newfoundland and at some stations in the Labrador Sea (Table 2.2). At some of these stations peridinin concentrations were quite high (> 0.2 mg m^{-3}; Figure 4.2), but on average for the whole study area, the ratio of peridinin to total chlorophyll-*a* was much lower than that of fucoxanthin to chlorophyll-*a*, which would indicate the dominance of diatoms and prymnesiophytes over dinoflagellates at a large spatial scale (Figure 2.6).

Recent studies have demonstrated the important contribution of smaller cells, the nanoplankton (2 - 20 μm), to the total phytoplankton biomass in the ocean (Malone, 1980*a*; *b*; Jacques, 1989). In this study (Chapter 2) one of the prominent features of the pigment composition at different locations was the ubiquitous presence, in relatively high abundance, of 19-hexanoylxoyfucoxanthin, which is a pigment characteristic of prymnesiophytes (see Figures 2.3, 2.5 and 2.6). This phytoplankton group comprises a diverse number of species, some of which are members of the nanoplankton. Many prymnesiophyte cells pass through 2 μm filters, however, and are part of the picoplankton fraction (<2 μm). Some species of prymnesiophytes can form extensive and conspicuous blooms, such as the genus *Phaeocystis* which can produce large colonies of cells embedded in a mucilaginous exudate (Palmisano *et al.*, 1986; Cota *et al.*, 1994; Marra *et al.*, 1995), or the genus *Emiliania* whose cells are covered by calcareous plaques (cocoliths; Ackelson *et al.*, 1988; Brown and Yoder 1994). The small size of prymnesiophytes, in comparison with most diatoms and dinoflagellates, makes them more efficient at uptake of nutrients. On the other hand, they also have photosynthetic pigments able to absorb blue-green light. It has been hypothesized (van Leeuwe and Stefels, 1998) that prymnesiophytes can

readily transform fucoxanthin, a highly efficient light-harvesting pigment, into the less efficient 19-hexanoyloxyfucoxanthin derivative according to the light intensity to which the cells are exposed. This may provide another means, along with the xanthophyll-cycle, to reduce the flux of energy to the RCs and hence avoid photodamage at high irradiance. This extra flexibility in their capacity to adapt to a variety of light fields might be an important factor determining the widespread distribution of prymnesiophytes in the ocean.

The existence of free-living cyanophytes as a component of the phytoplankton community in the ocean was recognised two decades ago (Johnson and Sieburth, 1979). Since then, with the development of new techniques (e.g., flow cytometry), the importance of cyanophytes and that of another prokaryotic group, prochlorophytes (Chisholm *et al.*, 1988), has become more clear (Platt and Li, 1987; Partensky *et al.*, 1999). Some of the early studies suggested that cyanophytes were susceptible to photoinhibition and therefore could not grow at the high irradiances found at the surface of the ocean (Glover, 1985). This belief was in part due to the lack of success in culturing cyanophytes at high irradiance (Barlow and Alberte, 1985). Nevertheless, cyanophytes have been found growing at the surface and in the upper layers of the euphotic zone in the ocean (Iturriaga and Michell, 1986; Veldhuis and Kraay, 1990). Furthermore, Kana and Glibert (1987) carried out laboratory experiments which demonstrated that cyanophytes could grow at high irradiance (up to 2000 μmol quanta $m^{-2} s^{-1}$) if they were acclimated gradually from low to higher irradiances. Note, the contrast in the ability to photoacclimate in cyanophytes, which require a gradual (longer term) acclimation, and diatoms, which are able to survive sudden changes to extreme levels of irradiance.

There is now increasing evidence that a common feature of the vertical distribution of phytoplankton in the tropical open ocean is the numerical dominance of cyanophytes in the upper layer, followed by prochlorophytes slightly above the base of the euphotic zone, and finally picoeukaryotes at the base of the euphotic zone (Li, 1995; Shimada *et al.*, 1996; Partensky *et al.*, 1996; Bouman, 1998). This

vertical distribution can be explained mainly by the strategies of adaptation of different algae to different environments (e.g., light field and nutrient availability). Thus, the dominance of picoplankton (<2 μm) in the phytoplankton community of tropical oceans can be attributed largely to the higher surface-to-volume ratio of small cells, which favours the uptake of the scarce nutrients found in these areas (Chisholm, 1992). Among the picoplankton groups prokaryotes (cyanophytes and prochlorophytes) seem to be better suited to grow under low nutrient concentrations, while eukaryotes (e.g., prymnesyophytes, prasinophytes, chlorophytes) found in larger numbers at the base of the euphotic zone take advantage of the nutrient input from below the nutricline (Shapiro and Guillard, 1987). Furthermore, both cyanophytes and prochlorophytes, have high concentrations of photoprotective pigments, mainly zeaxanthin, which should certainly be beneficial, if not necessary, feature for life under the high irradiance found in the clear waters of the tropical oceans.

Cyanophytes contain phycobilins as the main light-harvesting-complexes which absorb light in the middle of the visible spectrum from the blue-green (~495 nm, phycourobilin), green (~550 nm, phycoerytrobilin), to the orange (~650 nm, phycocyanin). Water absorbs strongly in the red part of the light spectrum (Smith and Baker, 1983), so that a few meters below the sea surface the intensity of red light is very low. High intracellular concentrations of zeaxanthin, located in the cytoplasmic membrane in cyanophytes (Omata and Murata, 1983), also considerably reduce the amount of blue light reaching the thylakoids. Thus, the light available for photosynthesis (i.e. the light reaching the thylakoids) will be in the mid-visible range which can be harvested efficiently by phycobilins, especially phycoerythrin in the near surface layers. Cyanophytes, as postulated above, are usually more abundant in the upper zone of the water column, but they can be found throughout the euphotic zone. Cyanophytes at depth not only increase the intracellular concentration of phycobilins and chlorophyll-*a*, as an acclimation to low light (Kana and Glibert, 1987: Figure 2.7), but also change the proportions of phycoerytrobilin

(PEB) and phycourobilin (PUB), showing a higher PUB:PEB ratio (Vernet *et al.*, 1990; Wood *et al.*, 1998). This change in pigment composition may be due to the presence of different species (or strains) of cyanophytes possessing different proportions of PEB and PUB, rather than photoacclimation of surface populations (Ikeya *et al.*, 1994; Wood *et al.*, 1998). Thus, cyanophyte populations at depth tend to increase the absorption of light in the blue region of the spectrum to provide an adjustment to the prevailing spectral light at depth in open ocean areas. A change from low to high PUB:PEB ratio has also been attributed to chromatic adaptation of different strains of *Synechococcus* growing in coastal (prevailing green light) to oceanic (prevailing blue light) areas of the ocean (Wood, 1985; Wood *et al.*, 1998; 1999).

Prochlorophytes, which are predominant at depth, are more suited to absorbing blue light, since they have high concentrations of chlorophyll-*b* and especially divinyl-chlorophyll-*b* (Veldhuis and Kraay, 1990; Moore *et al.*, 1995; Figure 2.6). Prochlorophytes found in the upper layers of the euphotic zone, by contrast, have very low concentrations of divinyl-chlorophyll-*b*, and it has been reported that this vertical pattern is due to the presence of different strains of prochlorophytes with different pigment composition (Moore *et al.*, 1995; 1998).

At the lower limit of the euphotic zone picoeukaryotes are dominant. Several factors may contribute to this. First, as has already been mentioned, they need to utilise the higher levels of nutrients supplied from below the nutricline. Second, they possess high concentrations of blue-light absorbing pigments (e.g., chlorophyll-*a*, chlorophyll-*c*, chlorophyll-*b*) and carotenoids, which absorb in the green, and which can be present in concentrations high enough to increase the overall absorption (Wood, 1985; Glover *et al.*, 1986; Shapiro and Guillard, 1987; Warnock, 1990). On the other hand, eukaryotes do not have such high concentrations of photoprotective pigments as prokaryotes, which may also render them less able to live under the high irradiances of the upper layer of clear oligotrophic waters.

Li (1995) studied the abundance of three groups of ultraphytoplankton (i.e. <5 μm, encompassing prokaryotes, picoeukaryotes and part of the nanoplankton also) along a transect across the Central North Atlantic during fall and spring (the latter corresponding to the Canary transect described in this thesis). Some differences were observed in the abundance of different groups of ultraplankton with season. For example, prochlorophytes were found in greater numbers in the fall, although on a basin-average the numerical order of abundance was similar for both seasons: prochlorophytes > cyanophytes > eukaryotes (Li, 1995). Despite this marked numerical predominance of prochlorophytes, on a carbon basis, and also on the basis of contribution to total primary production, the order was different: eukaryotes > prochlorophytes > cyanophytes. In fact, in this part of the ocean, small eukaryotes were responsible for ~68% of the total primary production (Li, 1995).

As the number of reports of the widespread abundance of small phytoplankton cells in all type of environments in the ocean grew, the hypothesis was put forward that there is a "background" of small cells in all the world's ocean (Malone, 1980*a*; Yentsch and Spinrad, 1987; Yentsch, 1990). In the present study, average values of biomass (as chlorophyll-*a*) and the magnitude of the absorption and fluorescence signals were less variable in the small fraction than they were in the large fraction (Table 2.4), which is consistent with the idea of a more-or-less uniform distribution of small cells. On the other hand, this fraction showed an equal or greater variability than the large fraction in the characteristics of its absorption and fluorescence excitation spectra (Figure 2.11) suggesting that it is composed of a diverse phytoplankton population, or that any ubiquitous species have optical properties that are quite plastic.

Recapitulating, over the last few decades there has been a change in idea as to which are the most abundant groups of phytoplankton in the ocean, downwards in size from the netplankton (> 20 μm) to the nanoplankton (20 to 2 μm) and finally to the picoplankton (<2 μm). On the other hand, on average, over a variety of

environments. it seems that medium to large-size cells (i.e., nano and netplankton) make the largest contribution to phytoplankton biomass as suggested by Malone (1980*b*) and as shown here by the dominance of >2 μm to total chlorophyll (Table 2.4). It should be recognised. nevertheless. that despite the global dominance of certain net and nanoplankton groups to total biomass, large areas of the world's oceans (e.g.. ocean-basins) are dominated by a variety of small picoplankton cells. Hence. if we want to understand how different marine ecosystems function. we cannot focus our attention only on global quantities. We need also to understand why and how the different phytoplankton groups have evolved the specific characteristics that allow them to live under different environmental conditions. and further how these different phytoplankton communities interact with organisms from other trophic levels.

5.3 Arrangement of the photosynthetic apparatus in different algae: possible implications on the estimation of primary production

In the previous section the effect of pigment composition on the distribution of different groups of phytoplankton (see Chapter 2) was discussed. Pigments are not isolated inside cells. however. they are complexed with proteins forming specific parts of the photosynthetic apparatus (see Chapter 4). Pigment composition provides useful information on the ecological distribution of different species and it helps explain changes in the optical characteristics of phytoplankton which eventually can be applied in modelling exercises. Why then should we be concerned about the organisation of those pigments inside the cells? First of all. it is known that energy flows inside the photosynthetic membrane. and hence photosynthesis depends on its structural organisation. i.e.. the distribution of pigments among the different parts of the PSU (Butler. 1978: Larkum and Barrett. 1983: Falkowski and Raven. 1997). Based on what is known from the literature and the results from Chapters 3 and 4. one can speculate on how these variations in the arrangement

of the photosynthetic apparatus might affect calculations of primary production in the ocean.

If primary production is estimated *in situ*, for example, by means of incubation experiments with ^{14}C, in which algae are maintained at the depth from which they were collected, then the calculated production should be close to the true value (but see Marra, 1978; Cullen and Neale, 1993, for comments on artifacts of static incubations). In this case, whatever the arrangement of the photosynthetic apparatus of the phytoplankton was, it would be taken into account in the final result, since the cells would have photosynthesized according to the natural light field in which they had been growing. If production measurements are made exposing the samples to artificial light, distortions from the true value can be expected, unless extreme care is taken to simulate the natural light field as closely as possible. For example, Warnock (1990) found that *Synechococcus* incubated under green light had a higher photosynthetic efficiency at low light (α^B: initial slope of the production vs. irradiance curve normalised to biomass) than when they were incubated under white or blue light; that is because this strain (WH7803) is rich in phycoerythrin. Prézelin *et al.* (1989) found that natural samples containing cyanophytes had lower production rates when they were incubated in white light, than when they were incubated in blue-green light, more representative of the light conditions at the collection depth. This was because these cyanophytes growing deep in the euphotic zone were acclimated to absorb strongly in the blue-green (see Chapters 2 and 4, and previous section in the discussion). In addition, when action spectra (defined in Chapter 3) are recorded by estimating ^{14}C incorporation under monochromatic light without the addition of background light of a complementary wavelength, production is underestimated at wavelengths where part of the light is absorbed by pigments serving only PSI (Emerson, 1958; Larkum and Barrett, 1983). This effect is important in algae containing phycobilins, for example cyanophytes (Lewis *et al.*, 1988; Warnock, 1990), in which most of the chlorophyll-*a* is in PSI; that is, their PSUs have a "puddle" structure (see Chapters 3 and 4). This bias towards

representing only the action spectrum of PSII also occurs when the fluorescence excitation spectrum is used as a proxy for the action spectrum of photosynthesis (Neori *et al.*, 1988; Johnsen and Sakshaug, 1996; Figures 3.5 and 4.2).

From an oceanographic perspective, it is important to know how this bias towards the action spectrum of PSII might affect the estimation of primary production, considering the distribution of algae with different arrangements of the PSUs and the light field at which they grow. Cyanophytes have been taken here as an extreme case to evaluate this problem, since they represent the "puddle" model, with the maximum heterogeneity in the distribution of LHCs between photosystems (see Chapter 4). Species showing a "lake" arrangement of the PSU should, in principle, show no difference between the total action spectrum and that of PSII alone. Different species of cyanophytes have been found to constitute an important part of the phytoplankton population in the ocean (Prézelin *et al.*, 1989; Olson *et al.*, 1990; Glover *et al.*, 1985; Shimada *et al.*, 1996; Lewis *et al.*, 1988; Capone *et al.*, 1997). If these algae are abundant in the upper layers of the euphotic zone, where light intensity is high enough to saturate photosynthesis, primary production should be unaffected by considerations of the action spectrum, and there should be no bias in its modelled estimate. The action spectrum of photosynthesis represents the variation with wavelength of the rate of production at low monochromatic irradiance (see definition Chapter 3). When cyanophytes grow close to the surface, photosynthesis proceeds at a constant rate, defined by the assimilation number (P_m^B, see Platt and Jassby, 1976). On the other hand, cyanophytes are also found deep in the euphotic zone in the open ocean (Prézelin *et al.*, 1989; Takahashi and Hori, 1984), where the prevailing light is blue-green (see Morel *et al.*, 1993; Johnsen *et al.*, 1992; Prézelin *et al.*, 1989; Ikeya *et al.*, 1994). This type of light would be mainly absorbed by chlorophyll-*a*, which in cyanophytes transfers energy to the PSI, and by carotenoids, mostly photoprotective, which do not transfer energy to the RCs. To be able to fix CO_2, algae need both photosystems to operate in series. That is, there should be a balance between the energy reaching RCI and RCII.

PSII is able to transfer excess energy to PSI by a process known as spillover, but the opposite, transfer from PSI to PSII, does not occur (Butler, 1978; Govindjee, 1995). Hence, the question has been raised as to how cyanophytes photosynthesize in environments where the predominant light excites mainly PSI. Ikeya *et al.* (1994) have shown, that cultures grown under blue-green light increase considerably their concentrations of phycobilins, which serve PSII, such that the fraction of blue-green light they absorb reaches $\sim$ 36 - 44 % of the total light. It was also shown that some of the carotenoid pigments (probably β-carotene, see Goedheer, 1969) can transfer energy to PSI, in addition to the energy absorbed directly by chlorophyll-*a* in the PSI. All these effects combined, Ikeya *et al.* (1994) estimated that 80% of the blue-green light was being used in photosynthesis and that almost equal amounts of energy were transferred to PSI and PSII. A similar type of study was conducted by Johnsen *et al.* (1992), who showed the adaptation of a diatom and a prymnesiophyte to different spectral light conditions.

Even in the extreme case considered above (cyanophytes growing deep in the water column), both photosystems are likely to be actively harvesting light, and both should therefore, be represented in the action spectrum. Both are represented in the absorption spectrum (Kyewalyanga *et al.*, 1997) which, if corrected for the presence of photoprotective pigments, might provide a more realistic estimation of the action spectrum than the fluorescence excitation spectrum. Warnock (1990), in a study of photosynthetic characteristics of picoplankton, measured ^{14}C action spectra of two strains of *Synechococcus* and one prymnesiophyte (*Pavlova* sp.), as well as these of field samples from an Arctic site (chromophyte dominated phytoplankton community) and an oligotrophic site (picoplankton dominated phytoplankton community). He analysed, based on these results, the implications of the 'Emerson enhancement effect' (see Chapter 3) on the estimation of primary production. Warnock (1990), concluded that this effect, was significant in unialgal cultures of cyanophytes, but it was negligible in his field samples. This conclusion relied on

the fact that cyanophytes had only a small contribution to the total phytoplankton absorption and ^{14}C action spectra even at the oligotrophic site. Nevertheless, Warnock (1990) suggested that there might be circumstances when cyanophytes dominate the phytoplankton community in the ocean, and hence, the bias produced by using a PSII action spectrum in the estimation of primary production could be significant. Warnock (1990) also developed a rational two-photosystems model to estimate primary production, which would take into account differences in the energy distribution between photosystems. Other studies have compared the efficiency of phytoplankton photosynthesis estimated by different types of action spectrum. For example, Schofield *et al.* (1990) compared the quantum yields of photosynthesis in cultures of different species estimated using ^{14}C action spectra with and without complementary illumination; Wood (1985) reported the effect of chromatic adaptation on the estimation of primary production in cultures of different species incubated *in situ* at different depths in the ocean; Prézelin *et al.* (1989) showed the effect of using different spectral light sources in estimating primary production from natural samples; Kroon *et al.* (1993) compared the quantum yields of photosynthesis estimated by measuring charge separation at PSII (by variable pulse amplitude fluorometry), oxygen evolution, and ^{14}C, in a dinoflagellate exposed to different spectral lights.

The question of what is the best representation of the action spectrum of photosynthesis, however, merits further study. This study should comprise the measurement of different types of action spectra (e.g., ^{14}C incorporation, with and without complementary illumination; fluorescence excitation spectrum; and absorption spectrum, total and that of photosynthetic pigments) in different regions of the ocean, where species with different arrangements of the PSU (e.g., lake, puddle, and any intermediate model) are to be found. Spectral light field should be measured at the corresponding depths. Primary production should then be computed using these spectral measurements and contrasted with *in situ* measurements. Furthermore,

the effect of using different types of action spectra should be evaluated at different scales, e.g.: at a specific depth, as an integrated water column value, and as a seasonal, regional or global average.

The arrangement of the PSU might affect not only the action spectrum of photosynthesis, but also the estimation of primary production by remote sensing (using phytoplankton fluorescence emission) or by using the method of flash-fluorescence (e.g., pump-and-probe). Prediction of the effects of lake and puddle arrangements of the PSU on these sophisticated methods of estimating primary production is beyond the scope of this study, but it does deserve to be addressed thoroughly since these techniques (remote sensing and flash-fluorescence) are being used increasingly for the estimation of primary production in the ocean. Finally, beyond the possible effect of the arrangement of the PSU on quantitative studies of primary production in the ocean, the variability in the structure of the photosynthetic apparatuses constitutes a prime reason why different species of phytoplankton are adapted to live and photosynthesize in different environments in the ocean. Thus, from ecological and evolutionary considerations, the physiological adaptations of the PSU in different species of algae are *per se* important and fascinating areas of study.

5.4 Conclusions

In this thesis it has been demonstrated that phytoplankton communities from different regions of the North Atlantic exhibit significant differences in their optical properties (absorption and fluorescence excitation spectra), which can be explained in part in terms of photoadaptation and photoacclimation. Spectral variation in the optical characteristics was also evident in the small-size fraction of phytoplankton, despite its having a more homogeneous biomass distribution in the ocean than the large-size fraction. In light of these observations it seems advisable that bio-optical variability should be incorporated into models used to estimate primary production and in algorithms used to retrieve the concentration of phytoplankton pigments from satellite measurements.

In this work it has also been shown that the shape of the fluorescence excitation spectrum is dependent not only on the state of photoacclimation (concentration of photoprotective pigments) but also on the organisation of pigments within the photosynthetic apparatus which varies between species. This variation was observed both in experiments with cultures of different species and in the field. The bias of the fluorescence excitation spectrum towards the action of PSII alone may affect its serving as a proxy for the action spectrum of photosynthesis. This study has provided evidence that the fluorescence excitation spectrum is biased towards the action of PSII. Whether this bias seriously limits the use of the fluorescence excitation spectrum as a proxy for the action spectrum of photosynthesis in the marine environment remains an open question, and merits further study.

APPENDICES

Appendix A: Estimation of the absorption and fluorescence excitation spectra

A.1 *In vivo* absorption

The *in vivo* absorption spectra were obtained following the quantitative filter technique (Yentsch, 1962; Kiefer and SooHoo, 1982; Mitchell and Kiefer, 1984, Kishino *et al.*, 1985). The optical density of particles on the filter ($D_{pf}(\lambda)$) was recorded between 350 and 750 nm. The blank was obtained by filtering a volume of filtered seawater from the given station (in a few cases distilled water), or the filtrate from the sample (in the case of culture experiments), similar to the volume filtered for the samples. The optical densitiy of the blank was subtracted from the optical density of the sample. The value measured at 750 nm was subtracted from the rest of the spectrum, assuming that this optical density was due to non-pigmented substances and that its effect was spectrally neutral (Sathyendranath *et al.*, 1987; Bricaud and Stramski,1990).

The values of optical densities measured on filters $[D_{pf}(\lambda)]$ were corrected for the pathlength amplification factor, β (Butler, 1962), which is defined as the ratio of the optical thickness of a diffusing material to its geometric thickness: i.e., β represents the amplification of the path of light through the glass fiber filter due to multiple scattering. The β factor is usually expressed (Kishino et al., 1985) as:

$$\beta = D_{pf}(\lambda)/D_p(\lambda). \qquad (A.1)$$

where D_p is the optical density of particles in suspension. To correct for the path-length amplification a quadratic equation, proposed by Mitchell (1990) was used:

$$D_p(\lambda) = A\,[D_{pf}(\lambda)] + B\,[D_{pf}(\lambda)]^2. \qquad (A.2)$$

The coefficients used for the field samples were those reported by Hoepffner and Sathyendranath (1992; A=0.31, B=0.57), for the culture experiments the coefficients were derived individually for each species (for *Thalassiosira weissflogii* A=0.35, B=0.20; for *Chaetoceros* sp. A=0.47, B=0.19; for *Synechococcus* sp. A=0.41, B=0.32).

In samples where procholorophytes were present, a specific set of coefficients was used to correct the pathlength amplification of optical density by prochlorophytes. The ratio of divinyl-chlorophyll-*a* (marker pigment for prochlorophytes) to total chlorophyll-*a* in the sample was calculated (F_{dv}). It was assumed that the same ratio applied to the contribution of optical density by prochlorophytes to the total optical density in the sample. Finally the fraction of optical density due to prochlorophytes was corrected using the specific coefficients determined by Moore *et al.* (1995; A_2=0.291, B_2=0.051) and the remainder was corrected using the coefficients for a mixture of phytoplankton reported by Hoepffner and Sathyendranath (1993):

$$D_p(\lambda) = F_{dv}[A_2\, D_{pf}(\lambda)+B_2\,(D_{pf}(\lambda))^2]+(1-F_{dv})[A\, D_{pf}(\lambda)+B\,(D_{pf}(\lambda))^2]. \quad (A.3)$$

The absorption coefficient of particles, $a_p(\lambda)$ (m^{-1}), was obtained as:

$$a_p(\lambda) = 2.3D_p(\lambda)/X. \quad (A.4)$$

where X (m) is the height in meters of the water column filtered, given by V/S (V =volume of seawater filtered (m^3) and S =filtering area of the filter (m^2)); and 2.3 is the conversion factor for transforming decimal logarithms to natural logarithms.

Corrections were made for detrital absorption either using the extraction method of Kishino *et al.* (1985), or the theoretical approach proposed by Hoepffner and Sathyendranath (1993). For the samples corrected using the extraction method (cultures of *Thalassiosira weissflogii* and *Chaetoceros* sp.) the total optical density of the particles was measured on the filter [$D_{pf}(\lambda)$] (example shown in Figure A.1a)

FIGURE A.1. Example of the corrections applied to the absorption spectra of phytoplankton. sample of *Thalassiosira weissflogii* grown at 20 μmol quanta $m^{-2} s^{-1}$. **a**) Optical densities of total particulate material on the filter ($D_{pf}(\lambda)$), detrital material on the filter ($D_{df}(\lambda)$), total particulate material in suspension (i.e., corrected for the pathlength amplification factor: $D_p(\lambda)$), detrital material in suspension (i.e., corrected for the pathlength amplification factor; $D_d(\lambda)$), and phytoplankton in suspension (i.e., $D_{ph}(\lambda) = D_p(\lambda) - D_d(\lambda)$). **b**) Representation of the *in vivo* absorption spectra of pigments present in *T. weissflogii*, and the total reconstructed spectrum of the diatom according to the method of Bidigare *et al.* (1990): $a_{ca}(\lambda)$: absorption spectrum of chlorophyll-*a*. $a_{cc}(\lambda)$: absorption spectrum of chlorophyll-*c*. $a_{ps}(\lambda)$: absorption spectrum of photosynthetic carotenoids. $a_{pp}(\lambda)$: absorption spectrum of photoprotective carotenoids. $a_r(\lambda)$: total reconstructed absorption spectrum. $a_{ph}(\lambda)$: absorption spectrum measured. **c**) Measured absorption spectrum normalised to 1 at 545 nm ($a^n_{ph}(\lambda)$) and portion of absorption from $a^n_{ph}(\lambda)$ corresponding to photoprotective pigments ($a^n_{pp}(\lambda)$) estimated according to Babin *et al.* (1996).

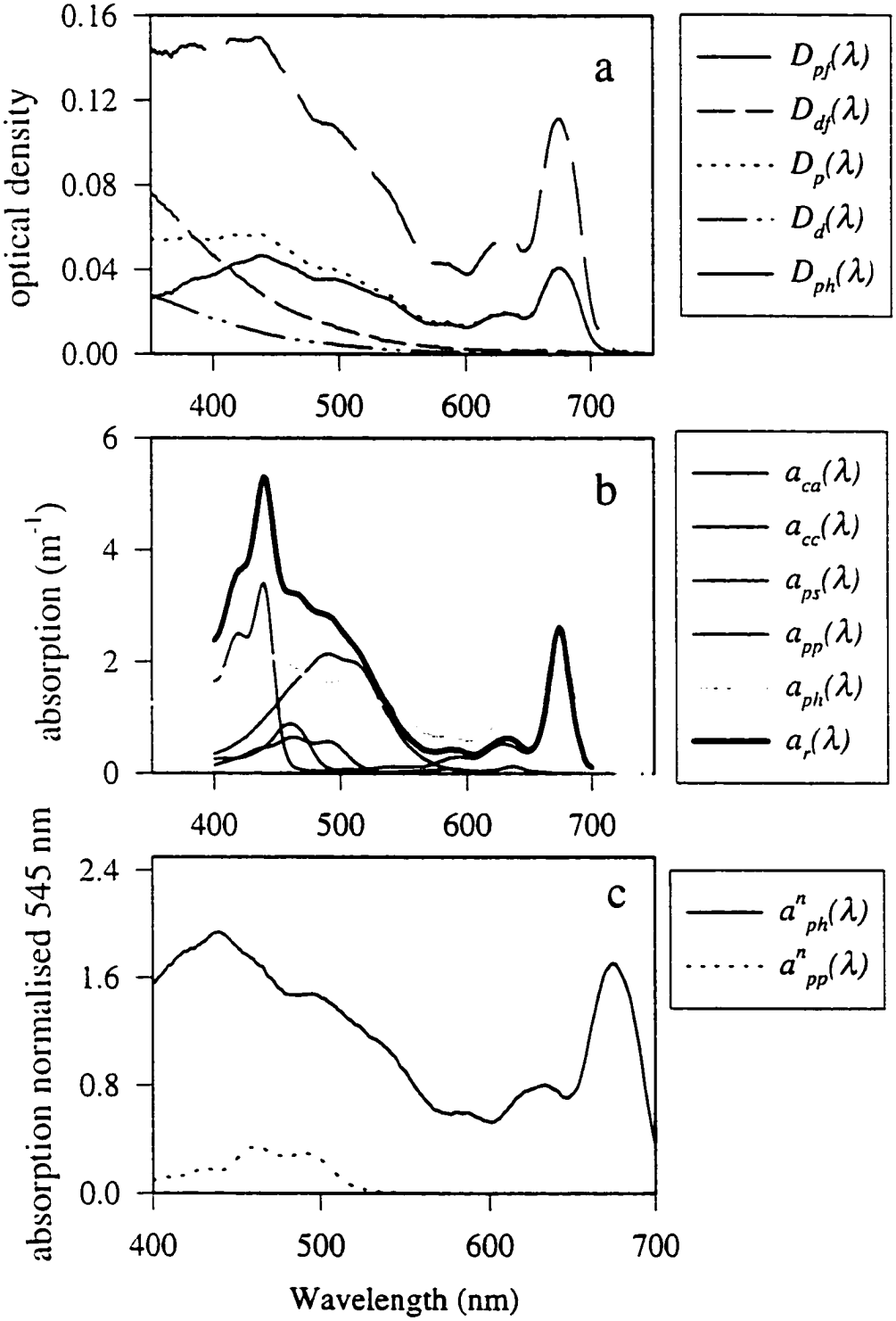
a
optical density
0.16
0.12
0.08
0.04
0.00
400
500
600
700
$D_{pf}(\lambda)$
$D_{df}(\lambda)$
$D_p(\lambda)$
$D_d(\lambda)$
$D_{ph}(\lambda)$
b
absorption (m^{-1})
6
4
2
0
$a_{ca}(\lambda)$
$a_{cc}(\lambda)$
$a_{ps}(\lambda)$
$a_{pp}(\lambda)$
$a_{ph}(\lambda)$
$a_r(\lambda)$
c
absorption normalised 545 nm
2.4
1.6
0.8
0.0
$a^n_{ph}(\lambda)$
$a^n_{pp}(\lambda)$
Wavelength (nm)

FIGURE A.1

and then pigments were extracted by passing passively through the filters 20 ml of a mixture of 90% acetone and dimethyl sulphoxide (DMSO) at a ratio of 6 : 4 (vol : vol: Hoepffner and Sathyendranath, 1992), followed by 10 ml of filtered seawater. The addition of seawater after the extraction by solvents is done to re-equilbrate the medium to the initial conditions. The optical density of detrital particles (i.e., particles without pigments) on the filter was measured $[D_{df}(\lambda)]$ (Figure A.1a). The blank was treated in the same way as the sample (extracted by solvents and washed with filtered seawater), and its optical density was subtracted from that of the sample.

The correction for the pathlength amplification factor on the filter measurements was applied individually to the total $[D_{pf}(\lambda)]$ and the detrital optical density $[D_{df}(\lambda)]$ to obtain the optical density of particles $[D_p(\lambda)]$ and detritus $[D_d(\lambda)]$ in suspension (Figure A.1a). The optical density of phytoplankton $[D_{ph}(\lambda)]$ (Figure A.1a) was obtained by subtracting the optical density of detritus from that of the total:

$$D_{ph}(\lambda) = D_p(\lambda) - D_d(\lambda). \tag{A.5}$$

Finally the optical density of phytoplankton was converted to absorption $[a_{ph}(\lambda)]$ using an equation similar to equation A.4 (Figure A.1b).

When the theoretical approach of Hoepffner and Sathyendranath (1993) was used (for field samples and for the culture of *Synechococcus* sp.) the optical density of particles on the filter was measured. This $[D_{pf}(\lambda)]$ was corrected for the pathlength amplification factor using equation A.2, or A.3 if the sample contained prochlorophytes, and this optical density in suspension $[D_p(\lambda)]$ was converted into absorption in suspension $[a_p(\lambda)]$ using equation A.4. The detritus correction was applied on $[a_p(\lambda)]$ (Figure A.2a). The theoretical approach of Hoepffner and Sathyendranath (1993) assumes an exponential shape for detrital absorption $[a_d(\lambda)]$ (Figure A.2a) of the form:

$$a_d(\lambda) = a_d(440)\exp[-q(\lambda - 440)]. \tag{A.6}$$

FIGURE A.2. Example of the corrections applied to the absorption spectra of phytoplankton. sample of *Synechococcus* sp. grown at 20 μmol quanta $m^{-2} s^{-1}$. **a**) Absorption spectra of total particulate material in suspension ($a_p(\lambda)$), detrital material in suspension (estimated according to Hoepffner and Sathyendranath. 1993: $a_d(\lambda)$), and phytoplankton in suspension (i.e., $a_{ph}(\lambda) = a_p(\lambda) - a_d(\lambda)$). **b**) Representation of the *in vivo* absorption spectra of pigments present in *Synechococcus*. and the total reconstructed spectrum of the cyanophyte according to the method of Bidigare *et al.* (1990): $a_{ca}(\lambda)$: absorption spectrum of chlorophyll-*a*. $a_{pp}(\lambda)$: absorption spectrum of photoprotective carotenoids. $a_r(\lambda)$: total reconstructed absorption spectrum. $a_{ph}(\lambda)$: absorption spectrum measured; notice that the absorption of spectrum of phycobilins could not be estimated because of lacking the concentration of these pigments. **c**) Measured absorption spectrum normalised to 1 at 545 nm ($a^n_{ph}(\lambda)$) and portion of absorption from $a^n_{ph}(\lambda)$ corresponding to photoprotective pigments ($a^n_{pp}(\lambda)$) estimated according to Babin *et al.* (1996).

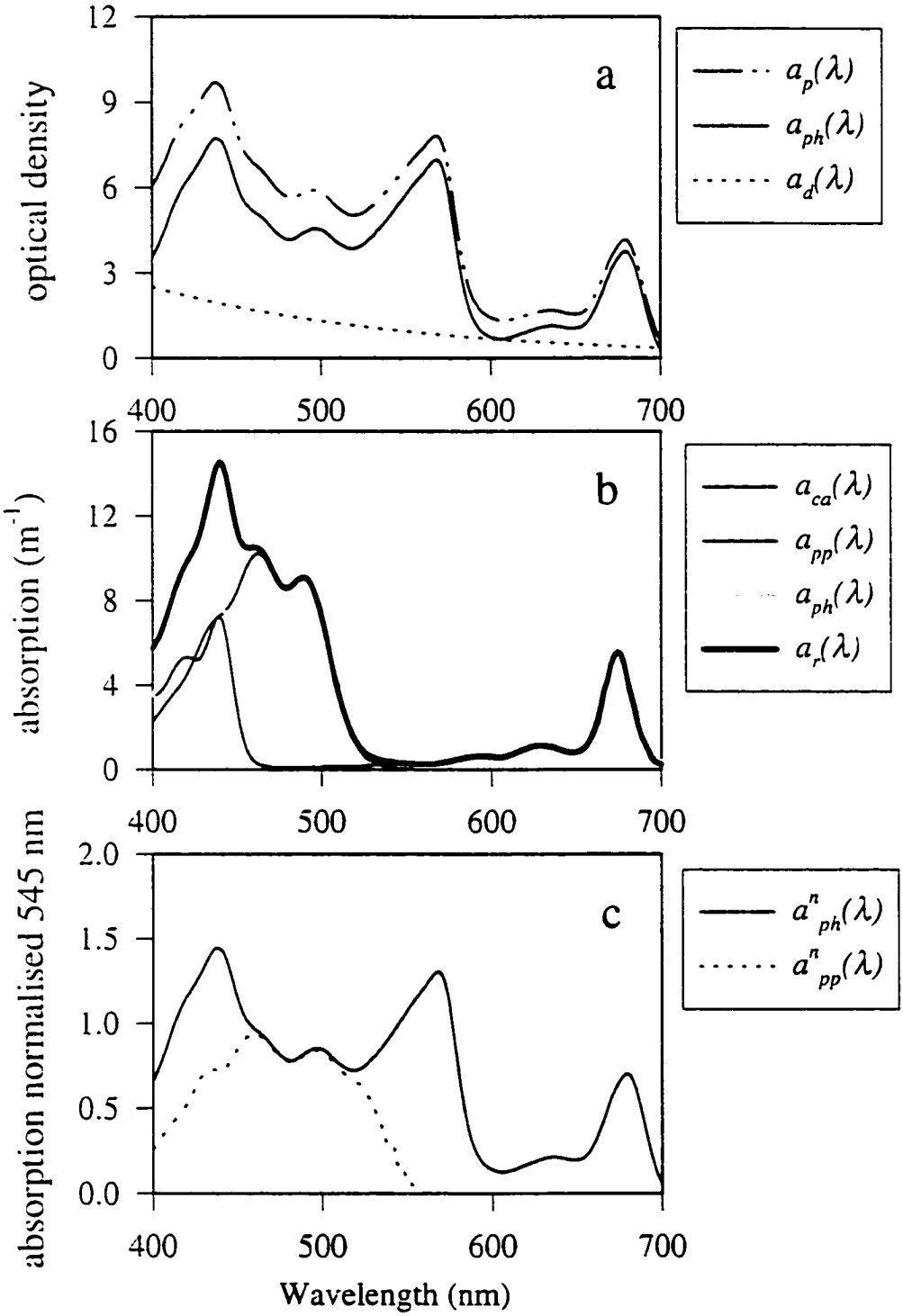
a
optical density
12
9
6
3
0
400
500
600
700
$a_p(\lambda)$
$a_{ph}(\lambda)$
$a_d(\lambda)$
b
absorption (m^{-1})
16
12
8
4
0
$a_{ca}(\lambda)$
$a_{pp}(\lambda)$
$a_{ph}(\lambda)$
$a_r(\lambda)$
c
absorption normalised 545 nm
2.0
1.5
1.0
0.5
0.0
$a^n_{ph}(\lambda)$
$a^n_{pp}(\lambda)$
Wavelength (nm)

FIGURE A.2

According to this method, the total absorption by particles can be defined as:

$$a_p(\lambda) = a_{ph}(440)a^n_{ph}(\lambda) + a_d(440)\exp[-q(\lambda - 440)]. \quad (A.7)$$

where, the absorption by particles, $a_p(\lambda)$, is known by measurements; the average shape of phytoplankton spectrum normalised to 440 nm, $a^n_{ph}(\lambda)$, was taken from Hoepffner and Sathyendranath (1993); the values of absorption by phytoplankton and detritus at 440 nm, $a_{ph}(440)$ and $a_d(440)$ respectively, and the exponent q, are solved by non-linear regression. Finally, the absorption coefficient of phytoplankton $[a_{ph}(\lambda)]$ (Figure A.2a) is obtained by subtracting the absorption of detritus from the total particle absorption according to:

$$a_{ph}(\lambda) = a_p(\lambda) - a_d(440)\exp[-q(\lambda - 440)]. \quad (A.8)$$

To compare the shapes of the absorption spectrum to that of the fluorescence excitation spectrum, $a_{ph}(\lambda)$ was normalised to 1 at 545 nm ($a^n_{ph}(\lambda)$; Figures A.1c and A.2c; see explanation for the selection of the wavelength of normalisation in section 3.5.1).

A.2 Estimation of the absorption by photoprotective pigments

The contribution of PP to the normalised absorption spectra was estimated using the method proposed by Bidigare *et al.* (1990) as modified by Babin *et al.* (1996). The absorption spectrum was reconstructed ($a_r(\lambda)$; see example in Figures A.1b, and A.2b) using pigment concentrations (p_i) and the corresponding specific *in vivo* absorption coefficients ($a^*_i(\lambda)$) given by Bidigare *et al.* (1990):

$$a_r(\lambda) = \sum_{i=1}^{n} p_i a^*_i(\lambda). \quad (A.9)$$

Similarly, an initial estimate of absorption by PP ($a_{pp}(\lambda)$) was also computed by multiplying the concentration of the sum of photoprotective pigments [PP] by the specific absorption coefficient of PP ($a^*_{pp}(\lambda)$) reported by Bidigare *et al.* (1990):

$$a_{pp}(\lambda) = [PP]a^*_{pp}(\lambda). \quad (A.10)$$

This method uses the shapes of absorption by pure pigments *in vitro* (slightly shifted, see Bidigare *et al.*, 1990), which are not the best representation of the absorption spectra of pigments forming pigment-protein-complexes *in vivo* (see Johnsen *et al.*, 1994). Furthermore, this reconstruction procedure does not account for packaging of pigments inside the cells. This last factor can be significant, especially in large cells grown at low irradiance, when both diameter and intracellular concentrations of pigments are high (Morel and Bricaud, 1981). Figures A.1b and A.2b show two examples of the discrepancies between reconstructed and measured absorption spectra for *Thalassiosira weissflogii* and *Synechococcus* sp., both grown at the lowest irradiance (20 μmol quanta $m^{-2} s^{-1}$). To avoid this problem, we used a procedure similar to that described in Babin *et al.* (1996) to obtain a corrected estimate of the normalised absorption by PP. The bias resulting from using fixed *in vitro* shapes of absorption by individual pigments to represent the *in vivo* ones still persists in the approach of Babin *et al.* (1996). This bias can only be assessed by measuring absorption from isolated pigment-protein-complexes from different species of phytoplankton. Nevertheless, this method (Bidigare *et al.*, 1990, as modified by Babin *et al.*, 1996) represents a simple first approximation of the contribution of absorption by photoprotective pigments to total phytoplankton absorption (Figure A.1c, and A.2c). The normalised absorption by PP ($a^n_{pp}(\lambda)$) was estimated as follows:

$$a^n_{pp}(\lambda) = \frac{a^n_{ph}(\lambda) * a_{pp}(\lambda)}{a_r(\lambda)}. \qquad (A.11)$$

In other words, the normalised phytoplankton absorption spectrum was multiplied by the ratio of the initial estimate of absorption by PP to the total reconstructed absorption at the same wavelength.

A.3 Fluorescence excitation spectra

The emission monochromator was set at 730 nm (Neori *et al.*, 1988; Hofstraat *et al.*, 1992), and the excitation spectra were recorded between 380 and 700 nm. For the

field samples (Chapters 1 and 2), a technical problem, probably related to the highly scattering properties of the filters (GF/F), allowed stray light to enter the emission detector and distorted the signal in the red part of the spectrum. Therefore, only the blue-green part of the spectra, from 400 to 600 nm, was considered for the analysis (e.g., Figure A.3a). The blank consisted of a clean GF/F filter, through which a volume of prefiltered seawater comparable to that of the samples had been filtered; for some oligotrophic stations distilled water was used for the blank filter since prefiltered seawater showed a fluorescence signal larger than that observed at other stations (coming probably from small cells passing through the GF/F filters). Filters were wetted with filtered seawater before reading.

For the field samples the fluorescence excitation spectra ($f(\lambda)$) were later corrected for distortions produced by the spectrum of the source (xenon) lamp, and the geometrical configuration of the instrument, according to the method of Culver *et al.* (1994): the excitation light incident at the sample position was measured with a radiometer (Biospherical Instruments QSL-100 4π collector) every 2 nm, and this spectrum was in turn corrected for spectral variations in the efficiency of the radiometer (information provided in the specifications of the radiometer). The average of three such spectra, normalised to 1 at 468 nm (maximum emission of the xenon lamp), was then interpolated every 1 nm, and used as the excitation-correction-spectrum ($C_x(\lambda)$) for the measurements (Table A.1; Figure A.3b). The excitation-corrected fluorescence spectrum ($f_{xc}(\lambda)$) was obtained as:

$$f_{xc}(\lambda) = f(\lambda)/C_x(\lambda). \qquad (A.12)$$

For the culture samples the excitation correction was automatically done by the instrument (e.g., Figure A.4a). The accuracy of the correction procedures was tested by comparing the corrected fluorescence excitation spectrum of pure chlorophyll-*a* in 90% acetone with its corresponding absorption spectrum, after normalising both spectra to 1 at 664 nm, which showed a good agreement (e.g., Figure 2.2). The excitation-corrected fluorescence spectra were smoothed using a 5 nm running

FIGURE A.3. Example of the corrections applied to the fluorescence excitation spectra of phytoplankton, sample of from station Canary 5, 20 meters. **a**) fluorescence spectrum without corrections ($f(\lambda)$), fluorescence spectrum corrected for the effect of the excitation lamp ($f_{xc}(\lambda)$), fluorescence spectrum corrected for the effect of the lamp and smoothed by a running average every 5 nm ($f_{sm}(\lambda)$). **b**) Spectrum of the correction for the effect of the lamp normalised to 1 at 467 nm ($C_x(\lambda)$); spectrum of the correction for differences between fluorescence of phytoplankton on a filter and in suspension normalised to 1 at 545 nm (shape correction: $C_s(\lambda)$). **c**) Fluorescence excitation spectra normalised to 1 at 545 nm with ($f_c^n(\lambda)$) and without shape correction ($f^n(\lambda)$).

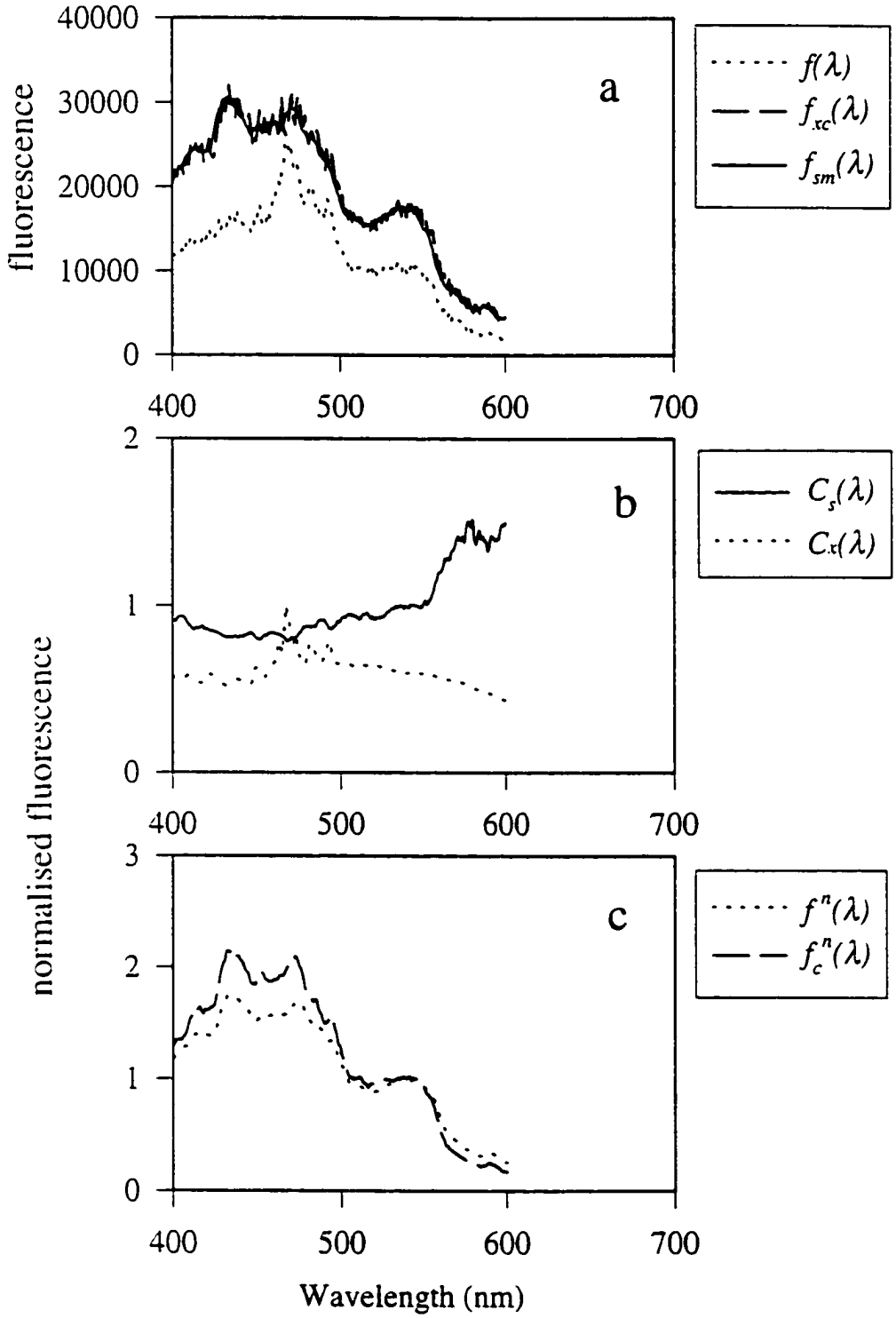
40000
30000
20000
10000
0
fluorescence
a
$f(\lambda)$
$f_{xc}(\lambda)$
$f_{sm}(\lambda)$
400
500
600
700
2
1
0
b
$C_s(\lambda)$
$C_x(\lambda)$
normalised fluorescence
3
2
1
0
c
$f^n(\lambda)$
$f_c^n(\lambda)$
Wavelength (nm)

FIGURE A.3

average ($f_{sm}(\lambda)$; Figure A.3a and A.4a). The smoothed fluorescence excitation spectra were then normalised to 1 at 545 nm ($f^n(\lambda)$, Figures A.3c, and A.4c).

Further testing using a culture of *Thalassiosira* sp. (isolated from a seawater sample from the Labrador Sea), showed slight but consistent differences in the shape between normalised spectra obtained in suspension and on the filter. Therefore, the ratio of the spectra on the filter to that in suspension was used as a shape-correction-factor ($C_s(\lambda)$), to correct the spectra of the field samples (Table A.1, Figure A.3b). This shape-correction factor was also calculated for each one of the species used in the laboratory experiments (Tables A.1, and example in Figure A.4b). Thus, the corrected fluorescence excitation spectra, normalised at 545 nm, ($f_c^n(\lambda)$; Figures A.3c and A.4c) were obtained as:

$$f_c^n(\lambda) = f^n(\lambda)/C_s(\lambda). \qquad (A.14)$$

FIGURE A.4. Example of the corrections applied to the fluorescence excitation spectra of phytoplankton, sample of *Thalassiosira weissflogii* grown at 20 μmol quanta $m^{-2} s^{-1}$. **a**) fluorescence spectrum already corrected for the effect of the excitation lamp ($f_{xc}(\lambda)$), fluorescence spectrum corrected for the effect of the lamp and smoothed by a running average every 5 nm ($f_{sm}(\lambda)$). **b**) Spectrum of the correction for differences between fluorescence of phytoplankton on a filter and in suspension normalised to 1 at 545 nm (shape correction: $C_s(\lambda)$). **c**) Fluorescence excitation spectra normalised to 1 at 545 nm with ($f_c^n(\lambda)$) and without shape correction ($f^n(\lambda)$).

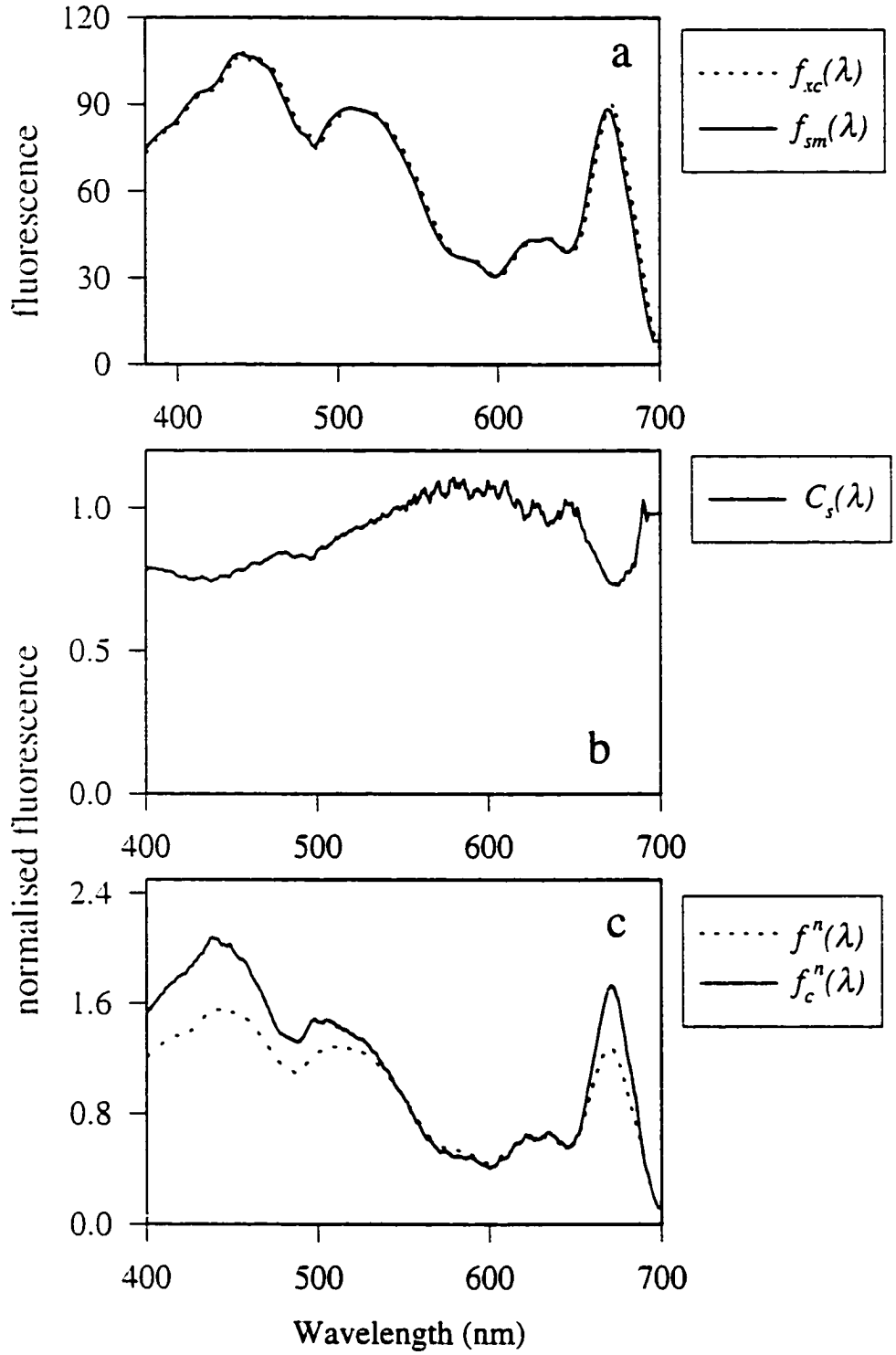
a
b
c
fluorescence
normalised fluorescence
Wavelength (nm)
$f_{xc}(\lambda)$
$f_{sm}(\lambda)$
$C_s(\lambda)$
$f^n(\lambda)$
$f_c^n(\lambda)$
120
90
60
30
0
1.0
0.5
0.0
2.4
1.6
0.8
400
500
600
700

FIGURE A.4

Table A.1 Values of the factors applied to correct the fluorescence excitation spectra of phytoplankton for: the effect of excitation lamp (C_x). and for differences between fluorescence on a filter and in suspension (C_s: field: for field samples. *T.w.*: for *Thalassiosira weissflogii*. *Ch.*: for *Chaetoceros* sp.. *Syn.*: for *Synechococcus* sp.). WL: wavelength (nm).

Table A.1

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
400	0.584	400	0.911	0.800	1.139	1.036
401	0.566	401	0.910	0.784	1.136	1.351
402	0.558	402	0.909	0.791	1.130	1.104
403	0.549	403	0.927	0.789	1.130	1.127
404	0.552	404	0.932	0.789	1.123	1.045
405	0.554	405	0.938	0.787	1.117	1.035
406	0.559	406	0.932	0.788	1.115	1.064
407	0.563	407	0.929	0.785	1.119	1.030
408	0.574	408	0.913	0.783	1.118	0.992
409	0.586	409	0.894	0.783	1.115	0.970
410	0.576	410	0.880	0.784	1.107	0.930
411	0.566	411	0.873	0.778	1.099	0.906
412	0.561	412	0.857	0.777	1.101	0.891
413	0.556	413	0.857	0.778	1.093	0.881
414	0.548	414	0.867	0.777	1.098	0.886
415	0.541	415	0.868	0.776	1.091	0.940
416	0.539	416	0.866	0.775	1.091	0.940
417	0.536	417	0.875	0.769	1.086	0.963
418	0.558	418	0.878	0.763	1.080	0.931
419	0.580	419	0.858	0.761	1.067	0.933
420	0.586	420	0.862	0.759	1.062	0.880
421	0.592	421	0.859	0.758	1.055	0.830
422	0.589	422	0.851	0.759	1.051	0.792
423	0.587	423	0.847	0.761	1.050	0.826
424	0.584	424	0.849	0.753	1.055	0.835
425	0.580	425	0.845	0.749	1.058	0.831
426	0.572	426	0.838	0.749	1.060	0.908
427	0.564	427	0.839	0.752	1.053	0.894
428	0.552	428	0.828	0.747	1.049	0.841
429	0.541	429	0.826	0.753	1.043	0.848
430	0.532	430	0.818	0.754	1.031	0.846
431	0.523	431	0.815	0.753	1.030	0.797
432	0.520	432	0.808	0.755	1.027	0.851
433	0.517	433	0.810	0.760	1.018	0.872
434	0.521	434	0.816	0.754	1.015	0.850
435	0.526	435	0.813	0.752	1.020	0.825
436	0.536	436	0.808	0.752	1.013	0.832
437	0.546	437	0.816	0.745	1.009	0.855
438	0.554	438	0.815	0.742	1.022	0.858
439	0.561	439	0.810	0.747	1.020	0.859
440	0.555	440	0.813	0.750	1.023	0.878
441	0.548	441	0.818	0.751	1.024	0.893
442	0.546	442	0.814	0.752	1.022	0.825
443	0.544	443	0.822	0.760	1.013	0.820
444	0.542	444	0.825	0.765	1.014	0.849
445	0.540	445	0.829	0.760	1.009	0.866
446	0.538	446	0.840	0.760	1.005	0.877
447	0.536	447	0.838	0.763	1.011	0.886

Table A.1 Contin.

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
448	0.579	448	0.828	0.759	1.012	0.908
449	0.623	449	0.812	0.757	1.005	0.927
450	0.629	450	0.802	0.768	1.002	0.926
451	0.635	451	0.796	0.774	1.000	0.905
452	0.618	452	0.799	0.778	0.996	0.905
453	0.600	453	0.806	0.781	0.985	0.938
454	0.587	454	0.816	0.784	0.986	0.984
455	0.573	455	0.824	0.784	0.978	1.074
456	0.595	456	0.823	0.780	0.976	1.140
457	0.617	457	0.835	0.783	0.975	1.217
458	0.627	458	0.838	0.786	0.980	1.142
459	0.637	459	0.839	0.791	0.980	1.116
460	0.655	460	0.835	0.795	0.985	1.094
461	0.673	461	0.834	0.806	0.990	1.030
462	0.729	462	0.830	0.806	0.990	1.037
463	0.785	463	0.828	0.808	0.986	1.079
464	0.751	464	0.824	0.808	0.978	1.044
465	0.717	465	0.815	0.805	0.978	1.037
466	0.858	466	0.810	0.805	0.970	1.061
467	1.000	467	0.796	0.804	0.969	1.029
468	0.947	468	0.785	0.809	0.969	1.039
469	0.894	469	0.794	0.814	0.979	1.003
470	0.822	470	0.800	0.820	0.980	1.038
471	0.750	471	0.805	0.819	0.995	1.020
472	0.787	472	0.804	0.827	1.002	1.044
473	0.824	473	0.809	0.828	1.008	1.043
474	0.764	474	0.816	0.834	1.003	1.080
475	0.703	475	0.833	0.835	1.006	1.044
476	0.675	476	0.842	0.840	0.992	1.027
477	0.646	477	0.861	0.841	0.988	1.007
478	0.653	478	0.874	0.845	0.980	0.990
479	0.661	479	0.874	0.841	0.985	0.965
480	0.719	480	0.878	0.843	0.975	0.967
481	0.776	481	0.874	0.846	0.977	0.993
482	0.770	482	0.870	0.847	0.972	1.014
483	0.763	483	0.876	0.840	0.973	1.113
484	0.732	484	0.878	0.838	0.969	1.076
485	0.702	485	0.877	0.830	0.982	1.117
486	0.683	486	0.900	0.827	0.986	1.119
487	0.663	487	0.912	0.827	0.991	1.120
488	0.665	488	0.914	0.830	0.996	1.035
489	0.668	489	0.912	0.833	0.999	1.108
490	0.701	490	0.914	0.832	0.999	1.055
491	0.735	491	0.896	0.837	0.997	1.075
492	0.762	492	0.884	0.832	0.983	1.071
493	0.788	493	0.862	0.831	0.984	1.093
494	0.742	494	0.863	0.828	0.989	1.074
495	0.696	495	0.863	0.825	0.989	1.129

Table A.1 Contin.

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
496	0.677	496	0.875	0.819	0.992	1.088
497	0.659	497	0.885	0.827	0.994	1.110
498	0.656	498	0.908	0.823	0.999	1.141
499	0.652	499	0.901	0.838	0.993	1.127
500	0.652	500	0.914	0.850	0.993	1.105
501	0.651	501	0.931	0.857	0.986	1.142
502	0.655	502	0.935	0.859	0.996	1.132
503	0.659	503	0.933	0.867	0.989	1.136
504	0.654	504	0.947	0.860	0.984	1.164
505	0.650	505	0.951	0.860	0.982	1.172
506	0.645	506	0.941	0.865	0.988	1.178
507	0.641	507	0.947	0.873	0.989	1.177
508	0.640	508	0.938	0.875	0.990	1.166
509	0.640	509	0.937	0.882	0.996	1.161
510	0.640	510	0.931	0.886	1.000	1.149
511	0.639	511	0.918	0.900	1.003	1.152
512	0.643	512	0.924	0.893	0.986	1.155
513	0.646	513	0.949	0.899	0.982	1.153
514	0.645	514	0.946	0.904	0.981	1.152
515	0.643	515	0.951	0.910	0.967	1.162
516	0.642	516	0.958	0.904	0.965	1.159
517	0.642	517	0.939	0.910	0.984	1.170
518	0.643	518	0.924	0.916	0.982	1.172
519	0.644	519	0.936	0.923	0.982	1.181
520	0.643	520	0.920	0.920	0.988	1.189
521	0.643	521	0.922	0.926	0.989	1.175
522	0.640	522	0.931	0.930	0.976	1.174
523	0.637	523	0.931	0.935	0.982	1.157
524	0.635	524	0.929	0.932	0.985	1.143
525	0.633	525	0.937	0.931	0.993	1.143
526	0.631	526	0.938	0.928	0.979	1.138
527	0.629	527	0.953	0.938	0.985	1.132
528	0.626	528	0.964	0.938	0.980	1.140
529	0.623	529	0.970	0.942	0.980	1.131
530	0.618	530	0.977	0.947	0.977	1.106
531	0.614	531	0.990	0.958	0.992	1.102
532	0.612	532	0.985	0.955	0.993	1.100
533	0.610	533	0.997	0.947	0.994	1.089
534	0.607	534	0.990	0.950	0.979	1.084
535	0.604	535	0.998	0.962	0.975	1.084
536	0.603	536	1.001	0.963	0.974	1.084
537	0.603	537	0.999	0.961	0.974	1.067
538	0.601	538	0.984	0.980	0.981	1.059
539	0.599	539	0.999	0.973	0.976	1.058
540	0.599	540	0.994	0.971	0.974	1.057
541	0.599	541	0.988	0.968	0.973	1.045
542	0.598	542	1.008	0.979	0.973	1.041
543	0.598	543	1.005	0.984	0.970	1.029

Table A.1 Contin.

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
544	0.599	544	1.001	0.995	0.991	1.012
545	0.600	545	1.000	1.000	1.000	1.000
546	0.598	546	0.998	1.000	0.994	0.987
547	0.597	547	0.992	1.005	0.986	0.970
548	0.595	548	0.991	0.994	0.985	0.976
549	0.594	549	0.996	0.998	0.976	0.967
550	0.592	550	1.018	1.007	0.976	0.953
551	0.590	551	1.033	1.005	0.988	0.955
552	0.590	552	1.009	0.991	1.008	0.953
553	0.590	553	1.027	1.020	1.012	0.939
554	0.588	554	1.039	1.025	1.030	0.941
555	0.587	555	1.060	1.014	1.028	0.946
556	0.582	556	1.087	1.026	1.006	0.940
557	0.576	557	1.134	1.047	0.994	0.939
558	0.573	558	1.154	1.014	0.994	0.939
559	0.571	559	1.197	1.030	0.980	0.923
560	0.570	560	1.202	1.039	0.980	0.912
561	0.570	561	1.213	1.061	1.004	0.901
562	0.566	562	1.240	1.054	1.018	0.881
563	0.562	563	1.286	1.070	1.021	0.868
564	0.560	564	1.276	1.045	1.033	0.868
565	0.557	565	1.288	1.037	1.048	0.863
566	0.557	566	1.279	1.020	1.035	0.861
567	0.556	567	1.327	1.054	1.037	0.878
568	0.556	568	1.333	1.052	1.035	0.883
569	0.556	569	1.356	1.066	1.023	0.879
570	0.552	570	1.395	1.085	1.008	0.881
571	0.548	571	1.419	1.093	1.024	0.885
572	0.545	572	1.397	1.062	1.008	0.885
573	0.542	573	1.394	1.045	0.995	0.897
574	0.537	574	1.415	1.049	1.000	0.923
575	0.532	575	1.382	1.036	0.992	0.949
576	0.527	576	1.443	1.052	0.983	0.983
577	0.523	577	1.503	1.051	0.997	0.997
578	0.518	578	1.502	1.096	1.012	1.025
579	0.512	579	1.473	1.097	1.001	1.059
580	0.508	580	1.516	1.106	1.013	1.086
581	0.504	581	1.398	1.070	1.027	1.098
582	0.502	582	1.369	1.086	1.005	1.164
583	0.500	583	1.407	1.070	1.007	1.192
584	0.497	584	1.454	1.086	1.030	1.254
585	0.494	585	1.405	1.090	1.031	1.296
586	0.492	586	1.417	1.099	1.016	1.363
587	0.490	587	1.389	1.065	1.037	1.418
588	0.483	588	1.389	1.062	1.041	1.466
589	0.476	589	1.329	1.032	1.040	1.422
590	0.473	590	1.358	1.039	1.055	1.455
591	0.470	591	1.420	1.041	1.074	1.532

Table A.1 Contin.

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
592	0.464	592	1.414	1.063	1.066	1.542
593	0.458	593	1.402	1.061	1.060	1.562
594	0.454	594	1.390	1.069	1.052	1.645
595	0.449	595	1.399	1.059	1.048	1.630
596	0.445	596	1.404	1.059	1.031	1.584
597	0.440	597	1.472	1.042	1.043	1.620
598	0.437	598	1.486	1.047	1.036	1.659
599	0.434	599	1.484	1.059	1.056	1.636
600	0.428	600	1.498	1.096	1.038	1.779
		601		1.073	1.059	1.807
		602		1.069	1.086	1.719
		603		1.074	1.138	1.642
		604		1.075	1.155	1.644
		605		1.044	1.188	1.528
		606		1.033	1.171	1.534
		607		1.045	1.138	1.536
		608		1.059	1.092	1.629
		609		1.080	1.049	1.571
		610		1.094	1.015	1.633
		611		1.094	1.003	1.593
		612		1.051	0.993	1.537
		613		1.045	1.018	1.542
		614		1.020	1.022	1.581
		615		1.011	1.021	1.529
		616		1.002	1.020	1.607
		617		1.032	1.007	1.575
		618		1.024	0.988	1.472
		619		1.015	0.966	1.501
		620		0.993	0.987	1.502
		621		0.959	0.997	1.553
		622		0.968	1.020	1.593
		623		0.974	1.021	1.677
		624		0.975	1.047	1.689
		625		0.992	1.038	1.747
		626		1.029	1.029	1.609
		627		1.004	1.037	1.734
		628		1.005	1.036	1.883
		629		1.017	1.033	1.776
		630		1.009	1.026	1.694
		631		0.981	1.035	1.738
		632		0.986	1.016	1.661
		633		0.962	1.000	1.544
		634		0.944	1.015	1.622
		635		0.938	1.040	1.644
		636		0.949	1.027	1.653
		637		0.955	1.024	1.568
		638		0.948	1.030	1.541
		639		0.960	1.011	1.504

Table A.1 Contin.

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
		640		0.969	0.997	1.492
		641		0.991	1.011	1.533
		642		0.964	1.020	1.682
		643		0.982	1.032	1.672
		644		0.997	1.052	1.763
		645		1.029	1.050	1.845
		646		1.015	1.061	1.777
		647		1.012	1.058	1.618
		648		1.014	1.072	1.567
		649		1.019	1.069	1.525
		650		0.977	1.067	1.497
		651		0.975	1.046	1.428
		652		1.001	1.073	1.460
		653		0.967	1.060	1.581
		654		0.941	1.029	1.535
		655		0.924	1.023	1.561
		656		0.919	0.998	1.690
		657		0.884	0.981	1.815
		658		0.882	0.987	1.823
		659		0.878	0.998	1.746
		660		0.862	1.005	1.812
		661		0.851	1.005	1.831
		662		0.845	0.982	1.639
		663		0.827	0.960	1.638
		664		0.813	0.943	1.867
		665		0.805	0.910	1.831
		666		0.789	0.888	1.723
		667		0.776	0.876	1.542
		668		0.763	0.864	1.454
		669		0.750	0.860	1.449
		670		0.743	0.856	1.332
		671		0.737	0.866	1.284
		672		0.735	0.866	1.356
		673		0.732	0.867	1.243
		674		0.737	0.854	1.171
		675		0.729	0.865	1.299
		676		0.732	0.872	1.262
		677		0.744	0.868	1.360
		678		0.749	0.873	1.325
		679		0.751	0.900	1.256
		680		0.775	0.908	1.193
		681		0.779	0.896	1.331
		682		0.773	0.915	1.117
		683		0.784	0.924	1.038
		684		0.806	0.933	1.008
		685		0.798	0.949	1.008
		686		0.840	0.989	1.008
		687		0.888	1.010	1.008

Table A.1 Contin.

WL	C_x	WL	C_s(field)	$C_s(T.w.)$	$C_s(Ch.)$	$C_s(Syn.)$
		688		0.916	1.043	1.008
		689		0.959	1.099	1.008
		690		1.029	1.187	1.008
		691		1.000	1.187	1.008
		692		0.954	1.187	1.008
		693		0.980	1.187	1.008
		694		0.980	1.187	1.008
		695		0.980	1.187	1.008
		696		0.980	1.187	1.008
		697		0.980	1.187	1.008
		698		0.980	1.187	1.008
		699		0.980	1.187	1.008
		700		0.980	1.187	1.008

Appendix B: Data from Chapter 3

Data on pigment concentrations, nitrate concentrations, temperature, density (σ_t), and optical properties (absorption and fluorescence excitation spectra) used in Chapter 3 are shown in Tables B.1, B.2 and B.3. Data on composition of the phytoplankton community and irradiance at different depths in station Canary 5 are shown in Table B.4.

Table B.1a Pigment concentrations ($mg\,m^{-3}$) in the two cruises (CR). Canary 1993 (Can.) and Labrador 1994 (Lab.). ST: station number. DE: depth (m). ca: chlorophyll-*a*. cc: chlorophyll-*c*. c3: chlorophyll-*c*3. cb: chlorophyll-*b*. va: dvinyl-chlorophyll-*a*. vb: divinyl-chlorophyll-*b*. pha: sum of phaeopigments-*a*. cd: chlorophyllide-*a*.

Table B.1a

CR	ST	DE	ca	cc	c3	cb	va	vb	pha	cd
Can.	1	1	0.114	0.012	0.001	0.007	0.037	0.003	0.000	0.000
Can.	1	20	0.110	0.012	0.001	0.007	0.032	0.002	0.000	0.000
Can.	1	100	0.181	0.028	0.014	0.042	0.037	0.034	0.000	0.000
Can.	2	60	0.232	0.024	0.007	0.046	0.067	0.015	0.000	0.000
Can.	2	80	0.336	0.033	0.014	0.102	0.100	0.069	0.000	0.000
Can.	2	100	0.192	0.024	0.012	0.049	0.031	0.032	0.000	0.000
Can.	4	1	0.286	0.017	0.000	0.046	0.000	0.000	0.000	0.000
Can.	4	10	0.305	0.021	0.000	0.052	0.000	0.000	0.000	0.000
Can.	4	20	0.379	0.022	0.000	0.062	0.032	0.000	0.000	0.000
Can.	4	30	0.358	0.022	0.000	0.063	0.036	0.002	0.000	0.000
Can.	4	50	0.274	0.039	0.016	0.070	0.000	0.000	0.000	0.000
Can.	5	1	0.172	0.014	0.000	0.024	0.000	0.000	0.000	0.000
Can.	5	10	0.285	0.025	0.006	0.042	0.000	0.000	0.000	0.000
Can.	5	20	0.316	0.028	0.000	0.051	0.000	0.000	0.000	0.000
Can.	5	30	0.733	0.073	0.020	0.166	0.068	0.012	0.000	0.000
Can.	5	40	1.130	0.138	0.044	0.265	0.070	0.013	0.000	0.000
Can.	5	50	0.645	0.081	0.029	0.177	0.048	0.010	0.000	0.000
Can.	5	75	0.188	0.022	0.009	0.036	0.000	0.000	0.000	0.000
Can.	61	1	0.148	0.008	0.000	0.016	0.000	0.000	0.000	0.000
Can.	61	35	0.523	0.040	0.010	0.100	0.039	0.003	0.000	0.000
Can.	61	45	0.720	0.079	0.025	0.148	0.025	0.010	0.000	0.000
Can.	61	55	0.655	0.069	0.025	0.145	0.021	0.009	0.000	0.000
Can.	7	1	1.011	0.140	0.009	0.037	0.000	0.000	0.000	0.033
Can.	7	5	1.269	0.184	0.014	0.035	0.000	0.000	0.000	0.024
Can.	7	10	2.824	0.497	0.041	0.063	0.000	0.000	0.000	0.139
Can.	7	15	3.254	0.650	0.069	0.113	0.000	0.000	0.000	0.224
Can.	7	20	0.859	0.199	0.048	0.028	0.000	0.000	0.000	0.128
Can.	7	25	0.698	0.147	0.038	0.024	0.000	0.000	0.000	0.064
Can.	8	1	0.246	0.040	0.004	0.012	0.000	0.000	0.000	0.008
Can.	8	10	0.465	0.074	0.006	0.020	0.000	0.000	0.000	0.027
Can.	8	22	6.020	1.660	0.564	0.000	0.000	0.000	1.310	1.378
Can.	9	60	0.230	0.021	0.006	0.024	0.089	0.012	0.000	0.000
Can.	9	70	0.490	0.041	0.016	0.153	0.190	0.079	0.000	0.003
Can.	9	80	0.493	0.049	0.021	0.166	0.161	0.093	0.000	0.000
Can.	9	100	0.216	0.028	0.014	0.051	0.044	0.039	0.000	0.000
Can.	11	60	0.133	0.016	0.000	0.014	0.048	0.004	0.000	0.000
Can.	11	80	0.316	0.043	0.017	0.096	0.168	0.075	0.000	0.010
Can.	11	90	0.480	0.084	0.046	0.097	0.146	0.098	0.000	0.021
Can.	11	100	0.415	0.068	0.038	0.103	0.138	0.133	0.000	0.012
Can.	12	50	0.111	0.012	0.001	0.009	0.043	0.003	0.000	0.000
Can.	12	70	0.321	0.051	0.026	0.056	0.069	0.020	0.000	0.000
Can.	12	90	0.253	0.041	0.023	0.052	0.067	0.032	0.000	0.000
Can.	12	100	0.240	0.034	0.019	0.049	0.052	0.030	0.000	0.000
Can.	13	45	0.349	0.072	0.026	0.059	0.100	0.023	0.044	0.018
Can.	13	55	0.593	0.156	0.062	0.096	0.074	0.028	0.082	0.042
Can.	13	65	0.454	0.090	0.050	0.078	0.000	0.000	0.000	0.014
Can.	13	75	0.222	0.041	0.025	0.027	0.026	0.023	0.000	0.000
Can.	13	100	0.106	0.023	0.014	0.008	0.000	0.002	0.000	0.003

Table B.1a Contin.

CR	ST	DE	ca	cc	c3	cb	va	vb	pha	cd
Can.	14	40	0.135	0.011	0.001	0.010	0.045	0.004	0.000	0.000
Can.	14	60	0.338	0.044	0.017	0.076	0.132	0.049	0.000	0.006
Can.	14	70	0.438	0.067	0.033	0.088	0.070	0.033	0.000	0.000
Can.	14	80	0.451	0.062	0.031	0.085	0.052	0.023	0.000	0.000
Can.	14	100	0.201	0.028	0.017	0.040	0.041	0.050	0.000	0.000
Can.	15	1	0.201	0.026	0.002	0.019	0.000	0.000	0.000	0.000
Can.	15	20	0.227	0.033	0.007	0.021	0.021	0.000	0.000	0.000
Can.	15	45	0.439	0.086	0.034	0.068	0.000	0.000	0.000	0.000
Can.	15	55	0.377	0.062	0.029	0.048	0.000	0.000	0.000	0.003
Can.	15	65	0.202	0.033	0.018	0.024	0.000	0.000	0.000	0.000
Can.	15	75	0.141	0.017	0.009	0.011	0.000	0.000	0.000	0.000
Can.	17	1	0.701	0.132	0.034	0.073	0.000	0.000	0.000	0.015
Can.	17	10	0.700	0.118	0.032	0.071	0.000	0.000	0.000	0.013
Can.	17	25	1.690	0.377	0.064	0.178	0.000	0.000	0.000	0.029
Lab.	2	50	0.102	0.032	0.004	0.000	0.000	0.000	0.000	0.003
Lab.	2	65	0.341	0.089	0.001	0.000	0.000	0.000	0.000	0.009
Lab.	2	80	0.301	0.091	0.002	0.000	0.000	0.000	0.077	0.048
Lab.	2	100	0.197	0.053	0.001	0.000	0.000	0.000	0.057	0.027
Lab.	3	1	5.726	1.111	0.044	0.269	0.000	0.000	0.000	0.386
Lab.	3	10	4.703	1.321	0.045	0.243	0.000	0.000	0.353	0.518
Lab.	3	20	7.229	1.603	0.051	0.202	0.000	0.000	0.338	0.518
Lab.	3	30	4.027	0.752	0.000	0.098	0.000	0.000	0.000	0.000
Lab.	3	40	1.020	0.195	0.011	0.045	0.000	0.000	0.098	0.000
Lab.	3	60	0.287	0.052	0.010	0.029	0.000	0.000	0.000	0.000
Lab.	5	1	0.661	0.119	0.026	0.094	0.000	0.000	0.000	0.011
Lab.	5	10	0.534	0.101	0.024	0.099	0.000	0.000	0.000	0.012
Lab.	5	20	0.513	0.092	0.021	0.075	0.000	0.000	0.000	0.009
Lab.	5	30	0.527	0.105	0.024	0.075	0.000	0.000	0.000	0.006
Lab.	5	40	0.350	0.067	0.019	0.065	0.000	0.000	0.000	0.004
Lab.	5	60	0.135	0.024	0.006	0.026	0.000	0.000	0.000	0.000
Lab.	6	1	0.834	0.176	0.028	0.025	0.000	0.000	0.000	0.014
Lab.	6	10	1.207	0.256	0.055	0.031	0.000	0.000	0.000	0.024
Lab.	6	20	0.866	0.185	0.054	0.000	0.000	0.000	0.085	0.025
Lab.	6	30	0.300	0.061	0.021	0.006	0.000	0.000	0.219	0.005
Lab.	6	50	0.217	0.047	0.015	0.004	0.000	0.000	0.248	0.004
Lab.	6	75	0.225	0.044	0.015	0.004	0.000	0.000	0.212	0.004
Lab.	7	1	0.982	0.149	0.014	0.146	0.000	0.000	0.000	0.017
Lab.	7	10	1.141	0.164	0.016	0.174	0.000	0.000	0.000	0.024
Lab.	7	20	1.156	0.183	0.021	0.153	0.000	0.000	0.000	0.062
Lab.	7	30	0.709	0.112	0.016	0.142	0.000	0.000	0.000	0.013
Lab.	7	40	0.372	0.044	0.011	0.102	0.000	0.000	0.000	0.000
Lab.	7	60	0.207	0.031	0.011	0.067	0.000	0.000	0.000	0.000
Lab.	8	1	0.530	0.097	0.023	0.042	0.000	0.000	0.000	0.012
Lab.	8	10	0.590	0.105	0.026	0.051	0.000	0.000	0.000	0.014
Lab.	8	20	0.425	0.082	0.019	0.039	0.000	0.000	0.000	0.009
Lab.	8	40	0.486	0.094	0.024	0.033	0.000	0.000	0.000	0.011
Lab.	8	60	0.386	0.072	0.030	0.029	0.000	0.000	0.000	0.005
Lab.	8	80	0.110	0.016	0.009	0.000	0.000	0.000	0.000	0.000

Table B.1a Contin.

CR	ST	DE	ca	cc	c3	cb	va	vb	pha	cd
Lab.	9	1	0.793	0.133	0.000	0.133	0.000	0.000	0.000	0.011
Lab.	9	10	0.743	0.166	0.030	0.115	0.000	0.000	0.000	0.012
Lab.	9	20	0.707	0.121	0.026	0.098	0.000	0.000	0.000	0.011
Lab.	9	40	0.263	0.041	0.012	0.060	0.000	0.000	0.000	0.000
Lab.	9	50	0.142	0.019	0.004	0.025	0.000	0.000	0.000	0.000
Lab.	9	75	0.069	0.011	0.001	0.012	0.000	0.000	0.000	0.000
Lab.	9	100	0.067	0.019	0.000	0.008	0.000	0.000	0.000	0.000
Lab.	10	2	1.516	0.306	0.055	0.073	0.000	0.000	0.000	0.040
Lab.	10	10	1.807	0.358	0.063	0.095	0.000	0.000	0.000	0.065
Lab.	10	17	1.837	0.354	0.062	0.102	0.000	0.000	0.000	0.033
Lab.	10	27	1.796	0.387	0.071	0.088	0.000	0.000	0.000	0.127
Lab.	10	37	0.305	0.060	0.016	0.031	0.000	0.000	0.000	0.006
Lab.	10	56	0.076	0.000	0.005	0.017	0.000	0.000	0.000	0.019
Lab.	11	1	2.804	0.613	0.108	0.111	0.000	0.000	0.000	0.099
Lab.	11	10	2.902	0.580	0.101	0.110	0.000	0.000	0.000	0.074
Lab.	11	20	1.771	0.411	0.095	0.059	0.000	0.000	0.119	0.084
Lab.	11	30	0.379	0.075	0.024	0.044	0.000	0.000	0.027	0.000
Lab.	11	40	0.348	0.069	0.027	0.045	0.000	0.000	0.029	0.000
Lab.	11	55	0.370	0.063	0.024	0.048	0.000	0.000	0.000	0.004
Lab.	11	80	0.146	0.025	0.012	0.029	0.000	0.000	0.000	0.000
Lab.	12	1	5.400	1.515	0.000	0.143	0.000	0.000	0.864	0.859
Lab.	12	10	3.375	0.726	0.000	0.094	0.000	0.000	0.000	0.091
Lab.	12	20	1.343	0.285	0.000	0.046	0.000	0.000	0.172	0.030
Lab.	13	1	6.514	1.631	0.000	0.167	0.000	0.000	0.000	1.508
Lab.	13	5	7.331	1.679	0.000	0.199	0.000	0.000	0.000	1.425
Lab.	13	10	7.356	1.659	0.000	0.184	0.000	0.000	0.000	1.149
Lab.	13	20	1.547	0.345	0.026	0.086	0.000	0.000	0.000	0.028
Lab.	13	40	0.258	0.042	0.014	0.075	0.000	0.000	0.000	0.000
Lab.	14	1	1.771	0.658	0.073	0.084	0.000	0.000	0.308	0.315
Lab.	14	20	1.681	0.667	0.069	0.080	0.000	0.000	0.305	0.332
Lab.	14	40	1.579	0.704	0.072	0.064	0.000	0.000	0.369	0.376
Lab.	14	60	1.923	0.759	0.080	0.064	0.000	0.000	0.450	0.351
Lab.	14	70	1.029	0.455	0.048	0.032	0.000	0.000	0.243	0.276
Lab.	14	80	0.335	0.093	0.015	0.015	0.000	0.000	0.000	0.028
Lab.	14	100	0.175	0.027	0.006	0.014	0.000	0.000	0.000	0.015

Table B.1b Pigment concentrations ($mg\,m^{-3}$) in the two cruises (CR). Canary 1993 (Can.) and Labrador 1994 (Lab.). ST: station number. DE: depth (m). fu: fucoxanthin. he: 19- hexanoyloxyfucoxanthin. pe: peridinin. bu: butanoyloxyfucoxanthin. cis: cis-neoxanthin. d+t: diadinoxanthin+diatoxanthin. ze: zeaxanthin. $\alpha + \beta$: $\alpha + \beta$-carotene.

Table B.1b

CR	ST	DE	fu	he	pe	bu	cis	d+t	ze	α+β
Can.	1	1	0.018	0.020	0.000	0.009	0.000	0.018	0.029	0.000
Can.	1	20	0.016	0.021	0.000	0.009	0.000	0.015	0.027	0.000
Can.	1	100	0.017	0.052	0.000	0.076	0.000	0.009	0.010	0.004
Can.	2	60	0.020	0.050	0.000	0.023	0.000	0.009	0.036	0.008
Can.	2	80	0.020	0.054	0.000	0.063	0.012	0.008	0.035	0.010
Can.	2	100	0.018	0.039	0.000	0.061	0.000	0.008	0.009	0.002
Can.	4	1	0.018	0.037	0.000	0.009	0.000	0.026	0.110	0.024
Can.	4	10	0.021	0.029	0.000	0.016	0.000	0.012	0.125	0.020
Can.	4	20	0.019	0.034	0.000	0.019	0.000	0.018	0.163	0.028
Can.	4	30	0.022	0.031	0.000	0.019	0.000	0.010	0.133	0.025
Can.	4	50	0.042	0.059	0.000	0.032	0.000	0.010	0.017	0.007
Can.	5	1	0.000	0.033	0.000	0.012	0.000	0.026	0.066	0.010
Can.	5	10	0.023	0.037	0.000	0.011	0.000	0.013	0.100	0.016
Can.	5	20	0.025	0.049	0.000	0.017	0.000	0.022	0.138	0.021
Can.	5	30	0.065	0.141	0.027	0.054	0.072	0.023	0.183	0.039
Can.	5	40	0.134	0.262	0.000	0.113	0.072	0.040	0.167	0.012
Can.	5	50	0.100	0.150	0.000	0.078	0.065	0.023	0.090	0.035
Can.	5	75	0.028	0.031	0.000	0.024	0.000	0.006	0.010	0.000
Can.	61	1	0.007	0.021	0.000	0.000	0.000	0.030	0.046	0.007
Can.	61	35	0.041	0.084	0.000	0.029	0.050	0.021	0.154	0.042
Can.	61	45	0.096	0.227	0.000	0.080	0.018	0.019	0.101	0.038
Can.	61	55	0.100	0.188	0.000	0.078	0.046	0.015	0.089	0.019
Can.	7	1	0.159	0.058	0.000	0.000	0.000	0.261	0.000	0.017
Can.	7	5	0.202	0.088	0.000	0.000	0.000	0.056	0.263	0.041
Can.	7	10	0.620	0.101	0.000	0.034	0.000	0.086	0.527	0.098
Can.	7	15	0.972	0.179	0.108	0.053	0.000	0.114	0.494	0.115
Can.	7	20	0.571	0.048	0.064	0.000	0.043	0.050	0.034	0.014
Can.	7	25	0.496	0.021	0.000	0.000	0.000	0.045	0.021	0.005
Can.	8	1	0.066	0.029	0.026	0.000	0.000	0.035	0.000	0.000
Can.	8	10	0.168	0.023	0.037	0.000	0.000	0.058	0.006	0.007
Can.	8	22	4.539	0.178	0.000	0.000	0.000	0.387	0.000	0.130
Can.	9	60	0.042	0.041	0.000	0.020	0.000	0.009	0.084	0.006
Can.	9	70	0.036	0.112	0.000	0.056	0.033	0.010	0.084	0.029
Can.	9	80	0.045	0.112	0.000	0.089	0.034	0.021	0.058	0.026
Can.	9	100	0.031	0.056	0.000	0.070	0.000	0.009	0.013	0.004
Can.	11	60	0.009	0.032	0.000	0.011	0.000	0.010	0.058	0.004
Can.	11	80	0.048	0.059	0.000	0.038	0.000	0.013	0.060	0.016
Can.	11	90	0.153	0.094	0.000	0.075	0.000	0.019	0.037	0.013
Can.	11	100	0.101	0.062	0.000	0.097	0.000	0.019	0.035	0.015
Can.	12	50	0.010	0.031	0.000	0.008	0.000	0.008	0.046	0.000
Can.	12	70	0.029	0.150	0.000	0.068	0.000	0.016	0.028	0.006
Can.	12	90	0.029	0.114	0.000	0.072	0.000	0.012	0.021	0.005
Can.	12	100	0.019	0.082	0.000	0.054	0.000	0.009	0.018	0.005
Can.	13	45	0.088	0.078	0.000	0.067	0.000	0.020	0.054	0.007
Can.	13	55	0.250	0.156	0.000	0.120	0.000	0.032	0.045	0.008
Can.	13	65	0.175	0.083	0.000	0.108	0.000	0.015	0.023	0.008
Can.	13	75	0.087	0.032	0.000	0.070	0.000	0.008	0.006	0.000
Can.	13	100	0.047	0.014	0.000	0.039	0.000	0.005	0.000	0.000

Table B.1b Contin.

CR	ST	DE	fu	he	pe	bu	cis	d+t	ze	α+β
Can.	14	40	0.000	0.028	0.000	0.008	0.000	0.007	0.052	0.000
Can.	14	60	0.025	0.091	0.000	0.047	0.000	0.012	0.060	0.010
Can.	14	70	0.033	0.187	0.000	0.102	0.000	0.018	0.043	0.016
Can.	14	80	0.023	0.142	0.000	0.088	0.000	0.014	0.031	0.005
Can.	14	100	0.048	0.023	0.000	0.061	0.000	0.006	0.011	0.005
Can.	15	1	0.011	0.070	0.000	0.027	0.000	0.032	0.034	0.000
Can.	15	20	0.020	0.072	0.000	0.032	0.000	0.024	0.037	0.000
Can.	15	45	0.058	0.241	0.032	0.102	0.000	0.023	0.022	0.000
Can.	15	55	0.046	0.137	0.000	0.109	0.000	0.015	0.010	0.000
Can.	15	65	0.030	0.070	0.000	0.070	0.000	0.009	0.005	0.000
Can.	15	75	0.018	0.028	0.000	0.033	0.000	0.002	0.000	0.000
Can.	17	1	0.123	0.207	0.068	0.072	0.000	0.095	0.036	0.014
Can.	17	10	0.115	0.207	0.065	0.076	0.000	0.080	0.040	0.000
Can.	17	25	0.377	0.315	0.518	0.107	0.000	0.282	0.038	0.028
Lab.	2	50	0.041	0.000	0.027	0.000	0.000	0.013	0.000	0.000
Lab.	2	65	0.147	0.018	0.000	0.008	0.000	0.012	0.000	0.000
Lab.	2	80	0.205	0.000	0.000	0.000	0.000	0.021	0.000	0.000
Lab.	2	100	0.123	0.000	0.000	0.001	0.000	0.016	0.000	0.000
Lab.	3	1	3.197	0.245	0.135	0.057	0.173	0.666	0.000	0.120
Lab.	3	10	2.987	0.207	0.000	0.000	0.186	0.390	0.000	0.094
Lab.	3	20	4.286	0.000	0.000	0.000	0.000	0.527	0.000	0.128
Lab.	3	30	2.003	0.156	0.000	0.000	0.000	0.148	0.000	0.068
Lab.	3	40	0.527	0.000	0.000	0.000	0.000	0.046	0.000	0.019
Lab.	3	60	0.119	0.006	0.000	0.000	0.054	0.016	0.000	0.000
Lab.	5	1	0.166	0.160	0.067	0.022	0.039	0.100	0.014	0.012
Lab.	5	10	0.144	0.105	0.047	0.030	0.000	0.081	0.015	0.007
Lab.	5	20	0.162	0.118	0.047	0.027	0.054	0.071	0.012	0.009
Lab.	5	30	0.180	0.131	0.052	0.026	0.030	0.061	0.013	0.000
Lab.	5	40	0.146	0.055	0.019	0.022	0.000	0.033	0.008	0.000
Lab.	5	60	0.064	0.013	0.000	0.000	0.000	0.008	0.000	0.000
Lab.	6	1	0.229	0.017	0.000	0.016	0.027	0.036	0.000	0.000
Lab.	6	10	0.378	0.018	0.000	0.025	0.040	0.064	0.000	0.000
Lab.	6	20	0.279	0.000	0.000	0.036	0.000	0.040	0.000	0.009
Lab.	6	30	0.134	0.000	0.000	0.000	0.000	0.029	0.000	0.000
Lab.	6	50	0.114	0.000	0.000	0.000	0.000	0.021	0.000	0.000
Lab.	6	75	0.110	0.000	0.000	0.000	0.000	0.020	0.000	0.000
Lab.	7	1	0.060	0.121	0.106	0.031	0.109	0.298	0.024	0.028
Lab.	7	10	0.068	0.127	0.118	0.036	0.127	0.318	0.026	0.047
Lab.	7	20	0.107	0.141	0.082	0.042	0.101	0.271	0.019	0.040
Lab.	7	30	0.058	0.140	0.069	0.026	0.043	0.109	0.012	0.019
Lab.	7	40	0.036	0.065	0.033	0.000	0.030	0.028	0.013	0.000
Lab.	7	60	0.041	0.046	0.000	0.020	0.007	0.017	0.009	0.000
Lab.	8	1	0.144	0.082	0.000	0.032	0.000	0.064	0.020	0.000
Lab.	8	10	0.171	0.096	0.018	0.040	0.000	0.077	0.019	0.009
Lab.	8	20	0.117	0.059	0.000	0.013	0.000	0.047	0.013	0.000
Lab.	8	40	0.172	0.092	0.000	0.036	0.012	0.058	0.019	0.000
Lab.	8	60	0.148	0.076	0.000	0.033	0.000	0.018	0.009	0.000
Lab.	8	80	0.064	0.020	0.000	0.023	0.000	0.007	0.000	0.000

Table B.1b Contin.

CR	ST	DE	fu	he	pe	bu	cis	d+t	ze	α+β
Lab.	9	1	0.172	0.224	0.087	0.044	0.057	0.139	0.024	0.018
Lab.	9	10	0.147	0.185	0.082	0.042	0.052	0.121	0.022	0.017
Lab.	9	20	0.161	0.203	0.079	0.042	0.044	0.120	0.022	0.014
Lab.	9	40	0.138	0.016	0.000	0.024	0.000	0.013	0.004	0.000
Lab.	9	50	0.052	0.006	0.000	0.003	0.000	0.005	0.000	0.000
Lab.	9	75	0.040	0.002	0.000	0.000	0.001	0.007	0.000	0.000
Lab.	9	100	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lab.	10	2	0.735	0.245	0.196	0.051	0.000	0.400	0.000	0.027
Lab.	10	10	0.839	0.297	0.233	0.061	0.000	0.428	0.000	0.033
Lab.	10	17	0.828	0.283	0.222	0.062	0.037	0.396	0.000	0.033
Lab.	10	27	0.853	0.326	0.245	0.072	0.000	0.285	0.000	0.033
Lab.	10	37	0.151	0.031	0.000	0.000	0.000	0.026	0.000	0.000
Lab.	10	56	0.030	0.000	0.000	0.000	0.000	0.002	0.000	0.000
Lab.	11	1	1.021	0.557	0.543	0.159	0.000	0.646	0.000	0.046
Lab.	11	10	1.035	0.580	0.600	0.100	0.045	0.653	0.000	0.055
Lab.	11	20	1.028	0.301	0.192	0.052	0.000	0.194	0.000	0.032
Lab.	11	30	0.218	0.035	0.000	0.012	0.000	0.019	0.000	0.000
Lab.	11	40	0.226	0.034	0.012	0.015	0.000	0.020	0.000	0.000
Lab.	11	55	0.217	0.033	0.000	0.039	0.000	0.018	0.000	0.000
Lab.	11	80	0.091	0.014	0.000	0.000	0.000	0.008	0.000	0.000
Lab.	12	1	3.726	0.000	0.000	0.000	0.000	0.593	0.000	0.131
Lab.	12	10	1.733	0.251	0.000	0.000	0.000	0.145	0.000	0.058
Lab.	12	20	0.661	0.000	0.000	0.000	0.000	0.059	0.000	0.016
Lab.	13	1	4.175	0.176	0.322	0.000	0.000	1.238	0.000	0.178
Lab.	13	5	4.414	0.201	0.344	0.000	0.000	1.124	0.000	0.000
Lab.	13	10	4.380	0.181	0.332	0.000	0.000	1.179	0.000	0.158
Lab.	13	20	0.822	0.040	0.056	0.000	0.000	0.079	0.000	0.024
Lab.	13	40	0.115	0.021	0.000	0.000	0.046	0.015	0.000	0.000
Lab.	14	1	1.525	0.134	0.000	0.018	0.049	0.201	0.000	0.030
Lab.	14	20	1.378	0.117	0.000	0.000	0.000	0.190	0.000	0.030
Lab.	14	40	1.500	0.105	0.000	0.000	0.053	0.175	0.000	0.018
Lab.	14	60	1.707	0.115	0.000	0.009	0.070	0.191	0.000	0.030
Lab.	14	70	0.984	0.048	0.000	0.000	0.034	0.105	0.000	0.021
Lab.	14	80	0.231	0.012	0.000	0.012	0.027	0.023	0.007	0.000
Lab.	14	100	0.072	0.010	0.000	0.000	0.005	0.006	0.000	0.000

Table B.2 Values of density (σ_t), temperature (Temp., °C), and nitrate concentrations (NO_3^-, μM) in the two cruises (CR), Canary 1993 (Can.) and Labrador 1994 (Lab.). ST: station number, DE: depth (m).

Table B.2

CR	ST	DE	σ_t	Temp.	NO_3^-
Can.	1	1	26.396	18.35	0.05
Can.	1	20	26.398	18.35	0.03
Can.	1	100	26.685	16.85	2.87
Can.	2	60	26.464	18.08	0.07
Can.	2	80	26.62	17.28	0.06
Can.	2	100	26.668	17.00	0.80
Can.	4	1	26.011	19.11	0.11
Can.	4	10	26.008	19.11	0.09
Can.	4	20	26.013	19.11	0.14
Can.	4	30	26.105	18.91	0.70
Can.	4	50	26.404	18.14	2.93
Can.	5	1	26.003	19.14	0.07
Can.	5	10	25.995	19.15	0.08
Can.	5	20	25.998	19.15	0.08
Can.	5	30	26.129	18.78	1.21
Can.	5	40	26.261	18.43	1.25
Can.	5	50	26.273	18.39	1.85
Can.	5	75	26.391	17.97	3.60
Can.	6	1	25.894	19.59	0.06
Can.	6	35	26.047	18.98	0.05
Can.	6	45	26.068	18.91	0.14
Can.	6	55	26.094	18.83	0.71
Can.	7	1	25.892	19.54	0.14
Can.	7	5	25.876	19.56	0.12
Can.	7	10	26.232	19.15	0.33
Can.	7	15	27.442	17.87	1.30
Can.	7	20	26.038	19.33	7.30
Can.	7	25	26.098	19.26	7.33
Can.	8	1	25.912	19.52	0.06
Can.	8	10	26.155	19.27	0.15
Can.	8	22	26.044	19.38	7.41
Can.	9	60	26.494	18.56	0.03
Can.	9	70	26.514	18.48	0.05
Can.	9	80	26.528	18.39	0.14
Can.	9	100	26.572	18.03	1.65
Can.	11	60	26.395	18.69	0.00
Can.	11	80	26.455	18.23	0.00
Can.	11	90	26.49	18.22	0.12
Can.	11	100	26.509	18.20	1.17
Can.	12	50	26.294	17.99	0.00
Can.	12	70	26.633	16.86	0.65
Can.	12	90	26.612	16.67	1.05
Can.	12	100	26.64	16.43	1.97
Can.	13	45	26.323	18.47	0.00
Can.	13	55	26.396	18.05	0.07
Can.	13	65	26.469	17.50	0.88
Can.	13	75	26.529	17.00	1.85
Can.	13	100	26.602	16.33	2.97

Table B.2 Contin.

CR	ST	DE	σ_t	Temp.	NO_3^-
Can.	14	40	26.154	18.82	0.00
Can.	14	60	26.368	17.93	0.00
Can.	14	70	26.400	17.96	0.02
Can.	14	80	26.419	17.86	0.21
Can.	14	100	26.470	17.33	2.00
Can.	15	1	26.151	18.50	0.00
Can.	15	20	26.187	18.49	0.00
Can.	15	45	26.362	17.74	0.60
Can.	15	55	26.520	17.08	1.10
Can.	15	65	26.550	16.87	1.52
Can.	15	75	26.560	16.82	1.63
Can.	17	1	25.995	13.17	0.00
Can.	17	10	26.038	13.22	0.03
Can.	17	25	27.407	11.79	0.03
Lab.	2	50	26.103	0.91	0.00
Lab.	2	65	26.274	-0.14	2.65
Lab.	2	80	26.474	-1.53	8.95
Lab.	2	100	26.510	-1.50	9.48
Lab.	3	1	25.125	0.41	0.03
Lab.	3	10	26.189	0.42	0.03
Lab.	3	20	26.200	0.29	0.10
Lab.	3	30	26.260	-0.27	6.55
Lab.	3	40	26.550	-1.29	8.44
Lab.	3	60	26.702	-1.37	9.68
Lab.	5	3	27.518	3.61	8.40
Lab.	5	10	27.554	3.60	8.40
Lab.	5	20	27.555	3.60	8.38
Lab.	5	30	27.561	3.56	9.20
Lab.	5	40	27.583	3.43	9.72
Lab.	5	60	27.658	3.10	10.91
Lab.	6	2	27.299	1.74	9.81
Lab.	6	10	27.310	1.80	11.51
Lab.	6	20	27.405	2.11	11.30
Lab.	6	30	27.427	2.15	11.51
Lab.	6	50	27.435	2.15	11.62
Lab.	6	75	27.450	2.23	11.80
Lab.	7	2	27.176	3.63	10.39
Lab.	7	10	27.469	3.62	10.33
Lab.	7	20	27.472	3.60	9.72
Lab.	7	30	27.495	3.37	9.93
Lab.	7	40	27.515	3.31	10.11
Lab.	7	60	27.574	3.32	10.96
Lab.	8	2	27.376	3.57	9.07
Lab.	8	10	27.550	3.58	9.00
Lab.	8	20	27.553	3.57	8.94
Lab.	8	40	27.553	3.57	7.18
Lab.	8	60	27.586	3.39	13.62
Lab.	8	80	27.671	3.13	15.62

Table B.2 Contin.

CR ST	DE	σ_t	Temp.	NO_3^-
Lab. 9	1	27.026	3.60	8.59
Lab. 9	10	27.515	3.60	8.62
Lab. 9	20	27.517	3.58	8.60
Lab. 9	40	27.519	3.58	12.94
Lab. 9	50	27.537	3.48	14.29
Lab. 9	75	27.672	2.96	13.83
Lab. 9	100	27.702	2.74	14.86
Lab. 10	2	26.967	0.88	8.81
Lab. 10	10	27.193	0.56	8.56
Lab. 10	17	27.243	0.45	8.80
Lab. 10	27	27.315	0.25	9.60
Lab. 10	37	27.513	1.02	10.95
Lab. 10	56	27.650	2.86	15.02
Lab. 11	2	27.021	1.28	6.68
Lab. 11	10	27.010	1.50	6.63
Lab. 11	20	27.066	1.57	9.58
Lab. 11	30	27.204	1.58	11.62
Lab. 11	40	27.274	1.45	11.65
Lab. 11	55	27.496	0.35	13.85
Lab. 11	80	27.578	1.29	14.34
Lab. 12	2	25.445	-1.00	0.00
Lab. 12	10	25.857	-1.11	0.70
Lab. 12	20	26.229	-1.26	5.59
Lab. 13	3	26.520	0.57	0.17
Lab. 13	5	26.299	0.55	1.84
Lab. 13	10	26.300	0.55	6.51
Lab. 13	20	26.784	-0.28	9.99
Lab. 13	40	27.248	-0.22	11.72
Lab. 14	3	27.322	4.69	8.36
Lab. 14	20	27.371	4.69	5.87
Lab. 14	40	27.373	4.69	---
Lab. 14	60	27.378	4.67	---
Lab. 14	70	27.403	4.45	---
Lab. 14	80	27.484	3.70	---
Lab. 14	83	27.495	3.62	---

Table B.3 Values of *in vivo* absorption and fluorescence of phytoplankton in the two cruises (CR). Canary 1993 (Can.) and Labrador 1994 (Lab.). $a_{ph}(\lambda)$: absorption coefficients at 440 nm. 545 nm. and 676 nm (m^{-1}): $a^n_{ph}(\lambda)$: phytoplankton absorption at 440 and 676 nm normalised to 1 at 545 nm (dimensionless): $f_c(\lambda)$: values of fluorescence excitation spectrum at 440 and 545 nm (relative units): $f^n_c(440)$: fluorescence excitation at 440 nm normalised to the value at 545 nm (dimensionless): ST: station number: DE: depth (m).

Table B.3

CR	ST	DE	$a_{ph}(440)$	$a_{ph}(545)$	$a_{ph}(676)$	$a^n_{ph}(440)$	$a^n_{ph}(676)$	$f_c(440)$	$f_c(545)$	$f^n_c(440)$
Can.	1	1	0.012	0.002	0.004	5.292	1.757	73678	20497	3.603
Can.	1	20	0.009	0.002	0.003	6.730	2.328	48475	18801	3.707
Can.	1	100	0.013	0.002	0.004	4.286	2.027	74994	21941	2.963
Can.	2	60	0.018	0.003	0.006	4.785	1.906	27195	5551	3.157
Can.	2	80	0.011	0.001	0.004	4.509	1.857	15962	3420	2.900
Can.	2	100	0.014	0.002	0.005	4.863	2.130	18300	4255	3.047
Can.	4	1	0.030	0.007	0.009	4.457	1.436	27554	16700	1.672
Can.	4	10	0.017	0.003	0.006	3.568	1.221	20223	9977	1.255
Can.	4	20	0.018	0.003	0.006	3.787	1.357	24762	13736	1.468
Can.	4	30	0.034	0.009	0.012	3.240	1.076	1721	1082	1.206
Can.	4	50	0.023	0.006	0.008	2.649	1.394	39885	26990	2.232
Can.	5	1	0.017	0.003	0.006	6.180	2.187	24815	7908	3.126
Can.	5	10	0.011	0.002	0.004	4.318	1.654	21695	11027	2.123
Can.	5	20	0.013	0.002	0.004	4.626	1.604	26108	15151	2.045
Can.	5	30	0.020	0.005	0.007	2.669	1.169	36511	18615	1.351
Can.	5	40	0.025	0.006	0.009	2.841	1.304	33483	16200	1.561
Can.	5	50	0.022	0.004	0.007	2.677	1.302	27796	25388	1.618
Can.	5	75	0.020	0.004	0.007	3.334	1.767	26431	23556	2.652
Can.	6	1	0.018	0.005	0.007	4.193	1.461	25028	7266	2.965
Can.	6	35	0.020	0.005	0.007	2.803	1.074	14004	3811	1.394
Can.	6	45	0.021	0.004	0.007	2.718	1.250	24885	5316	1.730
Can.	6	55	0.019	0.004	0.006	2.559	1.196	32411	7016	1.798
Can.	7	1	0.040	0.011	0.019	3.875	1.701	58857	23133	2.510
Can.	7	5	0.032	0.007	0.014	3.863	1.737	29894	12323	2.256
Can.	7	10	0.030	0.006	0.014	3.584	1.811	22444	9612	1.331
Can.	7	15	0.041	0.011	0.018	3.431	1.782	62498	23933	1.528
Can.	7	20	0.111	0.031	0.056	3.471	1.761	59546	46441	1.909
Can.	7	25	0.103	0.029	0.052	3.188	1.771	64602	44557	2.420
Can.	8	1	0.019	0.005	0.008	4.119	1.802	19150	6418	2.958
Can.	8	10	0.006	0.001	0.002	3.851	1.888	9226	2162	2.578
Can.	8	22	0.030	0.007	0.014	3.611	1.814	36771	14804	1.728
Can.	9	60	0.017	0.001	0.005	5.674	2.075	22561	4001	3.982
Can.	9	70	0.006	0.001	0.002	4.968	1.968	8490	1161	2.608
Can.	9	80	0.012	0.001	0.004	5.181	2.086	21985	3793	2.638
Can.	9	100	0.011	0.001	0.004	4.725	2.061	12552	2040	2.994
Can.	11	60	0.005	0.001	0.002	6.048	2.093	8353	1046	4.645
Can.	11	80	0.002	0.000	0.001	5.045	1.935	4832	601	3.181
Can.	11	90	0.002	0.000	0.001	4.616	1.912	3811	301	2.909
Can.	11	100	0.007	0.001	0.003	4.540	1.871	25114	4452	3.008
Can.	12	50	0.007	0.001	0.002	5.805	1.996	22634	3198	5.313
Can.	12	70	0.006	0.001	0.002	5.367	2.168	15647	2487	3.755
Can.	12	90	0.004	0.001	0.001	4.688	1.905	6813	500	3.243
Can.	12	100	0.004	0.001	0.001	4.305	1.816	10578	1243	3.653
Can.	13	45	0.005	0.001	0.002	4.464	1.857	17527	2600	3.286
Can.	13	55	0.005	0.001	0.002	4.606	1.947	7450	915	3.016
Can.	13	65	0.002	0.000	0.001	4.475	1.918	7198	996	2.808
Can.	13	75	0.025	0.006	0.010	3.957	1.860	86971	23839	3.115
Can.	13	100	0.034	0.007	0.014	3.994	2.019	32535	9385	2.889

Table B.3 Contin.

CR	ST	DE	$a_{ph}(440)$	$a_{ph}(545)$	$a_{ph}(676)$	$a^n_{ph}(440)$	$a^n_{ph}(676)$	$f_c(440)$	$f_c(545)$	$f^n_c(440)$
Can.	14	40	0.008	0.002	0.003	5.088	1.771	19624	3372	4.593
Can.	14	60	0.008	0.002	0.003	4.724	1.881	19958	3251	2.791
Can.	14	70	0.005	0.001	0.002	4.194	1.763	7441	1075	2.545
Can.	14	80	0.013	0.003	0.005	4.649	2.079	28176	5578	2.479
Can.	14	100	0.013	0.002	0.004	4.017	1.740	28932	6262	3.135
Can.	15	1	0.014	0.002	0.005	5.982	2.232	47837	9414	4.699
Can.	15	20	0.006	0.001	0.002	5.107	1.962	123312	77058	4.434
Can.	15	45	0.019	0.003	0.007	3.995	1.856	53213	10618	2.349
Can.	15	55	0.015	0.003	0.006	4.302	1.998	48395	10689	3.298
Can.	15	65	0.007	0.001	0.002	4.082	2.030	27348	10707	2.410
Can.	15	75	0.043	0.011	0.020	3.922	2.137	160728	64264	2.470
Can.	17	1	0.051	0.011	0.019	4.619	1.752	131862	33128	3.661
Can.	17	10	0.025	0.007	0.009	4.444	1.986	66666	15633	4.395
Can.	17	25	0.020	0.005	0.008	4.147	2.019	53349	11930	2.579
Lab.	2	50	0.003	0.001	0.001	3.769	1.722	157598	35452	2.817
Lab.	2	65	0.003	0.001	0.001	4.236	1.964	272230	64973	3.239
Lab.	2	80	0.005	0.002	0.002	3.160	1.850	475102	142628	2.704
Lab.	2	100	0.005	0.001	0.002	2.868	1.711	389737	100569	2.621
Lab.	3	1	0.115	0.030	0.062	3.796	2.030	1660335	574194	2.798
Lab.	3	10	0.050	0.009	0.018	3.520	1.903	1448465	318672	2.504
Lab.	3	20	0.051	0.009	0.018	3.353	1.814	1756760	358377	2.493
Lab.	3	30	0.172	0.051	0.095	2.382	1.624	2200724	927389	2.032
Lab.	3	40	0.122	0.038	0.067	2.401	1.549	2698572	1110377	2.545
Lab.	3	60	0.142	0.040	0.076	4.190	2.001	2185293	929665	3.613
Lab.	5	1	0.045	0.009	0.016	5.104	1.883	1909097	463212	3.912
Lab.	5	10	0.020	0.003	0.007	5.775	2.184	990834	163733	4.118
Lab.	5	20	0.022	0.003	0.007	5.061	1.919	930294	153557	3.785
Lab.	5	30	0.044	0.008	0.017	5.176	2.077	2233634	511935	3.711
Lab.	5	40	0.038	0.007	0.015	4.675	1.870	1985679	501090	3.752
Lab.	5	60	0.014	0.002	0.005	3.971	1.713	388228	62428	3.692
Lab.	6	1	0.046	0.010	0.022	4.925	2.336	772122	231779	3.342
Lab.	6	10	0.008	0.002	0.003	4.772	2.351	365649	62038	2.959
Lab.	6	20	0.047	0.010	0.024	4.729	2.265	686225	226351	3.012
Lab.	6	30	0.043	0.009	0.021	3.980	1.896	686373	221738	3.642
Lab.	6	50	0.008	0.001	0.003	4.177	1.742	459701	84307	3.480
Lab.	6	75	0.044	0.009	0.021	3.722	1.514	816724	278439	3.404
Lab.	7	1	0.070	0.012	0.028	5.533	2.201	3597290	1209122	3.154
Lab.	7	10	0.036	0.005	0.015	5.735	2.441	2582295	599893	2.904
Lab.	7	20	0.038	0.006	0.015	5.307	2.316	2322264	534208	2.890
Lab.	7	30	0.069	0.012	0.029	4.600	2.034	3465850	1165050	2.964
Lab.	7	40	0.084	0.016	0.036	4.664	1.869	3326502	1138121	4.061
Lab.	7	60	0.081	0.015	0.036	4.570	1.791	3331757	1175806	4.283
Lab.	8	1	0.030	0.006	0.013	5.327	2.264	1003588	286987	3.240
Lab.	8	10	0.017	0.004	0.006	5.000	2.070	666576	122095	3.176
Lab.	8	20	0.017	0.003	0.006	4.947	2.085	822766	154046	3.156
Lab.	8	40	0.029	0.006	0.012	4.322	1.834	753766	221801	3.305
Lab.	8	60	0.030	0.006	0.013	4.443	1.894	916797	270107	3.219
Lab.	8	80	0.017	0.003	0.006	3.946	1.895	631551	116110	3.753

Table B.3 Contin.

CR	ST	DE	$a_{ph}(440)$	$a_{ph}(545)$	$a_{ph}(676)$	$a^n_{ph}(440)$	$a^n_{ph}(676)$	$f_c(440)$	$f_c(545)$	$f^n_c(440)$
Lab.	9	1	0.047	0.009	0.018	5.602	2.188	2503386	600332	4.287
Lab.	9	10	0.036	0.006	0.010	5.391	2.126	2184336	360807	3.880
Lab.	9	20	0.028	0.004	0.010	5.563	2.168	1656110	272869	4.037
Lab.	9	40	0.052	0.010	0.020	4.409	1.997	2579625	683534	4.194
Lab.	9	50	0.046	0.008	0.018	4.643	2.121	1844428	454013	4.340
Lab.	9	75	0.025	0.004	0.009	4.459	2.467	1515417	249512	4.298
Lab.	9	100	0.030	0.004	0.011	4.192	2.532	1152315	210029	4.198
Lab.	10	2	0.072	0.015	0.034	4.739	2.227	3499914	1147919	2.819
Lab.	10	10	0.022	0.004	0.008	4.575	2.142	1360366	217383	2.747
Lab.	10	17	0.021	0.004	0.008	4.682	2.184	1146021	178431	2.741
Lab.	10	27	0.083	0.018	0.039	4.318	2.129	2376520	863661	2.630
Lab.	10	37	0.072	0.016	0.034	3.889	1.840	4119838	1397551	3.832
Lab.	10	56	0.023	0.004	0.009	3.728	1.887	1510769	255595	4.117
Lab.	11	1	0.064	0.013	0.031	4.732	2.348	3106354	1077566	2.799
Lab.	11	10	0.016	0.002	0.007	4.759	2.331	1171498	211440	2.819
Lab.	11	20	0.018	0.003	0.007	3.830	2.124	1180807	211096	2.797
Lab.	11	30	0.068	0.014	0.033	3.734	1.884	3484697	1145655	3.148
Lab.	11	40	0.042	0.011	0.024	3.754	2.035	2017891	691771	3.198
Lab.	11	55	0.049	0.013	0.026	3.917	2.023	3043370	1014393	3.299
Lab.	11	80	0.022	0.006	0.011	4.844	2.350	1603212	493689	3.696
Lab.	12	1	0.075	0.018	0.044	3.534	2.034	1835292	821755	2.147
Lab.	12	10	0.021	0.004	0.007	2.612	1.612	262484	42611	2.273
Lab.	12	20	0.046	0.018	0.027	2.693	1.584	1357899	591207	2.589
Lab.	13	1	0.152	0.040	0.087	3.862	2.191	1849079	862397	2.201
Lab.	13	5	0.022	0.003	0.007	3.850	2.153	629346	93734	2.059
Lab.	13	10	0.150	0.040	0.084	4.173	2.271	2511234	1082691	2.069
Lab.	13	20	0.178	0.045	0.099	3.281	1.833	2353131	1183244	2.563
Lab.	13	40	0.018	0.003	0.007	4.126	1.862	752786	133564	3.529
Lab.	14	1	0.088	0.023	0.046	3.819	1.979	1237350	431812	2.731
Lab.	14	20	0.021	0.004	0.008	3.935	2.105	888014	162323	2.619
Lab.	14	40	0.021	0.003	0.008	3.766	1.962	898231	175696	2.499
Lab.	14	60	0.090	0.024	0.046	3.787	1.956	1195194	417698	2.598
Lab.	14	70	0.082	0.022	0.042	3.746	1.976	1335073	469028	2.636
Lab.	14	80	0.020	0.004	0.008	3.249	1.643	527122	106311	2.510
Lab.	14	100	0.018	0.002	0.007	4.221	2.033	647814	125526	2.897

Table B.4 Values of irradiance (μmol quanta $m^{-2} s^{-1}$) and concentrations of different picoplanktonic groups (cells ml^{-1}) at different depths in station Canary 5.

Depth	I_z	cyan.	pro.	euk.
1	933	75808	2727	19069
10	435	76086	2868	17504
20	256	95472	5762	18193
30	132	126614	26372	18055
40	64	119717	24963	16927
50	34	70934	14934	11284
75	15	19896	4461	4081

cyan. : cyanophytes
pro. : prochlorophytes
euk. : eukaryotes
I_z : irradiance at depth z

Appendix C: Pigment data from Chapter 4

Data on intracellular pigment concentrations in the three different species of phytoplankton (*Thalassiosira weissflogii*. *Chaetocers* sp., *Synechococcus* sp.) analysed in Chapter 4 are shown in Tables C.1, C.2 and C.3.

Table C.1 Values of intracellular concentrations of pigments ($mg\,m^{-3}$) for *Thalassiosira weissflogii* grown at different irradiances (Irr., $\mu mol\,quanta\,m^{-2}\,s^{-1}$).

Irr.	ca	cc	fu	d+t	β-car
20	4198256	406843	1895298	221974	125607
50	4958983	473084	2319308	343709	147306
100	3446751	388133	1861397	416653	80197
250	3914255	281340	1642087	676084	139481
450	4328293	273517	1688103	1050770	178927

ca: chlorophyll-*a*
cc: chlorophyll-*c*
fu: fucoxanthin
d+t: diadinoxanthin+diatoxanthin
β-car: β-carotene

Table C.2 Values of intracellular concentrations of pigments ($mg\,m^{-3}$) for *Chaetoceros* sp. grown at different irradiances (Irr., $\mu mol\,quanta\,m^{-2}\,s^{-1}$).

Irr.	ca	cc	fu	d+t	β-car
20	13956235	3203964	9520113	665093	437183
100	10556179	2996860	8289863	1281341	455371

ca: chlorophyll-*a*
cc: chlorophyll-*c*
fu: fucoxanthin
d+t: diadinoxanthin+diatoxanthin
β-car: β-carotene

Table C.3 Values of intracellular concentrations of pigments ($mg\,m^{-3}$) for *Synechococcus* sp. grown at different irradiances (Irr., $\mu mol\,quanta\,m^{-2}\,s^{-1}$).

Irr.	ca	ze	β-car
20	17199263	8230909	2421491
50	12452444	8512258	1675042
100	8770259	8277349	530693

ca: chlorophyll-*a*
ze: zeaxanthin
β-car: β-carotene

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