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**Bio-optical observation and modeling of marine
biomass and primary production, for applications in
the coastal environment.**

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“He was alone, he was unheeded, happy, and near to the wild heart of life. He was alone and young and willful and wildhearted, alone amid a waste of wild air and brackish waters...”

(J. Joyce)

Bio-optical observation and modeling of marine biomass and primary production for applications in the coastal environment.

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ABSTRACT

Marine primary production (PP), i.e. the rate of carbon assimilated through photoautotrophic processes, is a key factor in the regulation of the global carbon cycle, with important potential feedback on climate. Phytoplankton represent 1% of the total terrestrial biomass but contribute 40% of global PP, through photosynthesis. Coastal environments are among the most productive areas of the planet. The development of predictive models, as well as the refinement of monitoring techniques in coastal areas are fundamental to address the issues of sustainability in a climatically/economically changing world. This study aims at using bio-optical techniques, which allow a synoptic view of larger portions of given marine ecological systems, to estimate biomass and primary production in temperate coastal waters. With the use of laboratory studies, *in situ* observations, and remote sensing (MODIS-Aqua), we have developed/refined, and applied/validated, certain bio-optical models, at the regional scale. The diatom *Skeletonema costatum* was chosen as a reference organism to model the effects of photo-acclimation on marine phytoplankton photosynthesis. We have implemented these effects in a semi-analytical model of primary production (Phyto-VFP), and applied it to a large 4-year dataset of oceanographic campaigns (SYMPLEX 1-4, N/O Urania_CNR), in the case I waters of the Channel of Sicily. The sub-regionalization of our datasets, on a trophic-level basis, has allowed us to reliably describe the annual cycle of primary production in this Mediterranean province. Synoptic estimates of biomass and primary production are even more suitably addressed by the use of satellite images, in large demand by administration agencies in recent years, as a tool to monitor water quality in coastal environments. In our study we have used three years of bio-optical observations to regionalize the standard MODIS-A algorithm for the Chesapeake Bay. We have developed a regional algorithm (OC3CB) with improved performance with respect to global OC4v4 and OC3M. We also describe an original approach to the use of MODIS-A observations of ocean color, with the development of a new product for MODIS-A (Lw510), especially conceived for coastal applications.

Osservazioni e modelli bio-ottici di biomassa e produzione marina, per applicazioni nell'ambiente costiero.

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RIASSUNTO

La produzione primaria (PP) marina, ovvero il processo di organicazione del Carbonio attraverso la fotosintesi, è un fattore fondamentale nel ciclo del Carbonio, con ritorno sui meccanismi regolatori del clima. Il fitoplancton marino rappresenta solo 1% della biomassa terrestre totale, ma è responsabile per il 40% della produzione primaria globale. Gli ambienti costieri sono tra le aree più produttive del pianeta. Lo sviluppo di modelli predittivi della PP marina, e il raffinamento delle tecniche di monitoraggio degli ambienti costieri, sono punti fondamentali per rispondere alle problematiche della sostenibilità durante gli odierni cambiamenti dei sistemi climatici/economici globali. L'interesse di questo studio risiede nella possibilità di stimare biomassa e PP in ambiente marino costiero con tecniche bio-ottiche, che consentono una descrizione quasi sinottica di un sistema ecologico. Le nostre osservazioni, di laboratorio, in mare e da satellite (MODIS-AQUA), hanno consentito di sviluppare, o affinare, e validare modelli bio-ottici di calcolo per biomassa e PP marine a scala regionale. Abbiamo scelto la diatomea marina *Skeletonema costatum* per modellizzare l'effetto della fotoacclimatazione sul processo fotosintetico del fitoplancton marino. Abbiamo implementato tali effetti in un modello semi-analitico di calcolo -Phyto-VFP, applicato su un vasto set di dati provenienti da quattro anni di campagne oceanografiche (SYMPLEX) nelle acque di Caso I del Canale di Sicilia. La sub-regionalizzazione dei nostri dati su base trofica ha consentito di descrivere adeguatamente il ciclo annuale della produzione primaria in questa provincia del Mediterraneo. L'osservazione sinottica di biomassa e PP si realizza ancor meglio con l'uso di immagini satellitari. In questo studio, abbiamo utilizzato un triennio di osservazioni bio-ottiche nella bassa Chesapeake Bay (U.S.A.) per sviluppare un algoritmo regionale di stima della biomassa. Tale algoritmo (OC3CB) effettua stime migliori degli algoritmi satellitari globali, ed è inoltre utilizzabile con radiometria satellitare MODIS-A, grazie allo sviluppo, ulteriore, di un nuovo prodotto radiometrico per MODIS-A (Lw 510), pensato appunto per applicazioni in ambiente costiero.

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on water transparency, i.e. the effect of water absorption components on light penetration in the water column)

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Definitions, symbols, and units of notations used in this study

Definitions	Symbols	Units
<i>Biogeochemical variables</i>		
Fluorometric chlorophyll <i>a</i> concentration	<i>Chl-a</i>	mg m ⁻³
Fluorometric chlorophyll <i>c</i> concentration	<i>Chl-c</i>	mg m ⁻³
Photosynthetic carotenoids	<i>PSC</i>	mg m ⁻³
Non-photosynthetic carotenoids	<i>NPSC</i>	mg m ⁻³
Phaeopigments	<i>Pheo</i>	mg m ⁻³
Chlorophaeopigments	<i>CHL</i>	mg m ⁻³
Carotenoids	<i>CAR</i>	
<i>Fluorescence variables</i>		
Chl- <i>a</i> fluorescence in dark-adapted state	<i>F_o</i>	Arbitrary units
Chl- <i>a</i> fluorescence in the presence of actinic light	<i>F_o'</i>	Arbitrary units
Max Fluorescence	<i>F_{max}</i>	Arbitrary units
Fluorescence yield	<i>F_v/F_{max}</i>	Unitless (ratio)
Maximum quantum yield of PSII	<i>Y</i>	Unitless (ratio)
Effective quantum yield of PSII	<i>Y'</i>	Unitless (ratio)
<i>Photosynthesis/irradiance relationship (PE)</i>		
Photosynthesis/irradiance relationship	<i>PE</i>	mg C m ⁻³ d ⁻¹
Slope of the linear portion of PE	<i>α</i>	Unitless (ratio)
Electron transport rate	<i>ETR</i>	μE/m ² s
Irradiance where photosynthesis is maximum	<i>E_{max}</i>	μE/m ² s
Light saturation constant	<i>E_k</i>	μE/m ² s
Growth-light saturation constant	<i>K_E</i>	μE/m ² s
Growth-light efficiency	<i>α_g</i>	Unitless (ratio)
<i>Inherent Optical Properties (IOPs)</i>		
Absorption coefficient (<i>a</i>)	<i>a</i>	m ⁻¹
- water	<i>a_w</i>	m ⁻¹
- phytoplankton	<i>a_{ph}</i>	m ⁻¹
- colored dissolved organic matter (CDOM)	<i>a_g</i>	m ⁻¹
- non-pigmented particles	<i>a_d</i>	m ⁻¹
- particles	<i>a_p</i>	m ⁻¹
- CDOM + particle	<i>a_{pg}</i>	m ⁻¹
- CDOM + non-pigmented particles	<i>a_{dg}*</i>	m ⁻¹
- chlorophyll-specific phytoplankton absorption	<i>a_{ph}*</i>	m ² mg ⁻¹
- Exponential decay factor	<i>S</i>	nm ⁻¹
- CDOM	<i>S_s</i>	nm ⁻¹

Definitions	Symbol	Units
<i>Water column</i>		
Euphotic zone depth	Z_{eu}	m
Optical penetration depth	OPD	m
<i>Radiometric variables</i>		
Wavelength	λ	nm
Zenith angle	θ	Degree
Plane irradiance	E	W m ⁻² nm ⁻¹
Downwelling plane irradiance	E_d	W m ⁻² nm ⁻¹
Upwelling plane irradiance	E_u	W m ⁻² nm ⁻¹
Upwelling radiance	L_u	W m ⁻² nm ⁻¹ sr ⁻¹
Water-leaving radiance	L_w	W m ⁻² nm ⁻¹ sr ⁻¹
Normalized water leaving radiance	nL_w	W m ⁻² nm ⁻¹ sr ⁻¹
Upwelling radiance from bottom reflectance	L_{ub}	W m ⁻² nm ⁻¹ sr ⁻¹
Totally upward radiance leaving from the water	L_t	W m ⁻² nm ⁻¹ sr ⁻¹
Sky radiance	L_{sky}	W m ⁻² nm ⁻¹ sr ⁻¹
Rayleigh scattering	L_r	W m ⁻² nm ⁻¹ sr ⁻¹
Aerosol scattering	L_a	W m ⁻² nm ⁻¹ sr ⁻¹
Sun glint	L_g	W m ⁻² nm ⁻¹ sr ⁻¹
White capping effect	L_{wc}	W m ⁻² nm ⁻¹ sr ⁻¹
Fraction of L_{sky}/L_t	P	Unitless (ratio)
Irradiance reflectance	R	Unitless (ratio)
Remote sensing reflectance	R_{rs}	sr ⁻¹
Diffuse attenuation coefficient (K)	K	m ⁻¹
- downwelling plane irradiance	K_d	m ⁻¹
- upwelling plane irradiance	K_u	m ⁻¹
- upwelling radiance	K_{Lu}	m ⁻¹

CHAPTER 1.0

Introduction

Marine primary production (PP), i.e. the rate of carbon assimilated through photoautotrophic processes, is a key factor in the regulation of the global carbon cycle, controlling the partitioning of carbon between the large ocean reservoir and the relatively small atmospheric reservoirs. Phytoplankton represent only 1% of the total terrestrial biomass but contribute 40% of global PP [Falkowski et al., 2002] through photosynthesis. Fluctuations in productivity are the most relevant mechanism in the control of CO₂ content in the ocean [Broecker 1982; Broecker and Peng 1986] resulting in a potentially important feedback to climatic changes [Berger et al., 1989].

Quantification of marine primary production through estimates of phytoplankton photosynthetic rates is therefore a central theme in ecology. Estimates of marine PP are traditionally performed via measurements of radiocarbon (¹⁴C) uptake [Steemann and Nielsen, 1952] or soluble oxygen concentration [Williams, 1982; Langdon, 1984], the results given in mass of assimilated carbon per unit time per unit volume per unit of biomass, referred to as chlorophyll-a (Chl-a). Both techniques present disadvantages that have limited oceanographers ability to quantify large-scale contributions to marine PP. The ¹⁴C method requires prolonged isolation (from 3 to 12 hours) of discrete samples of seawater in a limited space (flasks or tanks). This can allow a number of variables (respiration, grazing, photoacclimation, metal toxicity) to introduce a non-quantifiable bias in the estimates derived. Some of these problems also apply to the measurements of oxygen evolution. Despite being a potentially faster method, the available oxygen sensors are far from sensitive enough to measure changes in oxygen concentration on the natural suspension of phytoplankton cells from oligotrophic or mesotrophic waters.

The extent of the transmission of solar radiation in the water varies accordingly to the components of the water. Under this principle, it is possible to retrieve general biogeochemical characteristics from a natural water body based on the observations of light penetration, without the necessity to measure fully its inherent optical properties. Jerlov (1951, 1976) pioneered this field of science, with a classification of marine waters into a number of different categories based on the spectral dependence of percent transmittance of downward irradiance in marine waters. He identified three basic types of oceanic waters - I, II, and III, and nine types of coastal waters (1 to 9), in order of decreasing transmittance. Because of the method he employed for his measurement of light transmittance, his results show poor agreement with the curves obtained with modern submersible spectroradiometers (Kirk, 1994). Pelevin & Rutkovskaya (1977) have proposed the use of the vertical attenuation coefficient for irradiance at 500 nm to classify marine waters. The value of K_d would indeed convey quite a lot of information about any such water, because it varies in a fairly systematic manner from one oceanic water to another (Kirk, 1994). Smith and Baker (1978), on the basis of their measurements of the spectral variation of the vertical attenuation coefficient (K_d) for irradiance in various ocean waters, have concluded that in regions away from terrigenous influences, the attenuation (apart from that due to water) is mainly due to the phytoplankton, and the various pigmented detrital products that co-vary with it. They suggest that for such ocean waters, the total content of chlorophyll-like pigments provides a sufficient basis for optical classification, since on the basis of the pigment content, the curve of K_d against wavelength can be calculated.

A classification which has been found useful in the context of remote sensing of the ocean is that into "Case I" and "Case II" waters, put forward by Morel and Prieur (1977) and further refined by Gordon and Morel (1983). Case I waters are those for which phytoplankton and their derivative products (organic detritus and dissolved yellow colour, arising by zooplankton grazing and phytoplankton decay) play a dominant role in determining the optical properties of the ocean. Case II waters are those for which an important or dominant contribution to the optical properties comes from re-suspended sediments from the continental shelf, or from particles and/or discharge. Case I waters can range from oligotrophic to eutrophic, provided only that the particulate and coloured materials characteristics of Case II waters do not play a

significant role. In Case II water phytoplankton and their derivative products may or may not be present in significant amount. Prieur and Sathyendranath (1981) proposed a simple scheme based on the relative importance of absorption by the three major components other than water: algal pigments, dissolved yellow materials, and particulate matter other than phytoplankton. As a numerical indication of importance, the absorption coefficient due to each component -C', Y', and P', is used respectively. Different waters are classified as C' type, Y' type, P' type, or hybrid types.

Integration of optical measurements with traditional techniques, such as estimation of chlorophaeopigments and particulate-matter concentration via filtration, results in empirical bio-optical relationships which are the basis for retrieval of products, such as chlorophaeopigments concentration, from remote sensing of ocean color (O'Reilly et al., 2003; D'Ortenzio et al. 2003; O'Reilly et al., 2000; Morel et Berthon, 1989). Bio-optical methods are the most advanced technique for investigating the distribution and physiological activity of marine phytoplankton. Among the acknowledged advantages of these techniques are: their rapidity, absence of alteration of the environment, and conveying a quasi-synoptic view of the monitored environment.

The choice to adopt a particular bio-optical algorithm to retrieve products (e.g. chlorophyll-a concentration) from the remote sensing of a certain oceanic region largely depends on the optical classification of that specific water body. On the other hand, spatial and environmental agencies around the world generally adopt standard bio-optical algorithms, which derive from the analysis of extensive (global) datasets, which encompass a wide range of optical and biological characteristics of the world's oceans (e.g.: 'GSM01' and 'OC4v4' for SeaWiFS sensors; 'OC3M' for MODIS-A sensors). As a result, biomass and primary production tend to be under-estimated in oligotrophic regions, like the Mediterranean Sea (D'Ortenzio et al., 2000), and, conversely, over-estimated in eutrophic regions, such as coastal productive areas.

A number of optical observation platforms, tools and devices have been developed in the last ten years for increasing the spatial and temporal resolution of biomass and primary production retrievals in the oceans. Submersible fluorimetric probes (Primprod 1.08, Istituto di Biofisica, University of Moscow, FRRF_Fast-tracka, Chelsea Inc.; Pulse Amplitude Modulated Fluorimeter_PAM, Walz), in water and

above-water multi- and hyper spectral radiometric sensors. Recently both the European Space Agency (ESA) and the USA National Aeronautic and Space Administration agency (NASA) have launched in space two new spectroradiometers, respectively MERIS (MEdium Resolution Imaging Spectrometer, on board Envisat satellite platform), and MODIS-A (Moderate Resolution Imaging Spectroradiometer, on board EOS' satellites 'Aqua' and 'Terra').

In order to exploit the new technology to its full potential, and to provide new insights on global carbon cycling and climate change effects, there is an urgent need for experiments to be performed both *in situ* and in the laboratory to increase knowledge in bio-optical assessment of marine biomass and primary production. A discrete set of experiments must therefore be performed using simultaneously both traditional and novel techniques, in the field (on natural population of phytoplankton), and in the laboratory (on cultivated cells). Work in the lab will allow the control of the experimental conditions, allowing separate hypothesis to be tested. Field studies will define the conditions upon which phytoplankton distribution varies, and how photosynthetic efficiency is influenced by biomass distribution over depth and time (of the day, and seasonal cycles).

Both aspects should provide input to the improvement of empirical or semi-analytical bio-optical models of biomass and productivity. In particular, the experimental plans should review the recent ecological modeling literature to fill the evident gaps. In fact, recent bio-optical models of primary production (Hartig et al. 1998; Kolber and Falkowski, 1993; Morel and Berthon, 1989) have failed to address relevant questions, such as the influence of seasonality and mixing of water masses on the physiology of phytoplankton, as well as the photo-acclimation process. We will move significant steps in this direction with the present work.

This study was carried out with funds from various national and international sources, in Italy, and in the USA, with a limited number of significant goals, to achieve an improvement of the retrievals of biomass and primary production in coastal waters: 1) definition of a model organism for modeling primary production in coastal waters; 2) refinement of a bio-optical model of marine primary production [Marcelli et al., 1997; Nardello, 1999; Marcelli et al., 2003; Nardello et al., 2003] with the implementation of

the effects of photo-acclimation on the photosynthetic performance of phytoplankton; 3) development a new bio-optical model for retrieval of biomass from above-water radiometric observations and remote sensing, for applications in optically complex coastal environments (i.e.: Mid Atlantic Bight, USA); 4) assessment of the necessity to overcome the traditional optical classification of seawater between Case I/II environments, especially for productive coastal environments.

CHAPTER 2.0

Studies of phytoplankton photo-acclimation process to improve primary production models for the marine coastal environment

2.1 BACKGROUND

The “Photosynthesis vs. Irradiance” relationship (generally referred to as: “PE” curve) represents the basis to model phytoplankton productivity (Platt et al. 1977; Fasham et al. 1990; MacIntyre et al. 2002). The photosynthetic performance of marine phytoplankton is affected, with respect to light, by two main regulatory processes: photo-adaptation, which is the genetic expression of these organisms of their aptitude to utilize different levels or spectral quality of irradiance (Morel, 1991; Antoine and Morel, 1996) and photoacclimation, which is a phenotypic response of photoautotrophic organisms to availability of light in the environment (Falkowski and La Roche, 1991). The PE curves can be parameterized (Fig.2.0), allowing comparisons of the phototrophic response of phytoplankton in different physiological and environmental conditions. Photoacclimation largely influences the primary production rate performed by phytoplankton in the marine environment (Anning et al., 2000), but most models for the PE curve neglect these responses (Zonneveld, 1997).

In the PE relationship, we distinguish different regions. During the initial light-limited phase, the relative electron transport flow is linearly and positively dependent on the availability of the incident PAR irradiance. With increase of actinic PAR irradiance levels, photosynthesis is eventually light-saturated. Upon exposure to higher irradiance levels, photosynthesis results impaired from a clogging of the photosynthetic pathway, unable to work out an excessive electron flow. This is the light-inhibited portion of the PE response.

A series of parameters are used to synthetically describe the PE response curve (Fig. 2.0). The slope of the initial linear portion of the PE relationship, represents the initial velocity at which P occurs. It is referred to as α , or “light utilization index”. The maximum value of the function PE relationship is called $P_{\max}$, and $E_{\max}$ is the corresponding irradiance level. A fundamental parameter, E_k , derives from the ratio $P_{\max}/\alpha$, and represents a comparable index of photoacclimation. The negative slope of the light-inhibited portion of the PE relationship is referred to as β , after Platt (1980). These terms have different significance according to how we describe the biomass of the primary producers: concentration of chlorophyll-a (Chl-a), number of cells, weight of the carbon mass, or number of cells per volume. Reviews of these terms, and of their implications to the process, have been recently issued (MacIntyre et al., 2002; Behrenfeld et al., 2004).

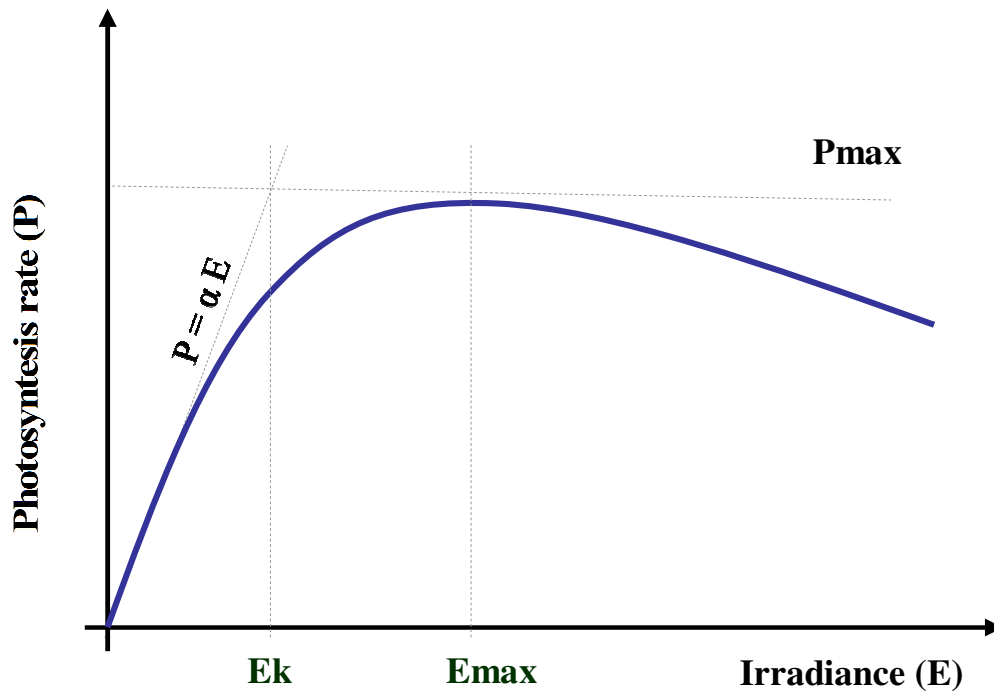


Fig. 2.0 - Parameterized theoretical PE curve, with indication of E_k (Light saturation constant), $E_{\max}$ (irradiance of maximum photosynthesis), $P_{\max}$ (maximum photosynthetic rate), and the linear portion of the photo-limited portion of the PE relationship, where $P = \alpha E$.

This study aims at showing the effect of photoacclimation on the diel cycles of the photosynthetic response of *Skeletonema costatum* (Greville) Cleve, with the adoption of optical measurements of *in vivo* chlorophyll-a variable fluorescence.

The correlation between fluorescence (FL), as measured through optical sensor at a wavelength $>670\text{nm}$, and Chl-a concentration between 0.01 and 10 mg/m^3 [Yentsch and Menzel, 1963] is extensively used today to retrieve phytoplankton abundance in the ocean [Falkowski and Raven, 1997]. The variation of *in vivo* chlorophyll-a fluorescence (FV) in response to actinic light, discovered by Kautsky in 1937, is correlated to photosynthesis as a competitive process [Samuelsson G., Oquist G., 1977; Weis and Berry, 1987; Genty et al., 1989; Kiefer and Reynolds, 1992]. There is strong evidence that photosynthetic yields measured with fluorescence techniques accurately reflect photosynthetic performance [Hawes et al., 2002] and could replace traditional methods to assess gross primary production [Hartig et al, 1998, Falkowski and Raven 1997: Antal et al., 1999; Antal et al., 2001]. The photosynthetic performance (P) is therefore approximated here by the velocity of the transport of electrons (ETR) in the photosynthetic pathway, as estimated from measurements of quantum yields (Y) of chlorophyll-a fluorescence.

We expect the parameters of the ETR/E curves to reflect photoacclimation, as they would when derived from more traditional measurements of the PE-curves (radiocarbon assimilation). For clarity, we will indicate the parameters of the PE curves derived from measurements of relative fluorescence yields as: α' , E_k' , P_{max}' . Few studies have detailed how the bio-optical and traditional photosynthetic parameters actually compare, but indications exist that they are very similar under certain conditions (Hawes et al., 2003).

We further expect that the variations of the PE parameters show a diel cycle (Bruyant et al., 2005), and that photoacclimation is also reflected in the light-harvesting capacity of this diatom, in terms of pigment content per cell, and pigment ratios (Sosik et al., 1989; Kirk, 1994; MacIntyre et al., 2002).

Based on our results, we will discuss the possibility to use the assessed phototrophic characteristics of *Skeletonema costatum* to improve the prediction of a bio-optical model of primary production (Phyto-VFP; Marcelli et al. 2003), in order to

account for the effects of space and time variations of light availability in the water column on phytoplankton photosynthesis.

2.2 METHODS

We performed a series of laboratory experiments to assess the diel variations of the PE parameters in batches of the eukaryote marine diatom *Skeletonema costatum* (Greville) Cleve, cultivated in three different states of photoacclimation. Photoacclimation is complete only when a condition of balanced growth has been achieved. Under balanced growth, the specific rate of change of all measures of cell mass is equal (Eppley 1981). For cells growing under a light:dark cycle, balanced growth is achieved when cell composition returns to the same value at 24-h intervals.

2.2.1 - Culture conditions

Culture conditions - Nutrient-replete axenic cultures of *Skeletonema costatum* (Greville) Cleve were cultivated in 12h light:dark cycle (light: h.09:00-21.00; dark: h.21:00–09:00). The cultivation medium was f/2 (Guillard; 1962), created from sterilized Mediterranean deep water (>200m) , enriched in nutrients (NO₃⁻ / PO₄²⁻ / SO₄²⁻), vitamins (biotin, chlorohydrate thiamin) and trace EDTA-complexes with metals (Zn, Mn, Mo, Co, Cu, Fe) (McLachlan, 1973).

Flasks containing the culture (Nunc 250ml, ventilated) were placed in an air-refrigerated cell, at the temperature of 18 °C ± 0.5. Growth light was provided by an array of six fluorescent tubes (Osram Sylvania, Cool-White) mounted together on a mobile stand, and connected to a timer. Light intensity was modulated for each experimental condition through the combined use of neutral filters (nickel screens, by Stork-Veco International) in front of the flasks, and distance of the lamps from the incubation cells. We experimented the growth rates of *Skeletonema c.* under six different light conditions: 25, 50, 100, 200, 300, 480-500 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (QSL100 quantameter , BioSpherical). Two batches of culture were kept at each light condition, for 6 days, and their growth monitored daily. We assumed the low-level fluorescence yields (Fo) as an indicator of biomass abundance. Fo was measured every 24 hours precisely to avoid endogenous variability of the signal, on 5 (five) replicates, by a Perkin Elmer LS650-10s spectrofluorometer, at excitation/emission wavelengths

438/682 nm. The specific growth rate of the population (r), expressed in units 1/day (1/d) , was calculated as:

$$r = \ln (F_t / F_o) / t \quad (2.1)$$

where:

F_o = the initial population biomass;

F_t = the population biomass at time;

t = time interval.

According to the results obtained, we were able to distinguish the ranges of PAR which correspond to limited, saturated and inhibited growth for *Skeletonema costatum*. A new set of nutrient replete batches of the culture was subsequently incubated at these irradiance intensities, which were found to represent the three basic states of the growth/irradiance relationship.

Each new batch was allowed seven days to develop a full acclimation to the experimental conditions. We began to perform measurements of the ETR_r/E relationship on the eighth day, collecting a fraction of the culture volume from each clone, every 2-3 hours (h. 06:00, 08:00, 10:00, 12:00, 15:00, 18:00, 20:00, 22:00, 24:00), for three days. All sampling activities were performed in a sterile environment to avoid contamination of the cultures.

2.2.2 -Pigments

The concentration of the liposoluble photosynthetic pigment chlorophyll-a (Chl-a), and accessory pigments chlorophyll-c (Chl-c), pheopigments (Pheo), total chloropheopigments (CHL), and total carotenoids (CAR) was determined after filtration of 30-40 ml of culture on Whatmann GF/F filters, fragmentation of the filters and 24h extraction in acetone (90% v/v). Following centrifugation, the acetone extracts were analyzed with a spectrophotometer (Uvikon 860, Kontron), according to Lazzara et al. (1997), using the equation sets given by Jeffrey & Humphrey (1975), and Lorenzen (1967), modified by Riemann (1978).

2.2.3 - The PAM measurement protocol for PE curve assessment

We measured the chlorophyll-a fluorescence yields of acclimated batches of *Skeletonema c.* through a pulse amplitude modulation fluorometer (PAM, Walz). A low-intensity blue probe-light (LED, with emission peak at 470 nm) provided excitation energy to PSII to measure chlorophyll fluorescence yield, in dark-adapted state (F_o) or in the presence of actinic light (F_o'). To perform PE curves, a xenon lamp (KL1500, Schott) provided actinic light, in the interval 0-1100 $\mu\text{E m}^{-2} \text{ s}^{-1}$, delivered in a stepwise progression of eleven levels (3, 15, 30, 40, 65, 90, 190, 270, 450, 650, 1100 $\mu\text{E m}^{-2} \text{ sec}^{-1}$), through manual alteration of the lamp potentiometer. After 90 seconds of exposure to each PAR level (acclimation), a second KL1500 lamp provided a saturation pulse of 800 $\mu\text{E m}^{-2} \text{ s}^{-1}$, for 800 ms. This flash determines the full saturation of reaction centers, which generates a large fluorescence signal from the PSII photo-systems (F_{max} , in dark conditions; F_{max}' , when actinic light is present). A delay of 100 ms was recognized in the discharge of the saturation flash, which was accounted for in the configuration for signals generation and detection. The detection unit of the fluorometer was equipped with a photo multiplier (PM101) for detection of low fluorescence emissions. The high sensitivity of this detector required the use of wavelength filters: a 3-mm thick high-pass filter (BG39, Schott, >700 nm) protected the detector from stray light; a short-pass dichroic filter (SP695, Walz) filtered the emission of the xenon lamps, to avoid saturation of the photomultiplier. The PAM 101-102-103 units controlled the electronics of the system, and allowed to synchronize the period of the light stimulations. The calculation and the recording of all the experimental signals were performed through the Wincont software (Walz). Maximum quantum yield of PSII (Y) is calculated, in dark conditions, as $(F_{\text{max}} - F_o) / F_{\text{max}}$. Effective quantum yield of PSII (Y'), referred to fluorescence emissions generated in presence of actinic light, derives from: $(F_{\text{max}}' - F_o') / F_{\text{max}}'$.

Samples collected from the culture were kept in a semi-dark condition until measurement time. Prior to any stimulation, they were allowed three minutes of total darkness to fully reduce photosystems. Three measures of maximum quantum yield were then performed on each sample, allowing 5 minutes in between each measure, for the sample to recover from the light-shock. Results were averaged. The PE curve was then performed according to a stepwise progression of eleven actinic irradiance levels

(as described above), allowing 90 seconds of acclimation to each light intensity. Three samples were run each time to allow significant statistics of the averages.

The effective quantum yield measurements (Y') were multiplied by the PAR intensity to produce an estimate of the relative electron transport rate (ETR) from PSII. The data were plotted against irradiance levels. We derived α' as the slope of the linear fit (intercept = 0) of our data in the range $0-50 \mu\text{E m}^{-2} \text{s}^{-1}$, which corresponded to the light-limited portion of the growth rate/growth light relation for the examined species. ETRmax was calculated as the average of ETR measurements at $270, 460, 650 \mu\text{E m}^{-2} \text{s}^{-1}$. The results of the ETR/E have been interpolated to produce a continuous curve, via an exponential fit, which mimics Platt's model (1980). For clarity, we will indicate the parameters of the PE curves derived from measurements of relative fluorescence yields as: α' , E_k' , P_{max}' .

2.2.4 - Statistical analysis

Quantum yields of PSII were measured three times for each sample, and results averaged. The light curves were also performed three times, renewing the sample after each curve; effective quantum yield of PSII resulting at each light intensity have been averaged. Daily spectrofluorimetric values of F_n , for the estimate of growth rates, resulted from averages of 5 measurements. We analyzed the variability of each data series through both the standard deviation (abbreviation in the text: stdev) and the ratio of the standard deviation by the mean value (abbreviation in the text: cvar). The periodicity of the diel variations in the PE parameters was tested with autoregression analyses (S-PLUS 6.0, Insightful) of the temporal series (62 h) of α' , ETRmax, and E_k' . Intervals (lags) between observations in the temporal series were 2 hours long. A few missing values were extrapolated by polynomial fitting of the temporal series.

2.3 RESULTS

2.3.1 - Limited, saturated and inhibited phase of growth.

Batches of *Skeletonema costatum* were cultivated at 25, 50, 100, 200, 300 and 500 $\mu\text{E m}^{-2} \text{s}^{-1}$. In the range 25-300 $\mu\text{E m}^{-2} \text{s}^{-1}$, the average growth rates of the culture showed a clear tendency to increase with light availability, from 0.70 div./d to 1.59 div./d (Tab. 2.1). The average rates slowed down dramatically to 0.87 div./d for batches cultivated at 500 $\mu\text{E m}^{-2} \text{s}^{-1}$. The variability of these measures (n=25) over 5 days is very low, between 1.6-5.7% (Tab. 2.1).

PAR ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Growth rate (div./d)	C.var.	n
25	0.70	0.05	5
50	1.22	0.02	5
100	1.51	0.05	5
200	1.58	0.05	5
300	1.59	0.06	5
480	0.87	0.05	5

Tab. 2.1 – Average growth rates (divisions/day) of nutrient-replete axenic cultures of *Skeletonema costatum*. Batches were cultivated under different PAR irradiance intensities (25, 50, 100, 200, 300, 480 $\mu\text{E m}^{-2} \text{s}^{-1}$), for seven days. Growth rates were subsequently measured every 24h, for five days. Number of sample (n) is 5 and the variability of the averages (cvar) is $\leq 6\%$.

Average values of the growth rates were distinct for each light condition. Plotted against irradiance levels, growth rates describe a typical growth/irradiance relationship (Fig. 2.1). The condition of light-limited growth is clearly revealed for cultures grown in the range 0-50 $\mu\text{E m}^{-2} \text{s}^{-1}$, where the relation between growth rate and light-acclimation intensity appears almost perfectly linear, and increasing (Fig. 2.1). A linear fit of these data points, passing through the origin of axes, shows a regression coefficient (r) always > 0.979 (data not shown). The slope of the relationship decreases significantly in the range 50-100 $\mu\text{E m}^{-2} \text{s}^{-1}$ and it eventually reaches a plateau, between 200 and 300 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Fig. 2.1). Growth rates of this diatom appear therefore light-saturated in the whole range 100-300 $\mu\text{E m}^{-2} \text{s}^{-1}$. We calculated a growth-saturation constant (KE) of ca. 60 $\mu\text{E m}^{-2} \text{s}^{-1}$. The number of division/day recorded for the batch acclimated at 500 $\mu\text{E m}^{-2} \text{s}^{-1}$

$^2 \text{ s}^{-1}$ produced a negative relationship in the range 300-500 $\mu\text{E m}^{-2} \text{ s}^{-1}$, which therefore corresponds to the growth-inhibiting light range. A more extensive experiment should especially reconsider the upper limit of this range, since we could not verify the growth/irradiance relationship of *Skeletonema costatum* in intermediate cultivation conditions, between 300-500 $\mu\text{E m}^{-2} \text{ s}^{-1}$.

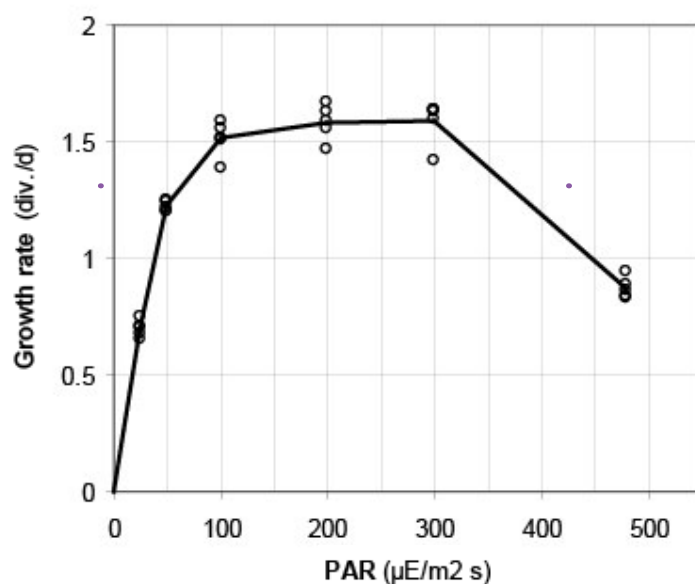


Fig. 2.1 - Growth rates (divisions/day) of nutrient-replete axenic cultures of *Skeletonema costatum*. Batches were cultivated under different PAR irradiance intensities (50, 100, 200, 300, 480 $\mu\text{E m}^{-2} \text{ s}^{-1}$), for seven days. Growth rates were subsequently measured every 24h, for five days. They are presented (o) here as a function of the acclimation irradiance intensity. The continuous line refers to the average value.

2.3.2 - Pigment composition

Samples from the preliminary phase showed chloropigments (CHL) concentration in the range 215-410 mg/m^3 . Differences in the pigment-pool composition of the various batches revealed the effect of photo-acclimation, with consideration of both pigments content per cell (Fig. 2.2.a) and pigment ratios (Fig. 2.2.b; Tab. 2.2).

PAR ($\mu\text{E}/\text{m}^2 \text{ s}$)	CHL (1) (mg/m^3)	CHL (2) (mg/m^3)	Chl-c (2) (mg/m^3)	CAR (2) (mg/m^3)	Chla-a (3) (mg/m^3)	Pheo (3) (mg/m^3)	N (cell/ml)
500	214.89	214.45	30.09	177.39	184.96	33.57	797,000
300	248.08	247.44	42.70	190.22	216.30	34.09	868,000
200	300.36	299.51	56.06	201.92	279.08	11.94	1,220,000
100	350.73	349.66	70.44	203.82	326.60	12.76	1,020,000
50	387.80	386.38	92.42	160.61	360.67	14.58	686,000
25	406.51	405.16	88.25	170.38	386.59	1.15	569,000

Tab. 2.2 - Average content of photosynthetic and accessory pigments for batches of *Skeletonema costatum* acclimated to different PAR intensities (25, 50, 100, 200, 300, 480 $\mu\text{E}/\text{m}^2 \text{ s}$). CHL=chlorophaeopigments; Chl-a= Chlorophyll-a; Chl-c= Chlorophyll-c; Pheo=Pheophytines; CAR=Carotenoids; N= number of cells per ml of culture.

The content of Chl-a per cell at 25 $\mu\text{E m}^{-2} \text{ s}^{-1}$ is nearly three fold that at 500 $\mu\text{E m}^{-2} \text{ s}^{-1}$, decreasing progressively from 0.71 pg/cell to 0.27 pg/cell, with an inverse relationship. Sosik et al. (1989) and Falkowski et al. (1985) report a similar three-fold variation, over a range of irradiance 10-600 $\mu\text{E m}^{-2} \text{ s}^{-1}$, for the diatom *Thalassiosira weissflogii*. According to the same authors, absolute values of Chlorophyll-a concentration in the cells were in the range ca. 13-3 pg Chl-a/cell, one order of magnitude higher than ours. This might be due to different culture density and to inter and intra specific differences (MacIntyre et al., 2002), e.g. the cell volume. The ratio Chl-c/Chl-a shows an opposite trend, from a maximum value of 0.23, at 25 $\mu\text{E m}^{-2} \text{ s}^{-1}$, to a minimum 0.16 at 500 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (Fig. 2.2.b), revealing a decreasing contribution of this accessory chlorophyll to the light-harvesting process, as acclimation PAR increases. The absolute values of Chl-c content per cell show similar trends to Chl-a variations (Fig. 2.2.a), but their absolute values are on average five times lower. The concentration of total carotenoids (CAR) is stable with increasing PAR. In cells acclimated to the lower PAR intensities, carotenoids are almost as abundant as chlorophaeopigments (44%). The ratio CAR/CHL raises steadily with increasing irradiance, up to 98% at 500 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (Fig. 2b), but only due to the decrease in Chl-a concentration. The effect of high levels of irradiance is also evident from the analyses of pheophytin concentration. The presence of this degraded material never represents more than 16% of the total CHL pool, but it remains below 5% for all the batches cultivated at PAR < 300 $\mu\text{E m}^{-2} \text{ s}^{-1}$. It increases to 13.4% and 15.4% respectively at 300 and 500 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (Tab. 2).

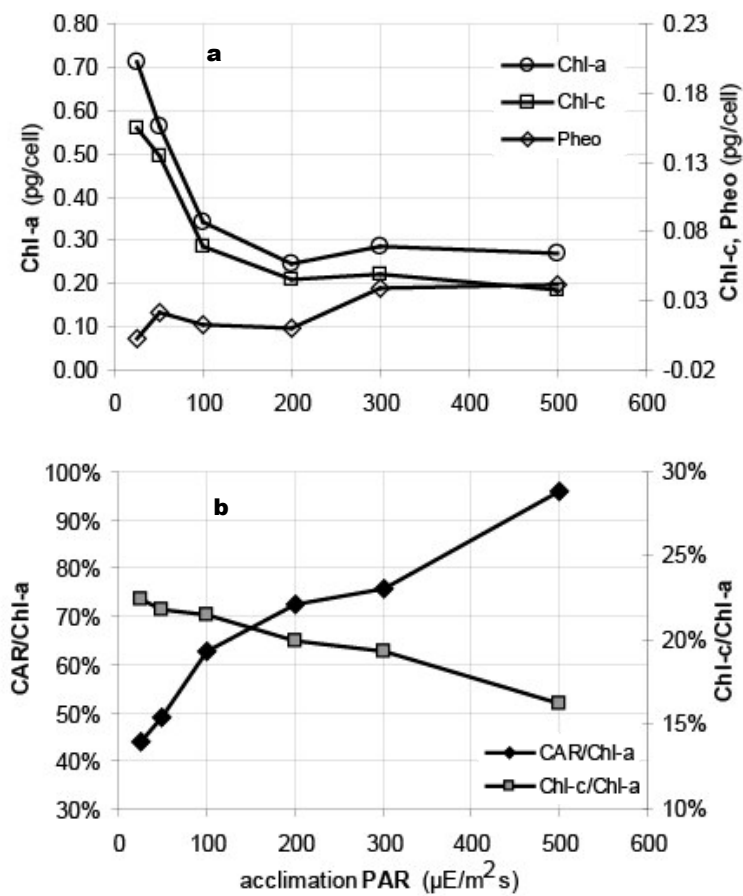


Fig. 2.2 - a) Average per cell content of Chlorophyll-a (Chl-a), Chlorophyll-c (Chl-c) and Pheophytins (Pheo) in *Skeletonema costatum* as a function of growth light conditions; **b)** Pigment ratios (%)

2.3.3 - PE-curve parameters: α' , E_k' , ETRmax.

Condition of photo-limited growth ($25 \mu\text{E m}^{-2}\text{s}^{-1}$)

Average ETRmax is $120.31 \mu\text{E m}^{-2}\text{s}^{-1}$, and varies by ca. plus or minus 20% ($95\text{--}157 \mu\text{E m}^{-2}\text{s}^{-1}$), over the experimental period (62 hours). Max values of ETR, around $160 \mu\text{E m}^{-2}\text{s}^{-1}$, were recorded each day at midday (Fig. 2.3.a), which is four hours after the onset of the light cycle. Minimum values are found during the night time (h24:00, h06:00), reduced by 40% (ca. $95 \mu\text{E m}^{-2}\text{s}^{-1}$) (Fig. 2.3.a), and with less variability (11–4%) (Tab. 2.3). Reaction centers should all be in the reduction state during the nighttime. This homogenous state can account for the minor variability we found in the nighttime measurements of the fluorescence yield (Tab. 2.3). E_k' -25 has an average value of $197 \mu\text{E m}^{-2}\text{s}^{-1}$ (Tab. 2.3), with significant periodicity for the 6th lag (1lag = 2h), and an autocorrelation factor (ACF) of -0.58 (Fig. 2.5). E_k' -25 reaches maximum values three hours after the beginning of each day (h 12:00).

The average value of α' 25 is 0.611, with very little variability (Tab. 2.3) over the total experimental period (cvar=1.48%), and for each sampling time (cvar< 5%, n=10-15). Besides being almost constant throughout the 24 hours, α' 25 is a rather high value, with respect to the other experimental conditions (Fig. 2.4.b). Given the constancy of α' , the diel variation of E_k' is rather related to that of ETRmax (Fig. 2.4.a). Autoregression analyses confirmed the absence of periodicity in α' , with autocorrelation factors always <0.5 (Fig. 2.5).

Condition of photo-saturated growth ($300 \mu\text{E m}^{-2}\text{s}^{-1}$)

The analyses of the PEcurves measured on the clone cultivated at light-saturating conditions showed a smaller α' than in records for cultivation intensities of $25 \mu\text{E m}^{-2}\text{s}^{-1}$ (Fig. 2.4.b). Its average value of 0.47 varies by 11% throughout the day, which is a rather small percentage in comparison with the variability of ETRmax and E_k' , by around 50% (Tab. 2.3).

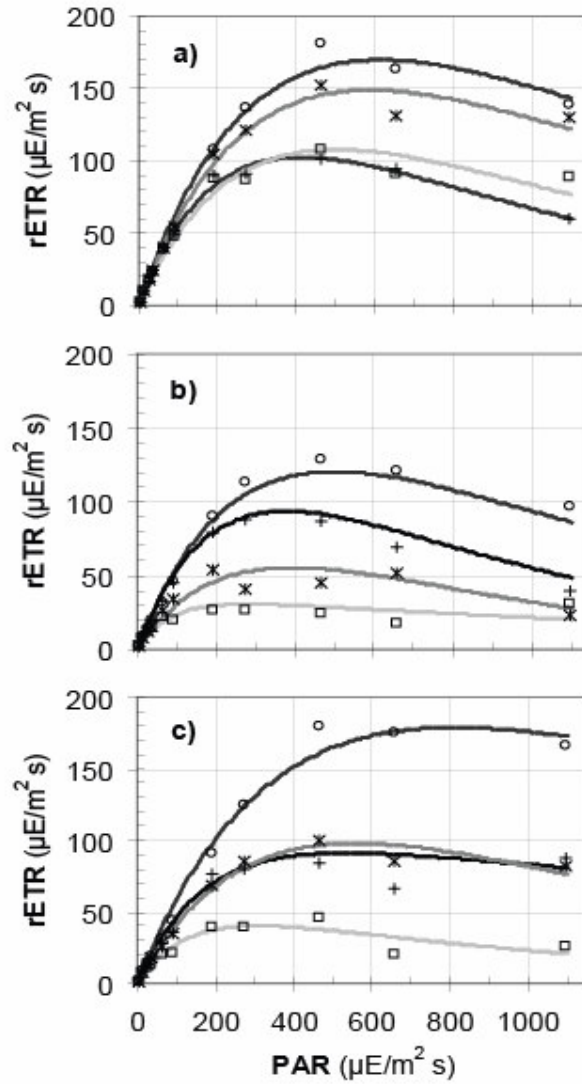


Fig. 2.3 – Curves of relative electron transport rate (ETR) as a function of increasing actinic light intensities. In the diagram, we report 3-day averages, measured at h06:00 (+), h12:00 (○), h18:00 (*), h24:00 (□). The continuous line is a fit from Platt model (1980). A) *Skeletonema c.* acclimated to photo-limiting light (25 $\mu\text{E m}^{-2} \text{ s}^{-1}$; B) batches acclimated to photo-saturating light (300 $\mu\text{E m}^{-2} \text{ s}^{-1}$; c) batches acclimated to photo-inhibiting light (500 $\mu\text{E m}^{-2} \text{ s}^{-1}$). Measures at h06:00 and h24:00 are relative to the dark period of the L:D cycle (12:12h).

The average values of ETRmax and E_k' , over the 62 hours, are $72.37 \mu\text{E m}^{-2} \text{s}^{-1}$ and $146.44 \mu\text{E m}^{-2} \text{s}^{-1}$, respectively (Tab. 2.3). These parameters are also lower than in the light-limited batches.

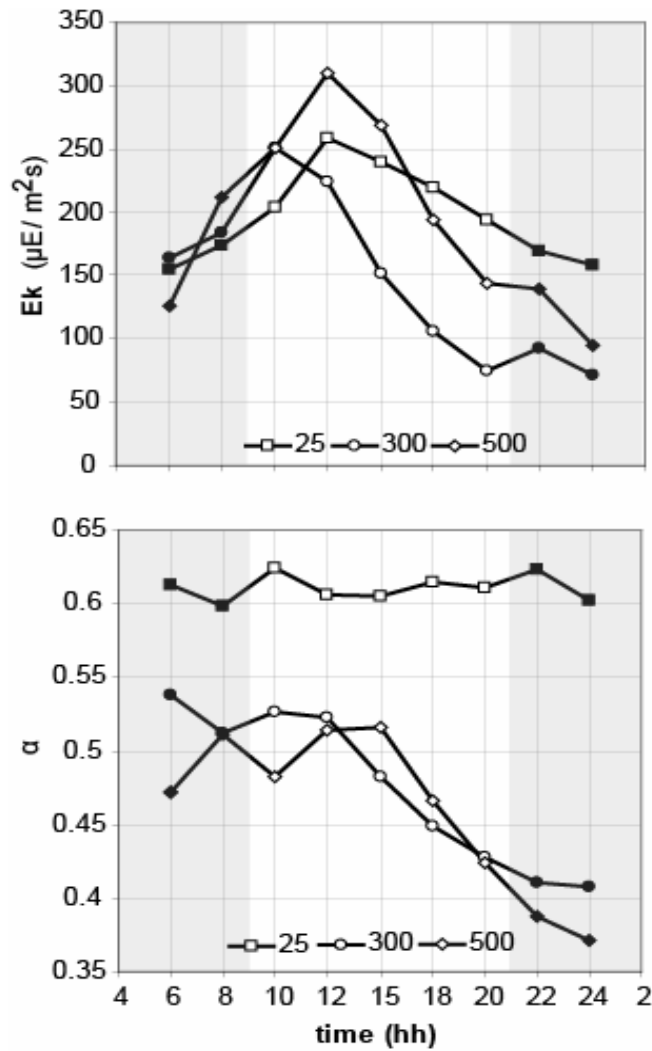


Fig. 2.4 a, b – Diel variations of the ETR/E parameters, for batches of *Skeletonema c.* grown at 25, 300, 500 $\mu\text{E m}^{-2} \text{s}^{-1}$. Each data point represent the average of the measurements, at a certain time of the day, over three days. Dark symbols refer to measurements performed during the dark period of the L:D cycle (12:12 hours). A) Photo-acclimation index of the ETR/E relationship, E_k' ; B) slope of the light-limited portion of the ETR/E relationship, α' .

In detail, ETRmax varies along the 24h, from minimum values around $30 \mu\text{E m}^{-2} \text{s}^{-1}$, at the onset of the dark cycle, to max values of $131.64 \mu\text{E m}^{-2} \text{s}^{-1}$, at h10:00, only one hour after the onset of light. The highest periodicity for α' and E_k' is on lag 6 (12 hours), with ACF=-0.61 and 0.579 respectively. In these experiments, variations of E_k' (fig. 4,b) are contributed by the summed effect of parallel variations of α' and ETRmax. For this reason, even with minor average values of ETRmax during the 24h, $E_k'_{300}$ can be as high as $E_k'_{25}$, around $150 \mu\text{E m}^{-2} \text{s}^{-1}$.

Condition of photo-inhibited growth ($500 \mu\text{E m}^{-2} \text{s}^{-1}$)

The average slope of the light-limited phase of the ETR/E relationship (α') is 0.46, similar to α'_{300} (tab. 2.3). Variation of α' during the day is slightly larger, from 0.37 at the end of the day (h24:00) to maximum values around 0.50 at midday. Periodicity is evident for lag 6 and 12, with ACF>0.5 (Fig.2.5.c). This parameter co-varies regularly with ETRmax, which shows an intermediate average value ($92 \mu\text{E m}^{-2} \text{s}^{-1}$) between ETRmax-25 ($120 \mu\text{E m}^{-2} \text{s}^{-1}$) and ETRmax-300 ($72 \mu\text{E m}^{-2} \text{s}^{-1}$) (tab. 2.3). This co-variation produces a larger maximal E_k' , at midday, than for the other batches. On the average of the measures performed in three days, the value $E_k'_{500}$ is very similar to $E_k'_{25}$ (Tab. 2.3), as a result of the combination of a low light-utilization efficiency and an intermediate ETRmax. All the three parameters show a clear periodicity at lag=6 (12h), with ACF > 0.5 (Fig. 2.5.c, f).

PAR	Time hh	α'	CVAR	r^2	n	Ek'	CVAR	ETRmax	CVAR	n
25	06	0.613	4.15%	0.997	10	155	21.4%	95	25.4%	2
	08	0.598	0.00%	0.998	10	173	20.7%	104	20.7%	2
	10	0.624	3.65%	0.998	15	204	17.7%	128	19.6%	3
	12	0.606	5.12%	0.996	15	258	6.7%	157	10.8%	3
	15	0.605	3.96%	0.998	15	240	2.7%	145	6.7%	3
	18	0.615	0.00%	0.998	15	219	7.0%	135	7.0%	3
	20	0.611	0.59%	0.998	15	194	3.8%	118	3.9%	3
	22	0.623	3.18%	0.997	10	169	7.0%	105	10.2%	2
	24	0.602	1.83%	0.997	15	158	2.6%	95	4.2%	3
average	daily	0.611	1.48%	0.997	120	197	18.69%	120	18.57%	24
300	06	0.538	4.34%	0.997	8	164	6.9%	88	11.2%	2
	08	0.512	2.52%	0.997	12	183	14.2%	94	14.8%	3
	10	0.526	2.38%	0.997	12	250	3.7%	132	1.5%	3
	12	0.523	1.17%	0.995	12	224	5.8%	117	6.8%	3
	15	0.482	7.43%	0.995	12	151	21.8%	74	28.2%	3
	18	0.448	4.51%	0.981	12	105	5.0%	47	5.6%	3
	20	0.428	6.07%	0.977	12	75	11.6%	32	17.2%	3
	22	0.410	5.77%	0.974	12	93	26.7%	38	24.5%	3
	24	0.408	0.62%	0.986	12	71	24.8%	29	25.4%	3
average	daily	0.475	11.02%	0.989	104	146	44.44%	72	52.54%	26
500	06	0.472	-	0.996	4	126	-	60	-	1
	08	0.512	0.14%	0.996	8	212	26.1%	108	26.3%	2
	10	0.482	-	0.996	4	251	-	121	-	1
	12	0.514	7.98%	0.993	8	310	11.0%	160	18.9%	2
	15	0.516	5.71%	0.994	12	268	5.6%	138	7.8%	3
	18	0.466	-	0.992	4	194	-	90	-	1
	20	0.424	5.05%	0.986	12	144	6.4%	61	1.4%	3
	22	0.387	0.15%	0.971	12	140	13.2%	54	13.1%	3
	24	0.371	5.71%	0.958	12	95	13.4%	35	15.9%	3
average	daily	0.460	11.85%	0.987	76	193	37.61%	92	46.34%	19

Tab.2.3 – Summary of the parameters derived from the ETR/E relationships for *Skeletonema costatum* acclimated to photo-limiting, photo-saturating and photo-inhibiting PAR irradiance, respectively 25, 300 and 500 $\mu\text{E m}^{-2} \text{s}^{-1}$. The curves were measured at two or three hours intervals, for 62h, allowing three replicates. The table presents the average values of the ETR/E parameters: α' , Ek' and ETRmax, with their variability (CVAR). Daily averages and variability are reported at the bottom of each experimental condition.

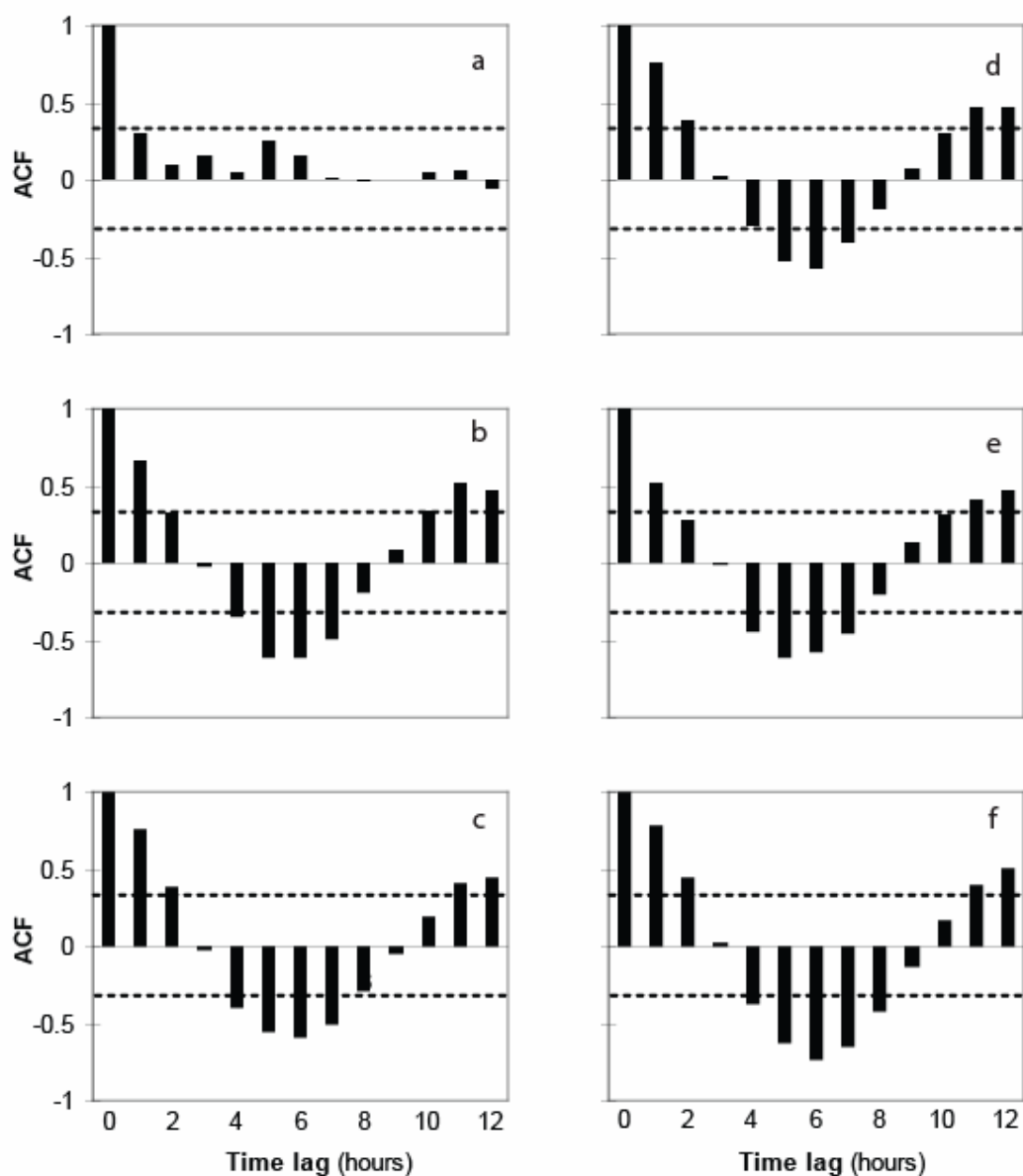


Fig. 2.5 – Analysis of the periodicity of the average diel variations of photosynthetic parameters in *Skeletonema c.*, derived from fluorometric PE curves, assessed every two hours, over a total 62-hour period. Batches of cells were cultivated with a 12-hour L:D cycle, in different light conditions, between 25 and 500 $\mu\text{E m}^{-2} \text{s}^{-1}$. The autocorrelation analyses is performed on a 24 hours range, with two-hour lags. Analyses on α' , at growth-light 25, 300 and 500 $\mu\text{E m}^{-2} \text{s}^{-1}$ are reporter in A,B and C, respectively. Analyses of corresponding E_k' values are in D, E and F. The threshold for significance in the autocorrelation factor (ACF) is ± 0.3 .

2.4 DISCUSSION

In our experiments, maximum growth rates of *Skeletonema costatum*, around 1.6 div./d, are found at growth-light intensities of 200-300 $\mu\text{E m}^{-2} \text{s}^{-1}$, with constant temperature of 18 degrees C. These conditions appear close to the optimum for this diatom. For the same species, Suzuki and Takahashi (1995) report optimal growth temperature at 25 °C, but maximum growth rates were 1.7 div./d, at 20 °C, with 200 $\mu\text{E m}^{-2} \text{s}^{-1}$ PAR. Under these conditions, the photoacclimation index E_k , was ca. 70 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Suzuki and Tagahashi, 1995), similarly to our observations of $E_k=60-70 \mu\text{E m}^{-2} \text{s}^{-1}$. Juneau and Harrison (2005) report μ_{max} (1/d) for *Skeletonema costatum* of 1.18, at 130 $\mu\text{E m}^{-2} \text{s}^{-1}$. In Nielsen and Sakshaug, (Limnol. Oce. 1993) *Skeletonema sp.* has a growth rate of 1.7-2 div/d at 15°C with L:D cycle 18:6 and in Anning et al. (2000) *Skeletonema sp.* has 1.6 div/d at 1200 $\mu\text{E m}^{-2} \text{s}^{-1}$. Langdon (1987) reports ca. 2.4 div./d for growth rates of *Skeletonema costatum*, between 200 and 480 $\mu\text{E m}^{-2} \text{s}^{-1}$ 30% higher than ours. He also calculates a growth-light efficiency (α_g) of 0.025 div. d-1/ $\mu\text{E m}^{-2} \text{s}^{-1}$ and a carbon specific $\alpha_g C$ of 0.18 d-1/ $\mu\text{E m}^{-2} \text{s}^{-1}$, calculated in the range 0-66 $\mu\text{E m}^{-2} \text{s}^{-1}$ of the growth/irradiance relationship. In our study, these values find a correspondence with an α_g = of 0.025 div. d-1/ $\mu\text{E m}^{-2} \text{s}^{-1}$, in the range 0-50 $\mu\text{E m}^{-2} \text{s}^{-1}$.

As a result of photo-acclimation, the eukaryotic cellular light-harvesting potential is expected to progressively reduce with growth light increase (Sosik et al., 1989; Kirk, 1994; MacIntyre et al., 2002). In our study, the contribution of chlorophylls to the cellular photosynthetic pigment pool of *Skeletonema c.* declines over the experimented growth-light interval (25-500 $\mu\text{E m}^{-2} \text{s}^{-1}$), both in Chl-c concentration, by 30%, and in Chl-a, by nearly 50%, with similar trends. As a consequence, the Chl-c/Chl-a ratio also decreases with increasing growth irradiance, as expected for many other species of diatoms (Jeffrey, 1972). In batch cultures of *Skeletonema c.*, this ratio was found to vary, from 0.53 at extremely low light intensity, to 0.18 at 130 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Falkowski et al., 1985). In our study, this ratio decreases comparably, from 0.23 at 25 $\mu\text{E m}^{-2} \text{s}^{-1}$ to 0.16 at 500 $\mu\text{E m}^{-2} \text{s}^{-1}$ (fig. 2b). Conversely, the total CAR to Chl-a ratio increases by nearly 50% with growth irradiance, but this is almost entirely due to the variation in Chl-a concentration, as total-carotenoids concentration per cell does not

show significant variation with acclimation PAR. According to the adopted methods, we could not distinguish here between photosynthetic accessory carotenoids (PSC) and photo-protective xanthophylls (PPC). We hypothesize that photosynthetic carotenoids are replaced by the photo-protective type at increased growth-light. Anning et al. (2000) describe for *Skeletonema c.* a rapid and opposite variation in the cellular content of PPC (diadinoxanthin and diatoxanthin) and PSC (fucoxanthin), upon a shift in acclimation light levels (from 1200 to 50 $\mu\text{E m}^{-2} \text{s}^{-1}$). According to these authors, this result supports the hypothesis that PSC will be synthesized from PPC, which act like precursors for fucoxanthin, as also suggested by other authors (Goericke and Welshmeyer, 1992). Phaeopigments increase over Chlorophyll concentration, as a function of growth light. The Ratio Phaeo/Chl-a is four times higher at 500 $\mu\text{E m}^{-2} \text{s}^{-1}$ than at 25 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Tab.1). This fact should account for degradation of Chl-a upon prolonged exposure to high light levels (500 $\mu\text{E m}^{-2} \text{s}^{-1}$), which are ca. 5 fold the assessed average KE (2.8 fold E_k') for this diatom species.

Photoacclimation modifies the shape of the PE curve by acting independently both on α and P_{max} (Behrenfeld et al. 2004). The contribution of these variables to the light-saturation index ($E_k = P_{\text{bmax}}/\alpha b$) should bring variation in the magnitude of E_k during the photoacclimation process. This makes of E_k a recognized photoacclimation index, and the effect of photoacclimation on the PE relationship is thus defined as “ E_k -dependent” (Behrenfeld et al. 2004). In our study, the average ($n=81$) light semi-saturation constant, E_k' , is very similar for batches grown at 25 and 500 $\mu\text{E m}^{-2} \text{s}^{-1}$; it is some 25% lower in the experiments at 300 $\mu\text{E m}^{-2} \text{s}^{-1}$. To use these values as a pure photoacclimation measure, we would find some difficulties: it would seem that our batches did not really experience such a different environment, or that acclimation did not modify their photosynthetic performance in a strong way. On the contrary, pigment analyses confirm that growth light influenced the pigment pool of the different batches. The similarity in the numerical values of E_k' may indicate a full acclimation, or an optimal steady state of photosynthesis. In this sense, an average value of E_k' might be considered as a photoadaptation index, or a synthetic expression of the phototrophic characteristics of phytoplankton groups, as modeled by phylogenesis. For comparison, in Bruyant et al. (2005), diel values of E_k' measured on *Prochlorococcus spp.*, range between ca. 200 and 450 $\mu\text{E m}^{-2} \text{s}^{-1}$, over a daily PAR variation on 0-1000 $\mu\text{E m}^{-2} \text{s}^{-1}$.

This diel variation leads the authors to confirm that Ek contains a photoacclimation information. In our case, despite differences in the absolute values, Ek' shows a similar diel excursion. We can conclude that Ek' is a photoacclimation index, which responds to variation of light availability quickly (we could only resolve the temporal scale down to hours).

The range of the variations of Ek', or say its average value over a natural excursion of actinic light conditions, appears instead as a good indicator of photoadaptation, rather than photoacclimation. Sun-adapted phototrophic microorganisms like *Prochlorococcus spp.* are found in surface waters throughout the planet and are often the most abundant group in high irradiance - low chlorophyll environments, like the subtropical Atlantic and Pacific Gyres (Andersen et al., 1996 Sweeny et al, 2003). Diatoms instead can dominate the poorest light environments, like the pack ice-ice assemblages, in Antarctica (Arrigo K., 2003; Thomas D.N., 2004, Lazzara et al., in press). In a series of experiments on nine phytoplankton species grown under identical conditions, *Skeletonema costatum* showed saturated photosynthetic rates at only 280 $\mu\text{E m}^{-2} \text{s}^{-1}$, far below the saturation intensities required by the other, sun-adapted species, around 1,300 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Juneau et Harrison, 2005). On these basis, we can adopt the average value of Ek' to quantify phototadaptation, i.e. the photosynthetic characteristic of a given species taxonomic group as defined by phylogenesis.

In our experiments, a clear indication of the onset of the photoacclimation process comes through the analyses of the pairs of Ek' and α' , or ETRmax and α' . α is the most reliable parameter when comparing fluorescence based PE parameters with traditional ones (Hawes et al., 2003). The value of α is proportional to the size of the antenna of PSII (Sauer et al., 2001; Juneau and Harrison, 2005). Shade-tolerant and shade-acclimated cells possess a larger α , to efficiently exploit low solar radiation for photosynthesis. Their antenna size is accordingly large, to absorb as much radiation as possible from a chronically oligophotic environment. On the average, in our experiments, α'_{25} is 0.610 (average daily variability ca. 3%), α'_{300} is 0.473 (cv=11%) and α'_{500} is 0.451 (cv=14%). The size of the PSII antenna can be represented here as the sum of the photosynthetic and accessory pigments, except for carotenoids, as the method did not allow discrimination between PSC and PPC. In our experiments, the concentration of these pigments decreases progressively from 0.87 pg/cell at 25 $\mu\text{E m}^{-2}$

s^{-1} , to 0.35 pg/cell at 500 $\mu E m^{-2} s^{-1}$. In addition, at 300 and 500 $\mu E m^{-2} s^{-1}$, α' and ETRmax appear highly correlated (respectively: $r= 0.902$, $n=26$; $r= 0.896$, $n=19$. Data not shown). At 25 $\mu E m^{-2} s^{-1}$, though, α' and ETRmax do not covary ($r=0.373$, $n=26$). In fact, α' is very stable (cvar = 2.5%) around its mean value, while ETRmax shows a typical trend: it increases during the final hours of the night, reaches a peak in the morning around midday, decreases to a minimum late in the photoperiod or at the beginning of the night (Prezelin 1992, Berhenfeld et al. 2004). Our analyses therefore agree with the definition of photoacclimation as a process that independently affects α and Pmax, when irradiance levels (E) are growth-limiting; the effect of non-limiting growth irradiance would rather generate Ek independent (sensu Berhenfeld) variations of the PE' parameters. According to Geider et al. (1985), irradiance is growth-limiting when it is inferior or equal to 2KE. Under these conditions, the maximum quantum efficiency of photosynthesis is also often constant in cultures (MacIntyre et al., 2002, Morel et al., 1987).

The diel changes in the biomass dependant light-limited and light saturated rate of photosynthesis usually show a rather high variability (Harding et al., 1981; Behrenfeld et al., 2004). The sampling frequency of our experiments was 2-3h to allow a proper characterization of the cycle amplitude and period for the variables α' , Ek', ETRmax. With the exception of α' at 25 $\mu E m^{-2} s^{-1}$, Ek' and α' varied with the time of the observations with a significant periodicity, which defines the rhythm of electron transport activity within the imposed diel 12:12 L:D cycle.

CHAPTER 3.0

REFINEMENT OF A BIO-OPTICAL MODEL (PHYTO-VFP) OF PRIMARY PRODUCTION, AND ITS REGIONALIZATION FOR APPLICATION IN CASE I COASTAL WATERS

3.1. BACKGROUND

The use of optical techniques to monitor temporal and spatial variation of seawater properties, in conjunction with traditional methods, is the most advanced method to assess marine biomass standing stock and primary production. A number of bio-optical models of primary production have been developed in the past twenty years (Kolber and Falkowski, 1993; Platt, 1989; Hartig et al., 1998, Morel and Berthon, 1989), but these all lack to account for the continuous photo-physiological adjustment (acclimation) that phytoplankton perform in their spatial dislocation and life cycle.

We have refined a model of PP designed in 1997 by Marcelli, Lazzara and D'Alcala', and thus developed the Phyto-VFP: Phytoplankton Variable Fluorescence Production model (Marcelli et al., 2003). This new model accounts for the variations of the main factors regulating the primary production process, i.e.: phytoplankton biomass, light availability in the water column, efficiency of energy conversion, and photoacclimation. The model also accounts for the light-field modifications in the marine environment, as a function of three space dimensions (latitude, longitude, depth), and of time. Phyto-VFP models the influence of light availability on the photophysiology of phytoplankton (acclimation), in consideration of the continuous spatial dislocation of microalgae. Estimates of PAR in the water column can either derive from empirical *in situ* measurements, above or below the water surface, or from the theoretical values of the energy flux at the sea surface as a function of sun

angle and elevation. The model's algorithms allow the estimate of the instantaneous primary production rate (PP), at every depth of the water column, as well as its integrated value, either over depth or time.

In a validation effort, we applied the Phyto-VFP model to an extensive dataset from four years of bio-optical oceanographic campaigns in the Channel of Sicily, on board R/V Urania (CNR), in the framework of the SYMPLEX (Synoptic Mesoscale Experiment) project, funded by the Italian Space Agency (ASI). The cruises allowed the survey of an extensive portion of the Channel of Sicily, in different seasons, during the years 1996-1999. SYMPLEX1 took place in the spring 1996 (April 12-May 12); SYMPLEX2 in mid-summer 1997 (July 21 –August 10); SYMPLEX3, again in springtime, in 1998 (March 28 – April 16); SYMPLEX4, in the fall of 1999 (October 21 – November 5). We have examined the available data from these campaigns with the scope to apply the PHYTO-VFP model. On the basis of the assessed biogeochemical properties of the study area, and with the use of a GIS system (Arcview 3.2) and other mapping software (ODV), we discriminated various bio-geographic subsets, or trophic regions. We applied the Phyto-VFP model to some sample stations from each subset, and used the seasonality of our oceanographic campaigns to describe a full-year cycle of primary production in the area.

3.2 METHODS

3.2.1 Study area

The Channel of Sicily is an intermediate basin, that connects the western and the eastern portions of the Mediterranean Sea. Using a model for water exchanges and sea level adjustments, Garret (1983) firstly stressed the importance of this channel in controlling the large scale circulation of the Mediterranean.

Many oceanographic surveys have been carried out in this area after the first investigations conducted by Saclant in 1972. As a consequence, the general characteristics of currents are relatively well known. Especially for the western side of the channel, the Strait of Sicily, various authors have estimated the fluxes and the temporal and spatial variability of the water masses (Ozturgut, 1975; Grancini and Michelato, 1987; Manzella et al., 1988; Manzella, 1994), though no definitive results have been obtained to date because of extreme variability and complexity of phenomena, occurring at different space and time scales.

The Channel of Sicily has a trapezoidal shape. The western side is 150 km wide with a mean depth of approximately 200 m. Two narrow sills of 430 m and 365 m make up the only deep connections to the western basin. The eastern side is much wider, extending for more than 500 km, but its mean depth does not exceed 200-300 m, except for the passage between Medina Bank and the Island of Malta (Eastern Sill, 560 m).

The channel is substantially a two-layer system. The fresher water of Atlantic origin (Modified Atlantic Water: MAW) lies in the upper layer, while the more saline Levantine Intermediate Water (LIW) occupies the lower one (Manzella et al., 1990). These two waters are separated by an intermediate transition layer, which varies in thickness between 30 m during winter and 100 m in summer (Morel, 1972).

The Atlantic water flow is composed by energetic mesoscale disturbances superimposed to the main current, subjected to seasonal variability (Bohm et al. 1998). During the summer, thermal fronts associated to upwelling phenomena along the South and East coast of Sicily are particularly evident (Fig.3.1). On the other

hand, LIW characteristics do not show particular seasonality. This water mass occupies the whole basin below 200 m (Ozturgut, 1975; Manzella et al., 1988), moving slowly westward (Grancini and Michelato, 1987). The characteristics of the water mass appear modified between the Eastern and Western part of the basin, probably due to the intense mesoscale activity observed on the Eastern side of the Channel by satellite imageries (Fig. 3.1), which calls for a significant vertical transport of salt and nutrients.

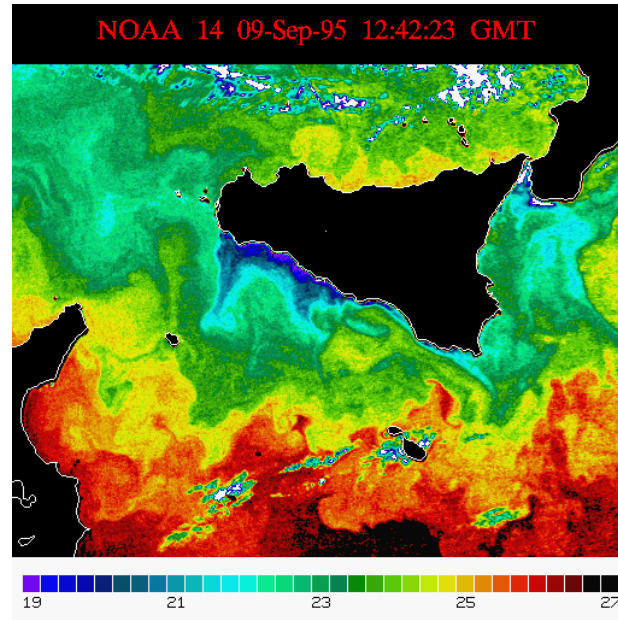


Fig. 3.1.: Channel of Sicily. Summer SST image of the Channel of Sicily

3.2.2 ALT/SYMPLEX experiment

The hydrological data analysed in this contribution were collected during the joint ALT and SYMPLEX cruises: ALT-SYMPLEX 1 (April 12th – May 13th, 1996) ALT-SYMPLEX 2 (July 20th – August 11th, 1997), ALT-SYMPLEX 3 (March 27th– April 20th, 1998), and ALT-SYMPLEX4 (October 21st – November 5th , 1999), on board R/V Urania (CNR), in the Sicily Channel.

The ALT experiment was part of a research project approved by the European Space Agency (ESA) in the framework of the Announcement of Opportunity for

ERS-1 and ERS-2. It had the objective of studying the relationship between altimetric sea surface signals and 3-D structures of the ocean. Similarly, the Synoptic Mesoscale Plankton Experiment (SYMPLEX) is part of the NASA/SeaWiFS program, as organized through the Announcement of Opportunity for SeaWiFS (Sea wide field of View satellite sensors). Its main goal was to study the relation between the surface fields visible by remote-sensing images of ocean color and the internal bio-physical dynamics of the ocean.

The ALT/SYMPLEX surveys were designed in order to satisfy the main objectives of the two experiments. The measurement were carried out in the eastern part of the Sicily Channel, where an intense mesoscale variability associated with a cold filament-like structure is observed in altimetric, infrared (Fig. 3.1), and colour satellite imagery. Observational strategy was based on repeated sampling at relatively high frequency (hours) of mesoscale eddies, when detected, or hydrodynamic structures, i.e. filaments, frontal instabilities, etc.

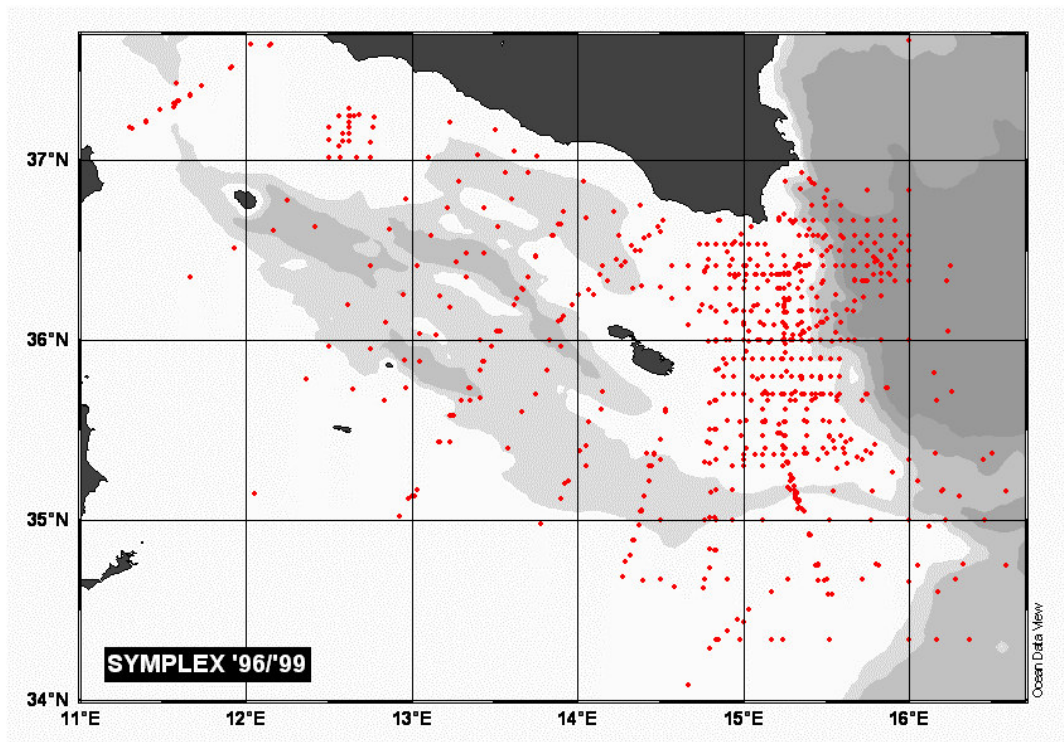


Fig. 3.2 Channel of Sicily. Map of the study area, with all (n=806) stations sampled during the SYMPLEX campaigns (red dots).

A wide and integrated set of hydrological, dynamical, optical, chemical and biological data was collected to characterize this region (Fig.3.2), through measurements of: temperature, salinity, density (CTD casts); underwater spectral irradiance field (Licor LI-1800UW, Satlantic OCI200); profiles of PAR (“Fotosonda Innamorati”, prototype, 1994); profiles of diffuse attenuation and reflectance; profiles of fluorescence of phytoplankton (SeaTech); phytoplankton lipophilic pigments distribution (spectrofluorometric analysis); *in vivo* analysis of phytoplankton variable fluorescence induced by DCMU (spectrofluorometric analysis). See table Tab.3.1. as a reference to the sampling activity in the different campaigns.

	SY1 (n)	SY2 (n)	SY3 (n)	SY4 (n)
CTD	222	202	181	201
Fluorometric profiles	222	202	181	201
Chl-a discrete samples	360	232	147	187
Radiometric profiles	146	9	17	11

Tab. 3.1. Number of stations (n) per sampling activity, in each SYMPLEX campaign (SY1, SY2, SY3, SY4).

A wider set of measurements were collected during these campaigns to meet the objectives of the ALT/SYMPLEX programs, but they will not be used in this study. This study only utilizes a portion of the larger data set provided by the ALT/SYMPLEX programs as a safe exercise-ground for the application of the Phyto-VFP model, beyond the original scopes of the SYMPLEX surveys.

3.2.3 Bio-optical data

Fluorescence profiles have been calibrated in chlorophyll-a (Chl-a) concentration (mg/m^3), with a linear regression between laboratory dosage of

acetone-extracted chlorophyll-a (Lazzara et., 1994) from the discrete samples, and the fluorometric observations at the corresponding station and depth by the SeaTech probe on board of the ship. To account for the expected variability in the performance of the fluorometric probe (Nardello, 1999) in different campaigns, we have grouped data in subsets, one per each leg of each campaign. We have further partitioned these clusters, to account for the difference in phytoplankton fluorescence yields in different actinic light conditions (photo-acclimation) (Claustre et al, 2006; Falkoswky and Raven, 1997). On the basis of the sampling time of day, the samples in each group have been further partitioned into a “photo-inhibited” (PI) and “non-photoinhibited” (NPI) case. Samples collected when surface PAR irradiance [PAR(0+)] was greater than $800 \mu\text{E}/\text{m}^2 \text{ s}^{-1}$ were assumed photo-inhibited (PI) (Nardello, 1999). Vice versa, samples were labeled as non-photo-inhibited (NPI) when $\text{PAR}(0+) < 800 \mu\text{E}/\text{m}^2 \text{ s}^{-1}$ (Nardello, 1999). Each subset was then regressed against dosed chlorophyll-a concentration. We report in table Tab.3.2. the coefficients (m= slope; q=intercept) of the linear regressions obtained, the regression coefficients (r^2), and the number of samples (n) regressed.

	SY1	SY1	SY1	SY2	SY2	SY2	SY2	SY2	SY2	SY3	SY3	SY3	SY4	SY4	SY4
	PI		NPI	leg1	leg1	leg1	leg2	leg2	leg2	PI		NPI	PI		NPI
m	0.02	0.01	0.03	0.69	0.53	0.76	0.41	0.38	0.41	0.05	0.05	0.05	0.14	0.13	0.16
q	0.37	0.39	0.36	16.27	16.57	16.13	3.95	4.13	3.82	0.85	0.80	0.91	2.34	2.34	2.37
r^2	0.74	0.87	0.66	0.79	0.91	0.75	0.73	0.74	0.74	0.87	0.90	0.86	0.86	0.88	0.84
n	360	100	260	129	38	91	97	42	55	186	74	112	151	82	69

Tab.3.2. Fluorometer calibration coefficients, in four SYMPLEX campaigns, according to “photo-inhibited” (PI) and “non-photoinhibited” (NPI) conditions, with linear relationship coefficients (m= slope; q=intercept), regression coefficient (r^2), the number of samples (n) per each regression.

Underwater irradiance profiles have been examined, and reconstructed with a logarithmic fit where data were missing or unreliable. Only 49 profiles were available at the end of this operation, from which we could derive the euphotic zone depth

(Z_{eu}), as the depth where $PAR(z) = 1\% PAR(0+)$. To retrieve this information in stations where no irradiance profiles were available, we used an iterative computation based on the empirical relationship between depth-integrated Chl-a concentration and Z_{eu} (Morel & Berthon, 1989; Morel, 1988; D'Ortenzio et al. 2000), in case I environments:

$$Z_{eu} = 568.2 * Ct_{tot} - 0.746 \text{ per } Z_{eu} < 102 \text{ m} \quad (3.1.a)$$

$$Z_{eu} = 200.0 * Ct_{tot} - 0.293 \text{ per } Z_{eu} > 102 \text{ m} \quad (3.1.b)$$

Where:

Ct_{tot} = depth-integrated chlorophyll concentration (mg/m^2) in the euphotic zone

The Chl-a profile is integrated with a stepwise progression over depth. These values are progressively inserted in equation 3.1.a to obtain progressively decreasing values of Z_{eu} . The process terminates when computed Z_{eu} is smaller than the integration depth (Morel & Berthon, 1989). It is on the basis of this relationship between Chl-a concentration and euphotic zone depth, analyzed over a large data set from the world's oceans, that Morel (1988) partitions seawater environments between Case I and Case II (Fig.3.3).

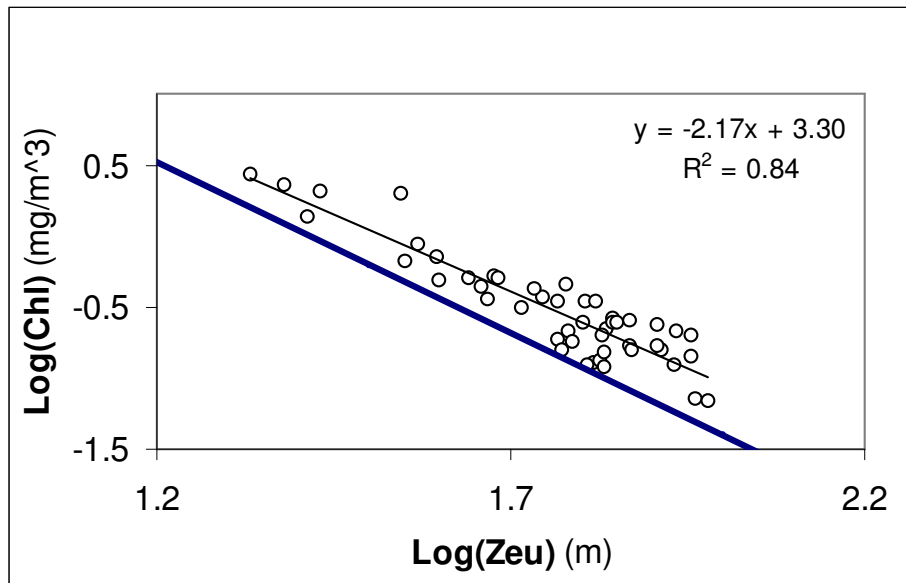


Fig. 3.3: Regression between chlorophyll concentration integrated over the euphotic zone depth (CHL; mg/m^2) and depth of the euphotic zone (Z_{eu} ; m). Number of samples = 49, $r^2=0.838$. The blue line represents the separation line between Case I (above the line) and Case II seawater environments, according to A. Morel (1988).

Spectrofluorometric analysis of *in vivo* chlorophyll-a fluorescence were performed with addition of DCMU to a suspension of concentrated (10x) seawater discrete samples. The profiles of variable fluorescence (F_v/F_m) obtained at each station were interpolated with a spline fit, and resolved to a 1 m interval.

3.2.4 The Phyto_VFP Model

The Phyto-VFP (Phytoplankton Variable Fluorescence Production) model delivers different estimates of primary production (PP):

- instantaneous: PP as a function of depth and time
- depth-integrated: PP integrated over depth, as a function of the time of the day
- time-integrated: PP integrated over 24 h.

In the first case, PhytoVFP estimates PP at a fixed time of the day, at every meter of the water column, starting from the surface down to a depth (z), where downwelling

planar irradiance (E_d) is 1% of E_d just below the sea surface ($E_d(0-)$). In the second case, the model progresses from the first run and integrates PP over the water column depth. We obtain the third type of calculation with the sum of such estimates over a 24h period.

The model architecture is based on four main components:

- I) Model of marine phytoplankton photo-physiology;
- II) Model of surface PAR
- III) Calculation of instantaneous PP rates
- IV) Calculation of integrated PP.

Component I - Marine phytoplankton photo-physiology

The foundation for this component of the PhytoVFP lies in the study reported in the previous chapter for the diatom *Skeletonema costatum*. We have parameterized the PE relationship of this organism, and modeled the variations of the PE parameters (α' , P_{max} , E_k'), for three different physiological conditions, as a function of the time of the day. Through a Fourier transformation, we decomposed the physiological signal of the PE parameters time series (62-hour experimental periods, with observation repeated every 2-3 hours), to show what frequencies it contained. As in the autocorrelation analysis reported in the previous chapter, α' resulted constant for each physiological condition explored (photo-limited, 25 $\mu E/m^2$ s; -saturated, 300 $\mu E/m^2$ s; -inhibited, 500 $\mu E/m^2$ s). P_{max} and E_k' showed a periodicity within the 24hours and we could distinguish the higher and fundamental harmonic peaks of the time series (Fig. 3.4). A detailed report on the Fourier transformation analysis is in the technical report Lazzara et al. (2002).

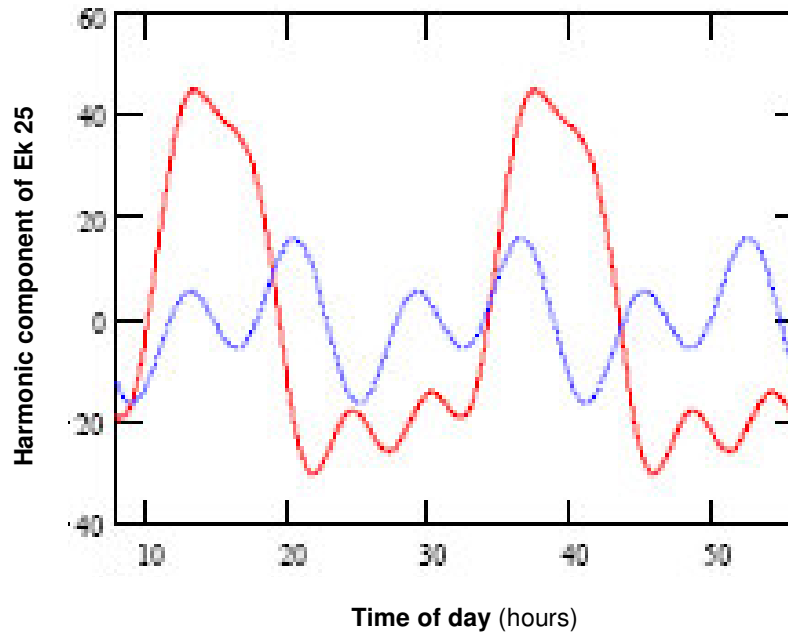


Fig. 3.4 – Fourier transformation for the time series of diel variation of E_k , at growth light intensity of $25 \mu\text{E}/\text{m}^2 \text{ s}$. The harmonic peaks of the time series (in red) could be distinguished from back ground noise (in blue).

Component II - surface PAR irradiance

This module estimates Par irradiance at the sea surface ($\text{PAR}(0+)$) as a function of time of the day, day of the year, geographic location.

Input data are:

t = time of the day (hours, minute)

geographic coordinates = Latitude North, Longitude East (decimal degrees)

c = conversion coefficient between Watt and $\mu\text{E}/\text{m}^2 \text{ s}$

Output data are theoretical values of $\text{PAR}(0+)$ at the specified time of the day and of the year, in a defined geographic location. A graphic of daily PAR excursion is also produced (example in Fig. 3.5). Algorithms in use are extracted from the software SOLEIRRA (Luca Massi, personal communication).

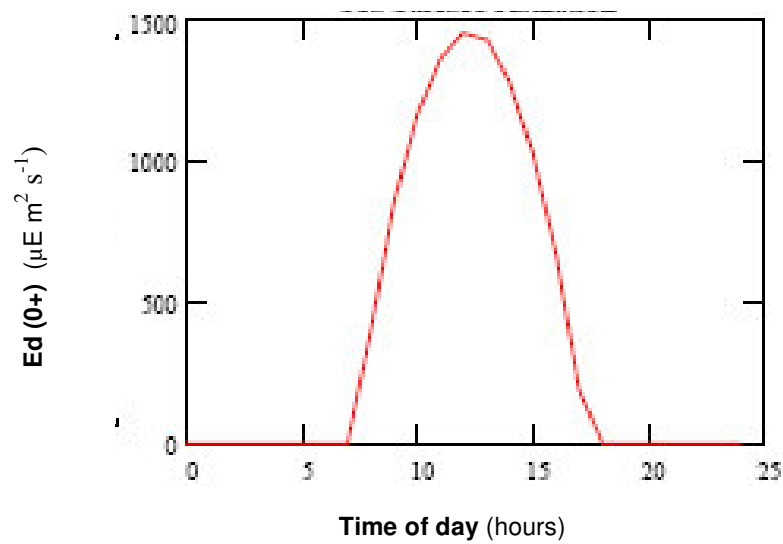


Fig. 3.5 Example of PhytoVFP output of surface daily PAR irradiance $\mu\text{E}/\text{m}^2 \text{ s}$.

Component III - Instantaneous primary production rates

This module estimates the rate of primary production (PP, $\text{mg C} / \text{m}^3 \text{ h}$) at a certain depth (z) and time of day. The results can be integrated over time (hours, days).

t= time of day in hours and minutes (hh,mm)

PAR= surface value of PAR ($\mu\text{E m}^2 \text{ s}^{-1}$)

Kd= vertical mean diffuse attenuation coefficient ($1/\text{m}$)

Ifinale= lower limit of irradiance level (generally = $1 \mu\text{E} / \text{m}^2 \text{ s}$)

a,b = irradiance thresholds for the functions $E_k(t,z)$, $\alpha(z)$, and $PP(t,z)$

r = higher limit of the function $Prod(t,z)$ in the saturation-irradiance range

σ = light utilization index

Fmax= profiles of maximum emission of Chl-a fluorescence (unitless)

Fv/Fm (=effph) = efficiency of Chl-a fluorescence (unitless)

Ed (=Irr) = profile of downwelling irradiance ($\mu\text{E} / \text{m}^2 \text{ s}$)

Output data are:

Production(t,z)= Primary production rate at a certain time of day

P(t)= Depth-integrated estimate of PP at a certain time of day (t)

Plots

Two modes can be set on: in “Tmode” we use of a theoretical irradiance profile; in “S mode”, the PAR profile (Irr) is empirical. Empirical profiles (Par, Fmax) are smoothed prior to any calculation. Primary production is then estimated by the following expression:

$$\text{produzione}(t,z)=\sigma \cdot \text{Prod}(t,z) \cdot \text{effph}(z) \cdot \text{clorofilla}(z) \quad (3.2)$$

The Prod(t,z) function is the new component of the PhytoVFP model. It accounts for the variation of the photosynthetic coefficients as a function of actinic irradiance and time, with the following algorithm:

$$\text{Prod}(t, z) = \begin{cases} [\tan(\alpha(z))I(z)] & \text{if } I(z) < Ek(t, z) \\ Pl(t, z) & \text{if } Ek(t, z) \leq I(z) \leq r - Ek(t, z) \\ \frac{Pl(t,z)^2}{I(z)} & \text{otherwise} \end{cases} \quad (3.3)$$

The function has been modeled considering the three main phases of the PE relationship: limitation, saturation, inhibition. Prod(t,z) depends in facts on three other functions: $\alpha(z)$, $Ek(t,z)$ and $Pl(t,z)$, which express the variation of photosynthetic parameters over time and light intensity conditions, as follows:

$\alpha(z)$ represents the slope of the linear portion of the PE relationship. We have modeled its variation in relation to light intensity/depth as follows:

$$\alpha(z) = \begin{cases} 0.614 & \text{if } 0 \leq I(z) < a \\ 0.475 & \text{if } a \leq I(z) < b \\ 0.447 & \text{otherwise} \end{cases} \quad (3.4)$$

Ek(t,z) represents the Par value where saturation of photosynthesis begins. It is expressed as a function of irradiance at depth z, and of the time of the day:

$$Ek(t, z) = \begin{cases} Ek25(t) & \text{if } 0 \leq I(z) < a \\ Ek300(t) & \text{if } a \leq I(z) < b \\ Ek500(t) & \text{otherwise} \end{cases} \quad (3.5)$$

Pl(t,z) is the value of Pmax during photo-saturation. It also depends on irradiance at depth z, and on the time of the day:

$$Pl(t, z) = \begin{cases} 0.705Ek25(t) & \text{if } 0 \leq I(z) < a \\ 0.514Ek300(t) & \text{if } a \leq I(z) < b \\ 0.479Ek500(t) & \text{otherwise} \end{cases} \quad (3.6)$$

Component IV - Integrated primary production

Diel primary production is eventually calculated with the use of a bi-dimensional matrix. In the two columns we find:

t= time of day at which we estimate PP(t) (hours, minutes)

produzione(t)= PP (mg C/m³ h)

The output is:

P = daily PP (mg C /m² d)

The algorithms contained in this module make produzione(t) a continuous function, allowing integration of prduzione(t) over time. An example is reported below. The photo-inhibition effect on PP, derived from the implementation of the photo-acclimation coefficients, is noticeable in the central hours of the day.

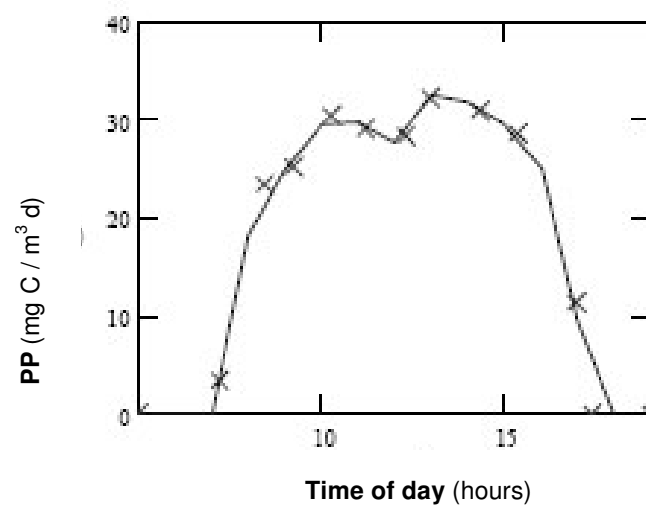


Fig. 3.6 Output from Phyto-VFP. Daily primary production rates as a (mass of carbon per volume of water, per day) as a function of time of the day.

3.3 RESULTS

We have analyzed the hydrological and biological data from the four SYMPLEX campaigns with geographical information software (ODV). The scope of this analysis was to define distinct geographical areas (regions) with peculiar trophic characteristics. The variables we utilized are: water temperature (T, degree C), Chlorophyll-a concentration (Chl-a, mg/m^3), depth of maximum Chl-a concentration ($Z_{\text{Chl-a max}}$, m), maximum Chl-a concentration ($\text{Chl-a}_{\text{max}}$, mg/m^3), euphotic zone depth (Z_{eu}), and integrated Chl-a over the whole euphotic zone (C_{tot} mg/m^2). Maps are shown in figures 3.7 - 3.17.

We have chosen the area where data from the four years overlap, $14^{\circ}00' - 16^{\circ}20'$ Long. E and $34^{\circ}30' - 37^{\circ}00'$ Lat. N, as the area of interest for our regionalizations. Over this area, we have defined sub-regions of different trophic level, based on a combination of factors: the value of integrated Chl-a concentration over the euphotic zone (C_{tot}) (SY1, SY3, SY4), or the depth and value of maximum chlorophyll-a concentration (SY2). In all cases we have defined the spatial extent of these sub-areas considering the presence of physical fronts, emerging from the hydrological analysis. The upper and lower values of the trophic level identified are summarized in Tab. 3.3.a, b

Campaign	SY1_0	SY1_1	SY1_2	SY3_0	SY3_1	SY3_2	SY4_0	SY4_1
C_{tot} (mg/m^3)	< 16	16-18	≥ 18	< 15	15 - 18	≥ 18	< 12.5	≥ 12.5

Tab. 3.3.a Ranges of Chlorophyll-a concentration integrated over euphotic zone depth (C_{tot}), defining different trophic levels in the area of interest ($14^{\circ}00' - 16^{\circ}20'$ Long. E and $34^{\circ}30' - 37^{\circ}00'$ Lat. N). We can distinguish three trophic sub-regions in SY1, and as many in SY2. We could only distinguish two levels during SY4. Each sub-region is identified by the campaign's name and a progressive number.

Campaign	SY2_0	SY2_1	SY2_2	SY2_3	SY2_4
Z_{Chl-a max} (m)	≥ 110	90 - 110	< 90	< 90	≥ 110
Chl-a_{max} (mg/m ³)	< 0.5	< 0.5	< 0.5	≥ 0.5	≥ 0.5

Tab. 3.3.b The combinations of maximum chlorophyll-a depth and maximum chlorophyll-a values define four trophic regions during SY2 (Summer 1997), over the selected area of interest (14°00' – 16°20' Long. E and 34°30' – 37°00' Lat. N). Each region is identified by the campaign's name and a progressive number.

Chl-a profiles for each region have been smoothed and averaged, to obtain one reference profile for each region (Figs. 3.18 - 3.21). Table 3.4 reports the average Chl-a value corresponding to each representative profile, the number of profile averaged, the maximum value of the average profile, and the standard deviation of the latter value.

	SY1_0	SY1_1	SY1_2	SY2_0	SY2_1	SY2_2	SY2_3	SY2_4
N	45	32	70	21	12	15	51	14
Chl-a_{0-200m avg} (mg/m ³)	0.08	0.11	0.17	0.08	0.08	0.09	0.14	0.33
Z_{Chl-a max} (m)	97	68	53	121	101	88	83	114
Chl-a_{max} mg/m ³	0.26	0.46	0.54	0.26	0.29	0.29	0.72	0.88
St. dev. mg/m ³	0.11	0.14	0.25	0.05	0.07	0.15	0.38	0.05

Tab. 3.4: Summary of the characteristics of each region-defining average Chl-a profiles. The table includes: the number of profiles averaged per region (N), the average (0-200 m) Chl-a value corresponding to each representative profile (Chl-a 0-200m avg, mg/m³), the maximum value of Chl-a in the average profile, and the standard deviation (St. dev) of the latter value.

	SY3_0	SY3_1	SY3_2	SY4_0	SY4_1
N	33	53	32	60	116
Chl-a 0-200m avg (mg/m ³)	0.13	0.18	0.28	0.08	0.09
Z_{Chl-a max} (m)	82	69	44	92	60
Chl-a_{max} mg/m ³	0.24	0.42	0.70	0.15	0.24
St. dev. mg/m ³	0.07	0.24	0.24	0.04	0.08

Tab. 3.4 (continuation from the previous page): Summary of the characteristics of each region-defining average Chl-a profiles. The table includes: the number of profiles averaged per region (N), the average (0-200 m) Chl-a value corresponding to each representative profile (Chl-a 0-200m avg, mg/m³), the maximum value of Chl-a in the average profile, and the standard deviation (St. dev) of the latter value.

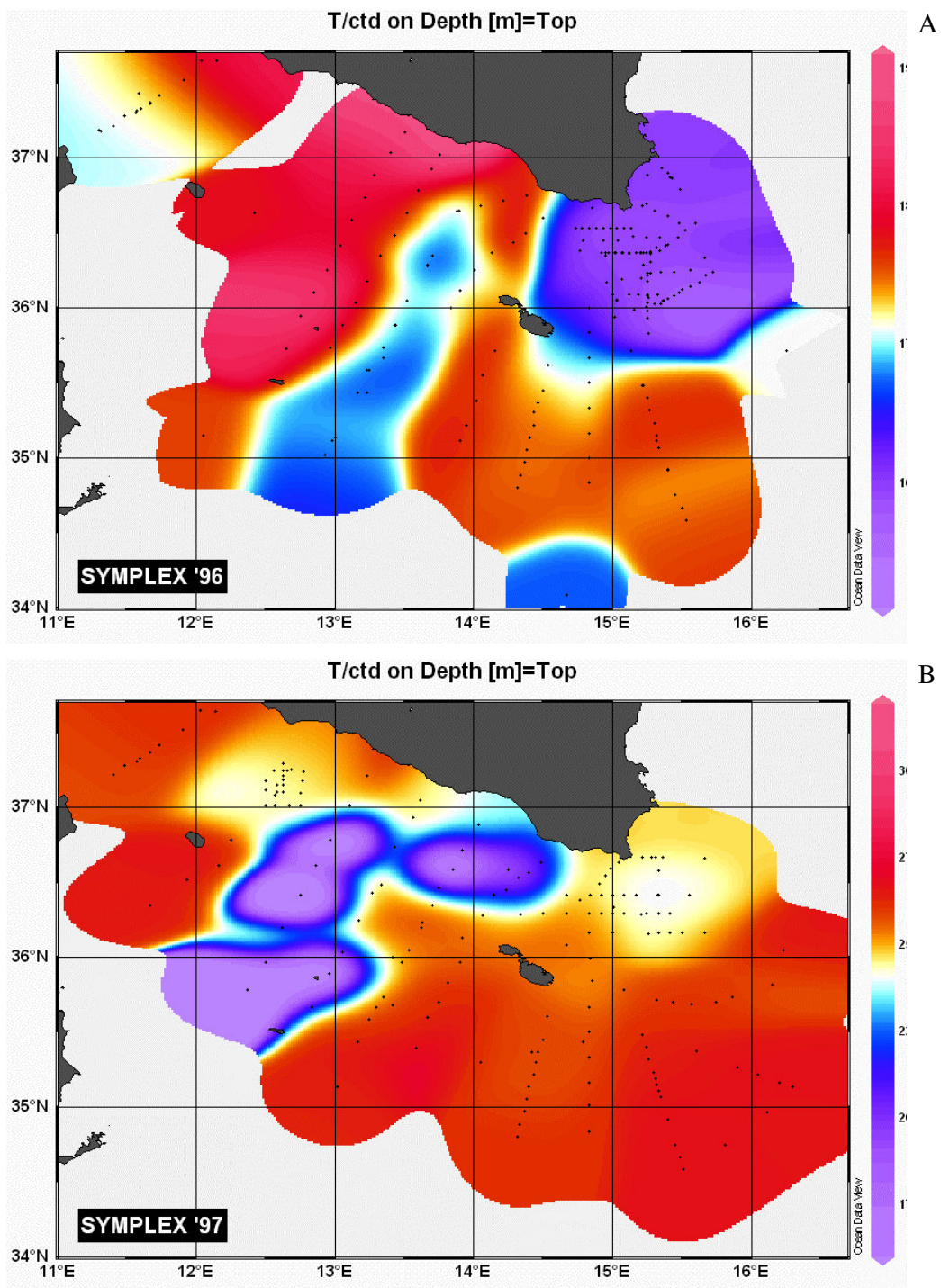


Fig. 3.7 (a,b) - Sub-surface seawater temperature (°C), in the Channel of Sicily, from CTD surveys, during a) SY1 (Spring 1996), b) SY2 (Summer 1997).

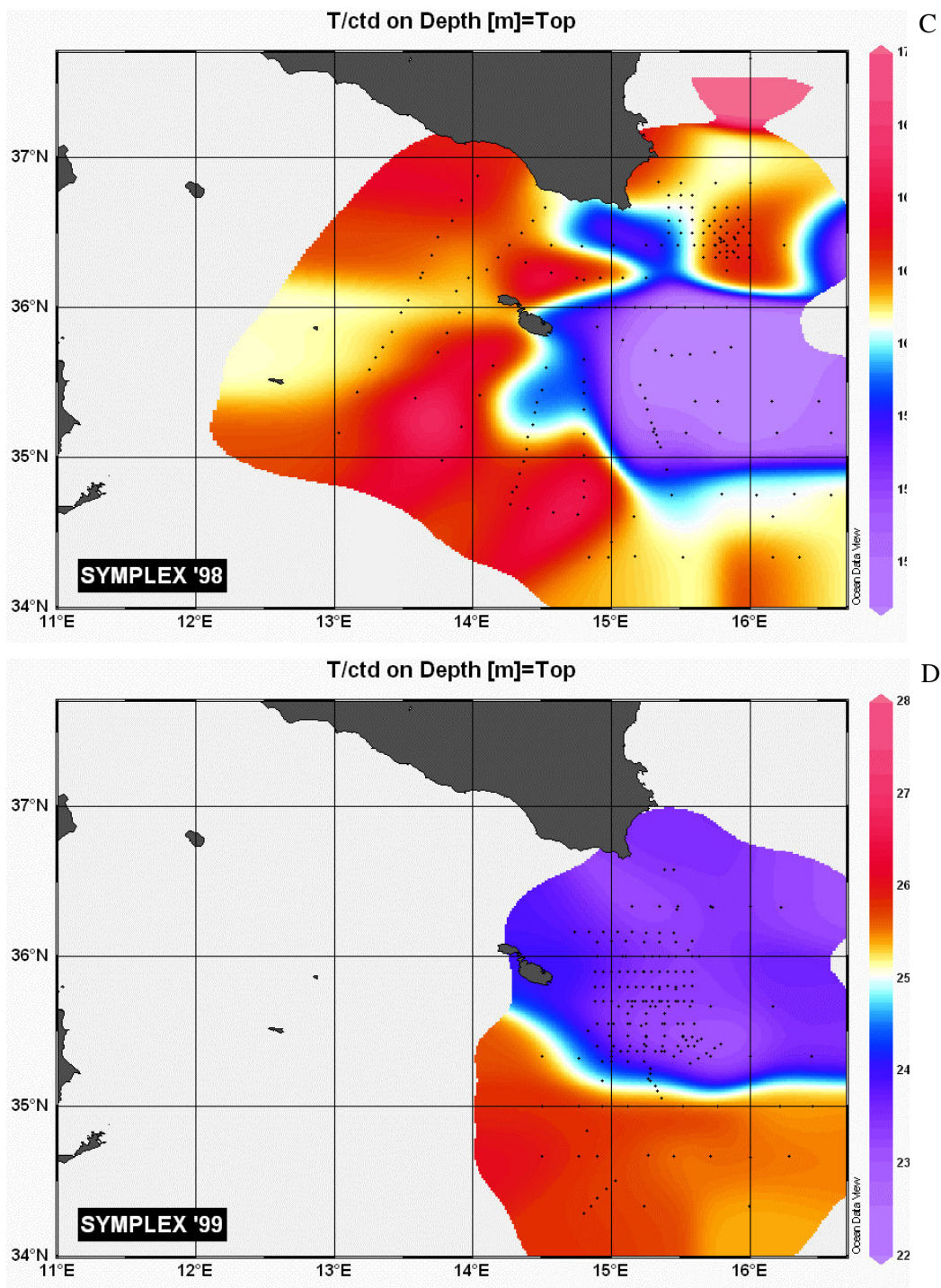


Fig. 3.7 (c,d) - Sub-surface seawater temperature (°C), in the Channel of Sicily, from CTD surveys, during , c) SY3 (Spring 1998) and d) SY4 (Autumn 1999).

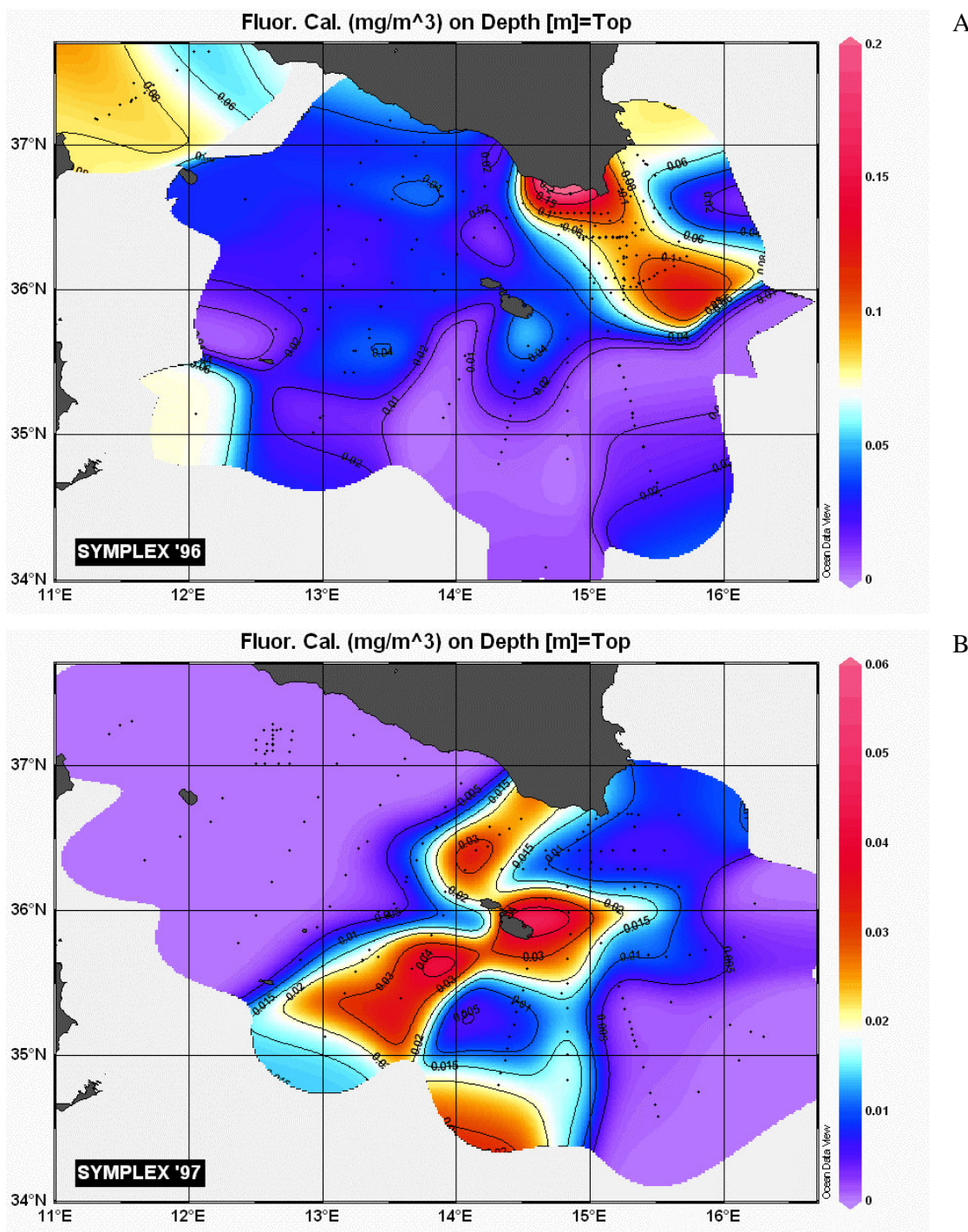
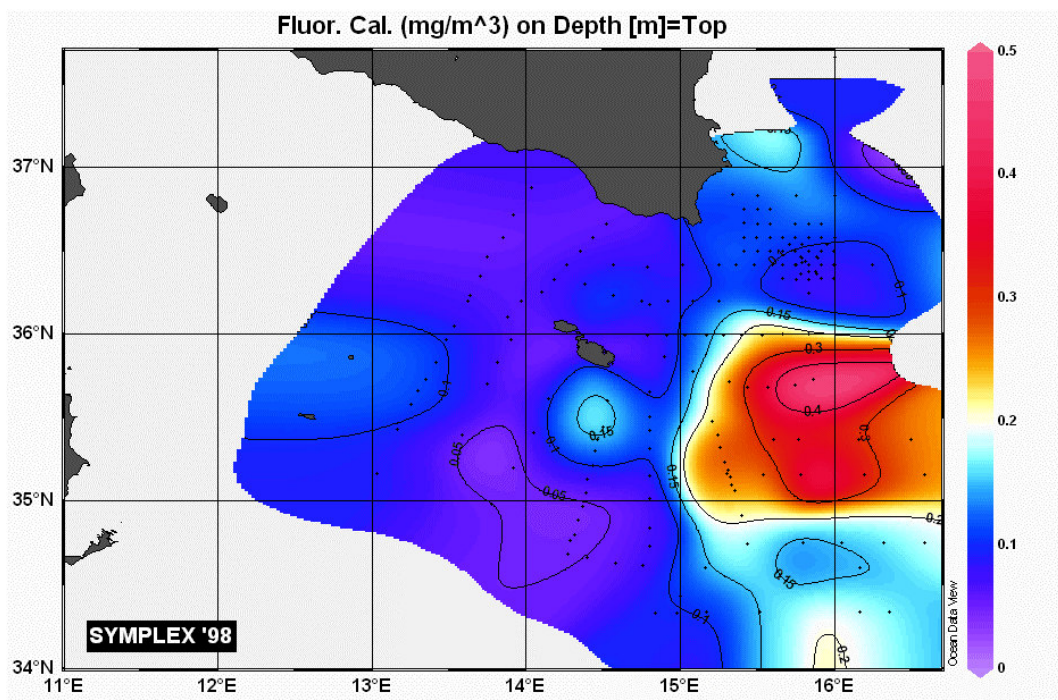
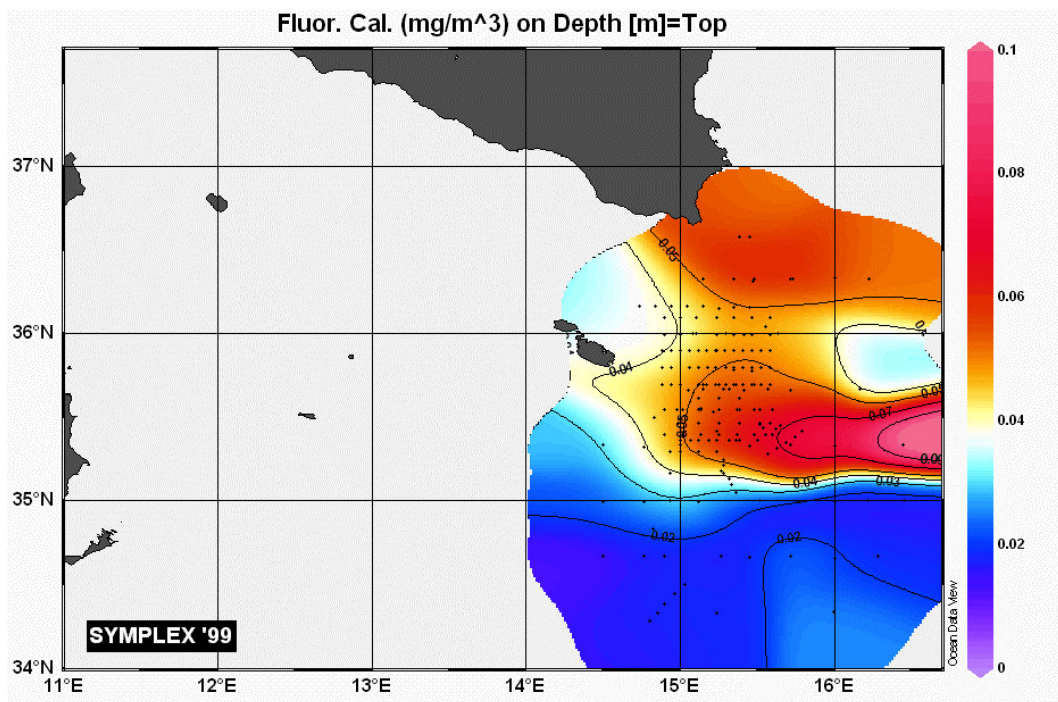


Fig. 3.8 (a,b)- Concentration of Chl-a (mg/m³) in the subsurface layer, in the Channel of Sicily, from calibrated fluorometric profiles, during a) SY1 (Spring 1996), b) SY2 (Summer 1997).



C



D

Fig. 3.8 (c,d) – Concentration of Chl-a (mg/m^3) in the subsurface layer, in the Channel of Sicily, from calibrated fluorometric profiles, during : c) SY3 (Spring 1998) ; d) SY4 (Autumn 1999)

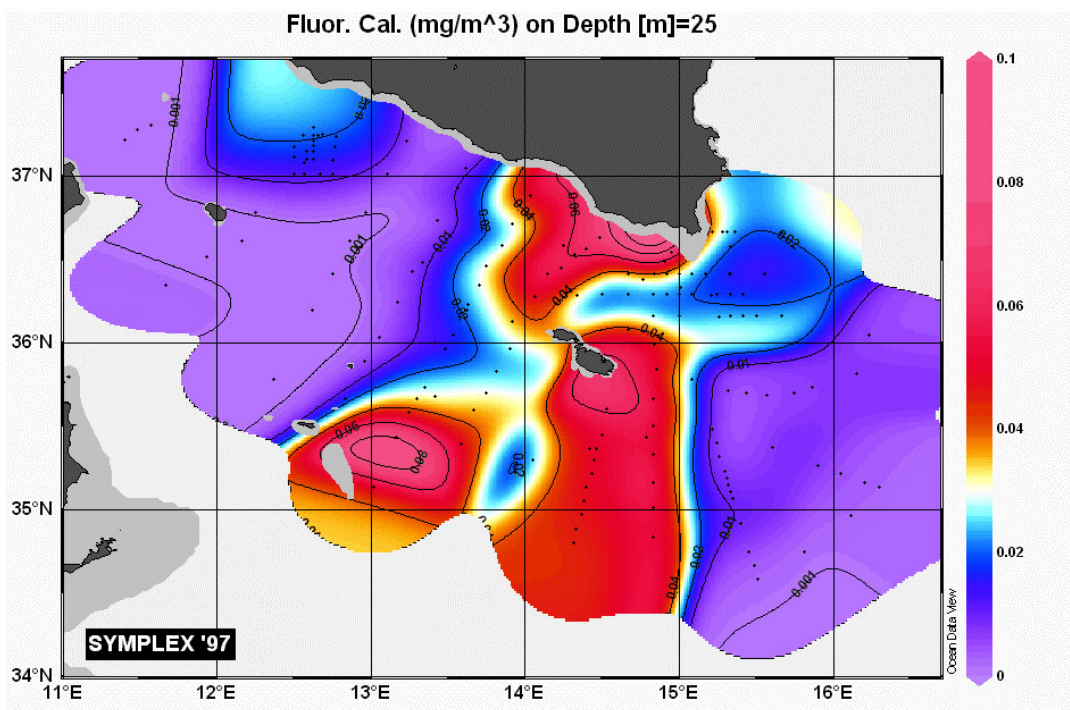
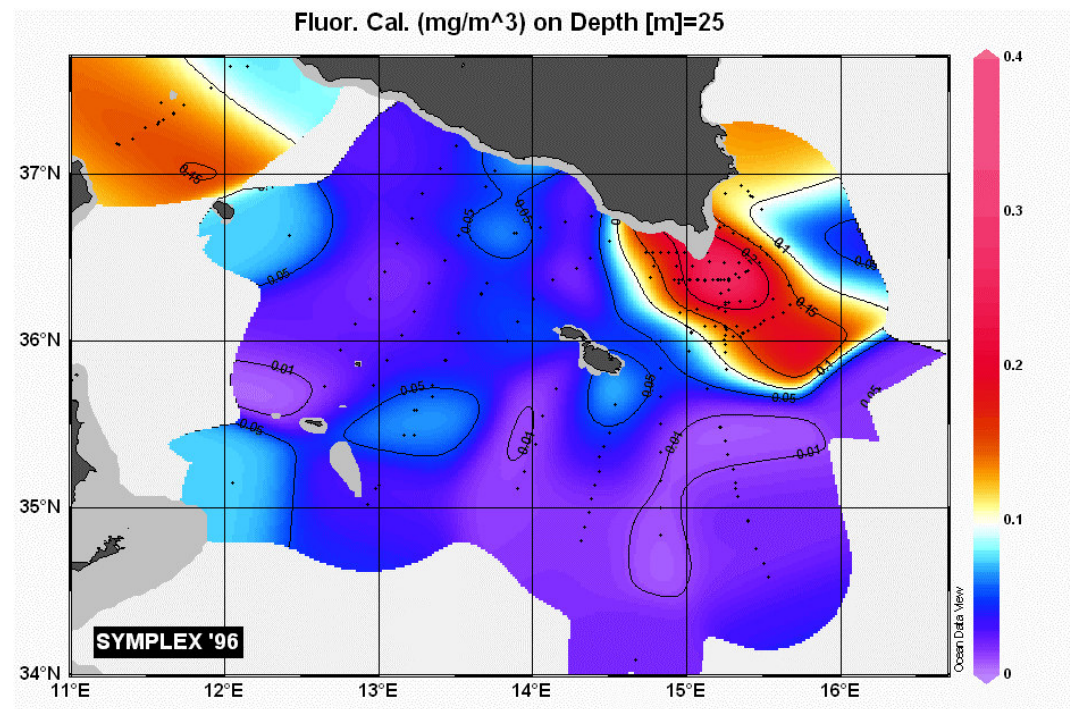
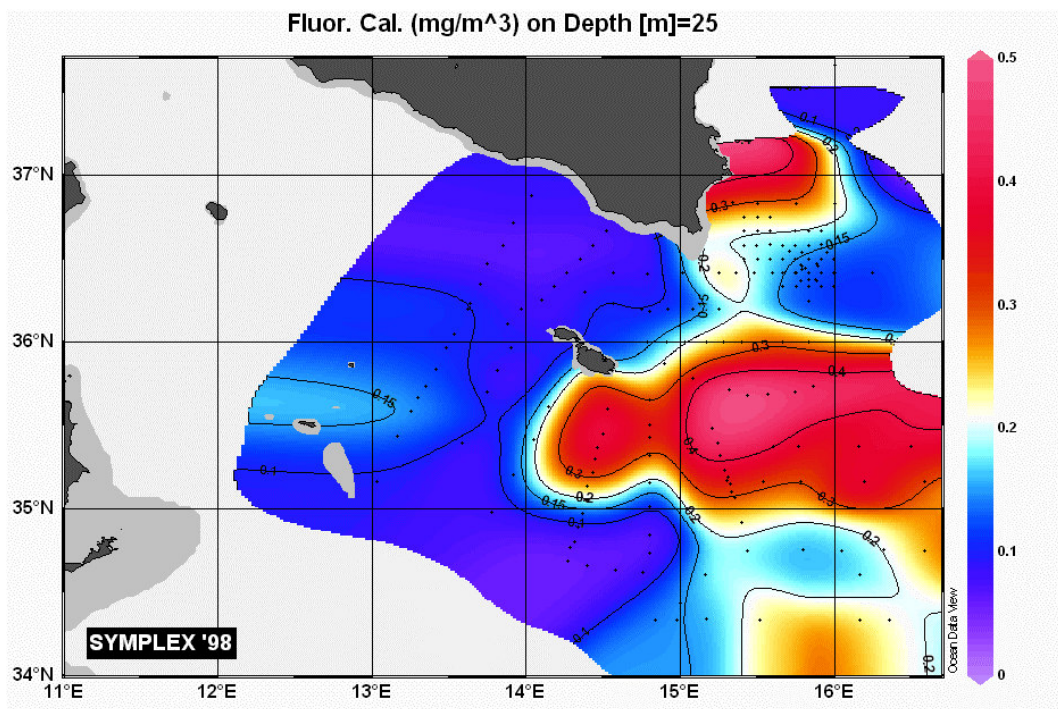
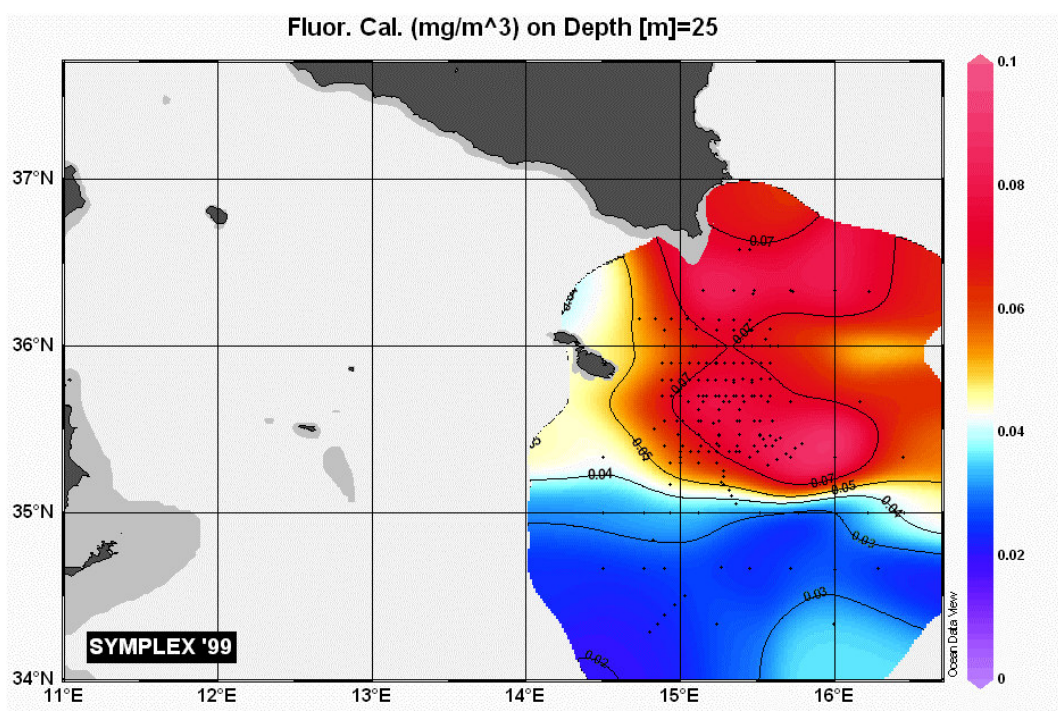


Fig. 3.9 (a,b) – Concentration of Chl-a (mg/m³) at depth 25 m, in the Channel of Sicily, from calibrated fluorometric profiles, during a) SY1 (Spring 1996), b) SY2 (Summer 1997).

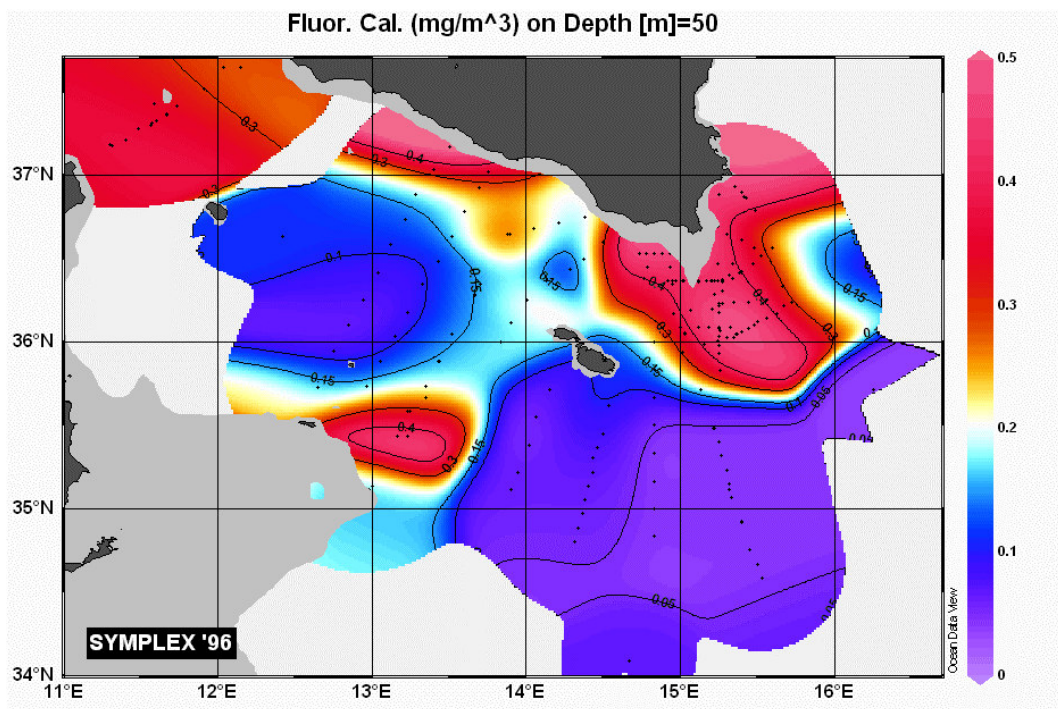


C

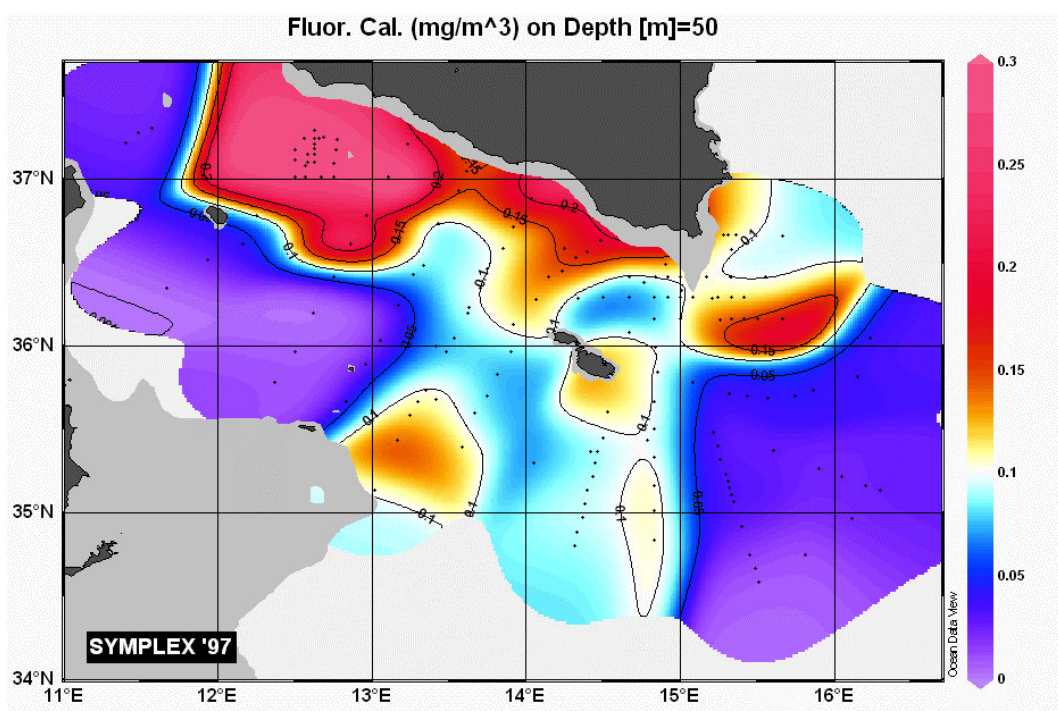


D

Fig. 3.9 (c, d)– Concentration of Chl-a (mg/m³) at depth 25 m, in the Channel of Sicily, from calibrated fluorometric profiles, during: c) SY3 (Spring 1998) and d) SY4 (Autumn 1999).

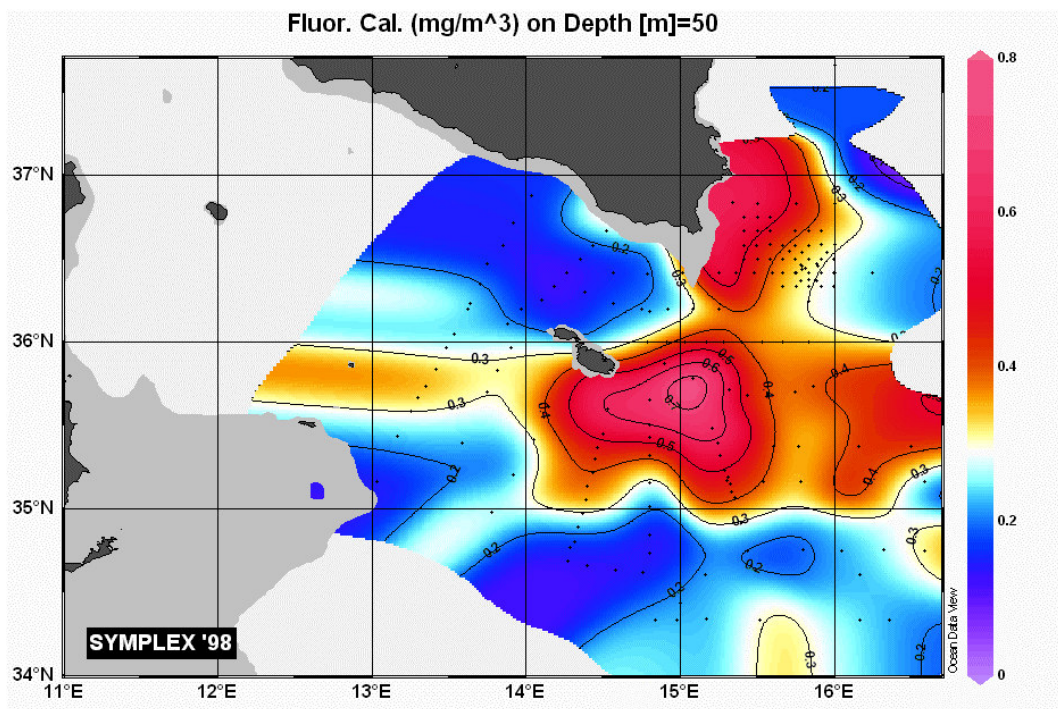


A

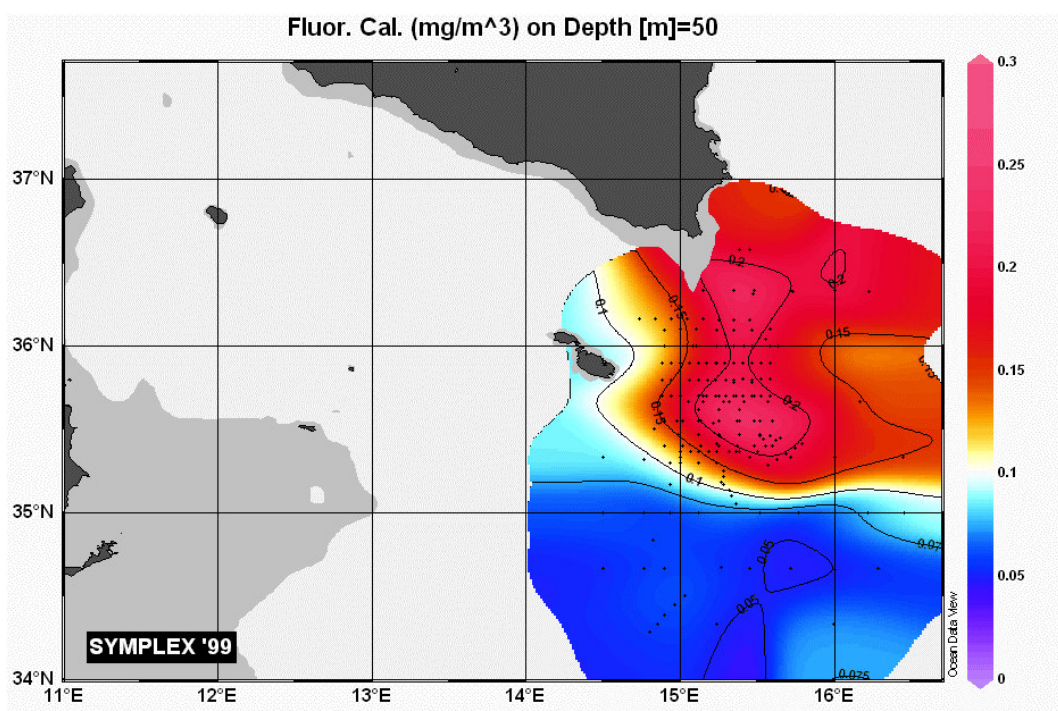


B

Fig. 3.10 (a,b) – Concentration of Chl-a (mg/m³) at depth 50 m, in the Channel of Sicily, from calibrated fluorometric profiles, during a) SY1 (Spring 1996), b) SY2 (Summer 1997).



C



D

Fig. 3.10 (c,d) – Concentration of Chl-a (mg/m³) at depth 50 m, in the Channel of Sicily, from calibrated fluorometric profiles, during: c) SY3 (Spring 1998); d) SY4 (Autumn 1999).

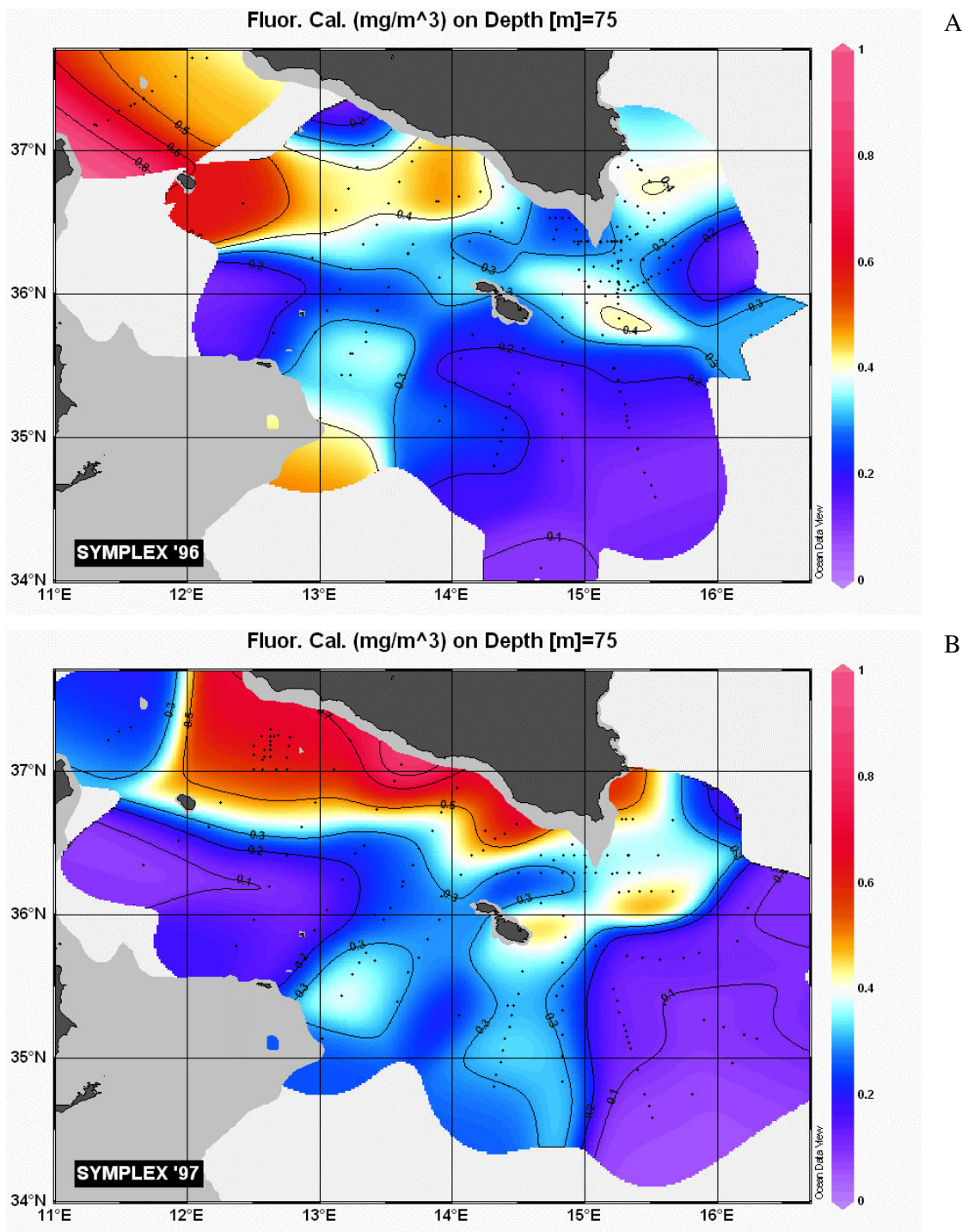


Fig. 3:11 (a,b) – Concentration of Chl-a (mg/m³) at depth 75 m, in the Channel of Sicily, from calibrated fluorometric profiles, during a) SY1 (Spring 1996), b) SY2 (Summer 1997)

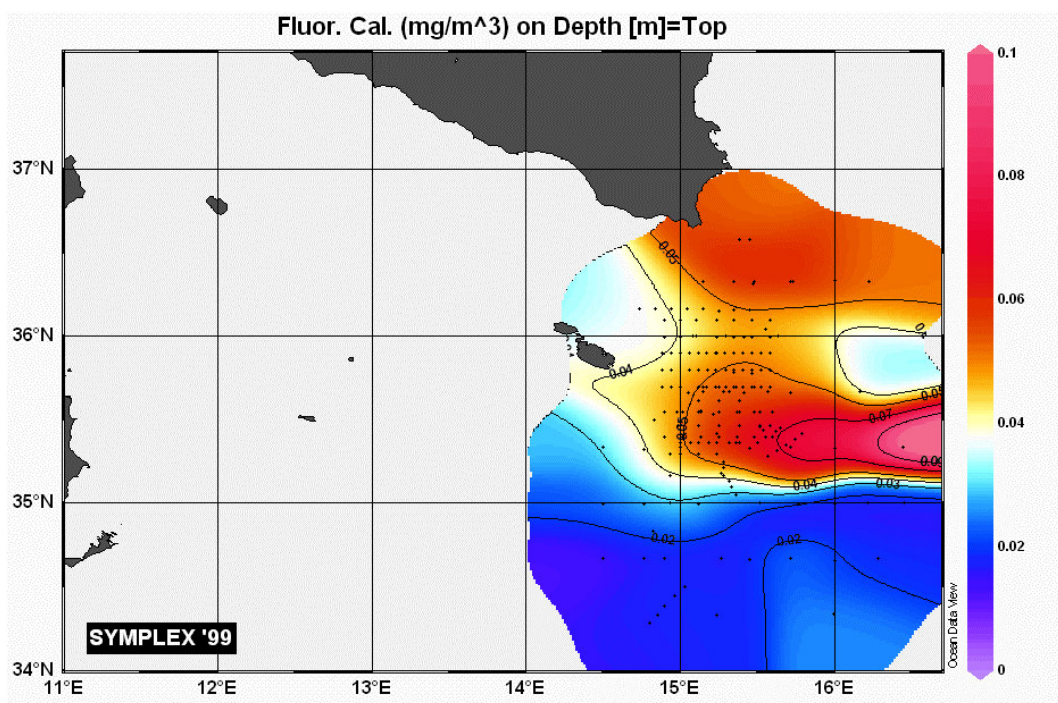
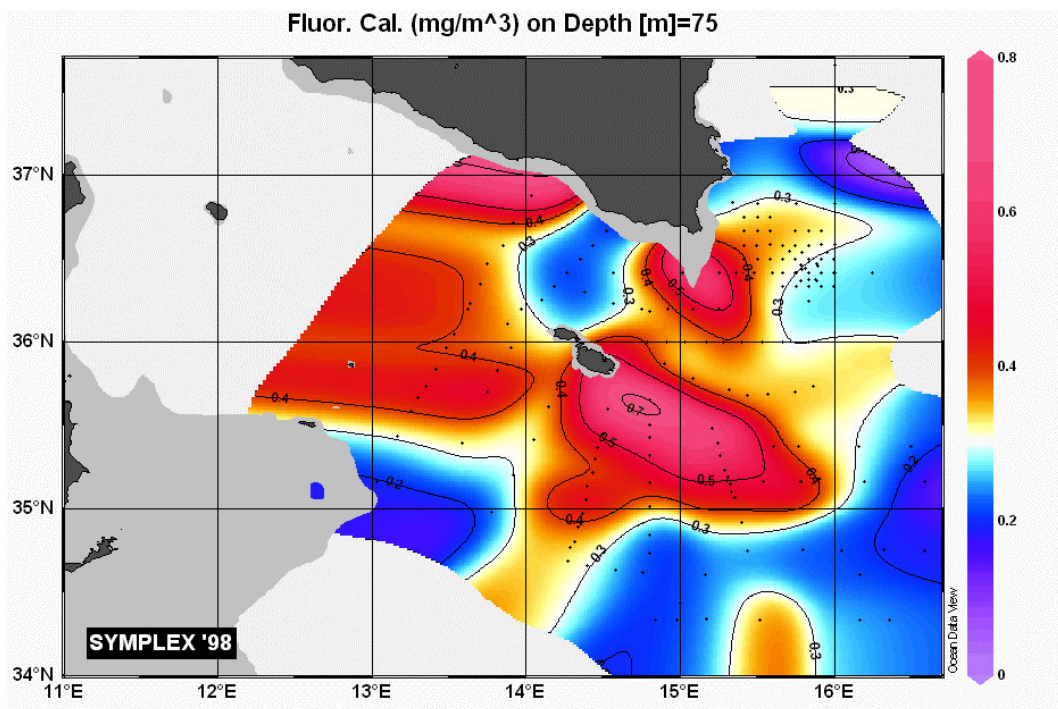


Fig. 3:11(c, d) – Concentration of Chl-a (mg/m³) at depth 75 m, in the Channel of Sicily, from calibrated fluorometric profiles, during : c) SY3 (Spring 1998) , d) SY4 (Autumn 1999).

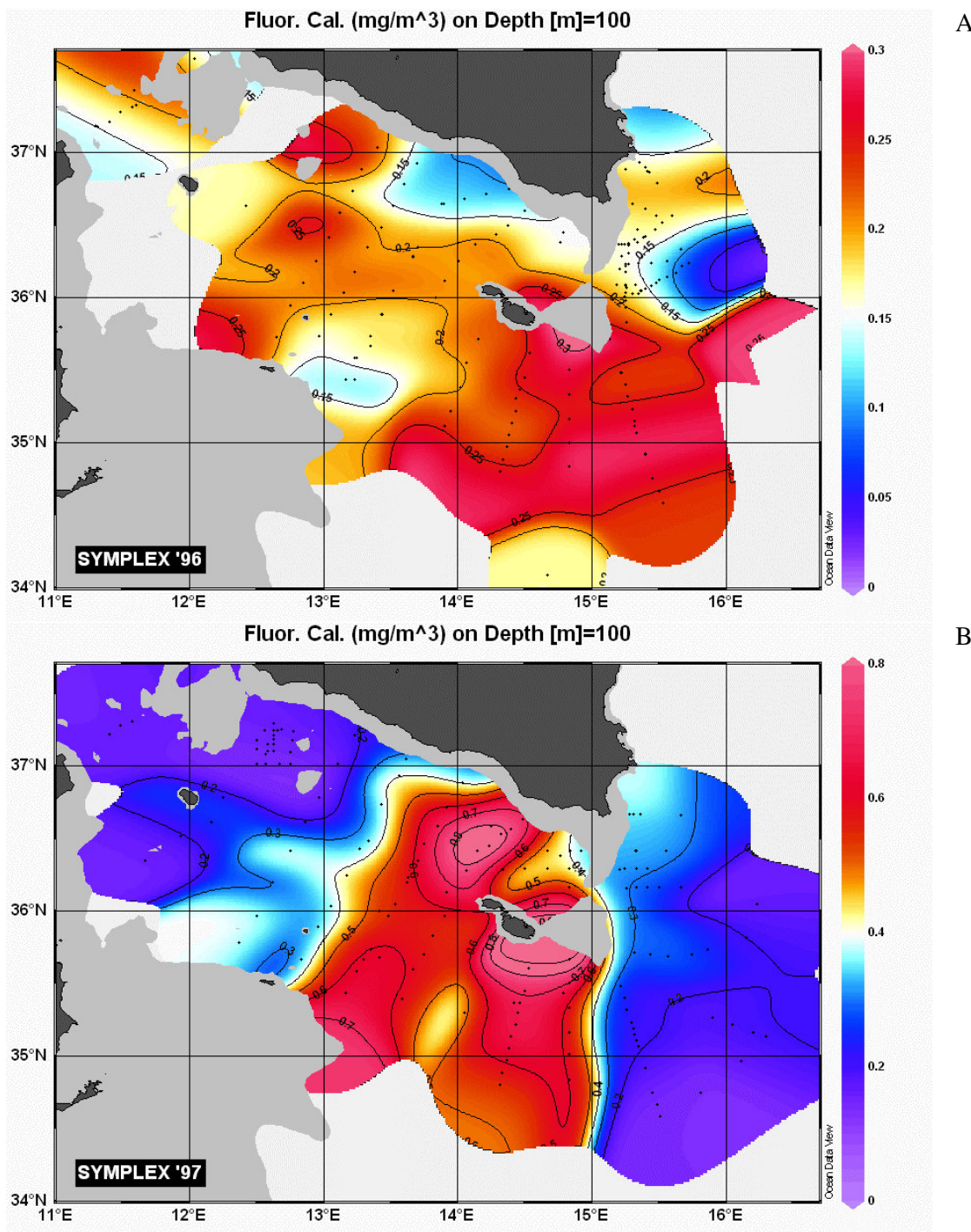


Fig. 3.12 (a,b) – Concentration of Chl-a (mg/m³) at depth 100 m, in the Channel of Sicily, from calibrated fluorometric profiles, during a) SY1 (Spring 1996), b) SY2 (Summer 1997),.

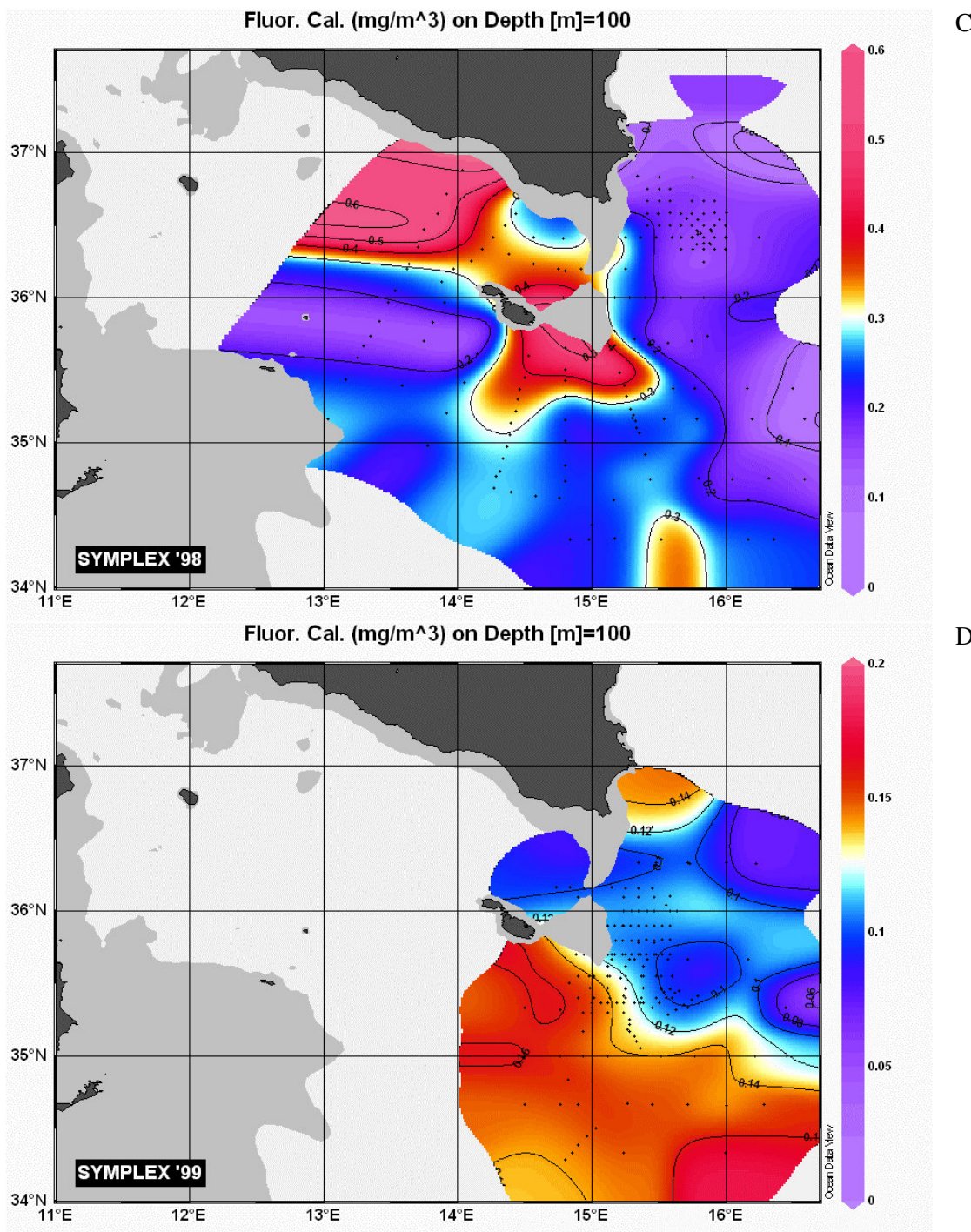


Fig. 3.12 (c, d) – Concentration of Chl-a (mg/m³) at depth 100 m, in the Channel of Sicily, from calibrated fluorometric profiles, during : c) SY3 (Spring 1998), d) SY4 (Autumn 1999).

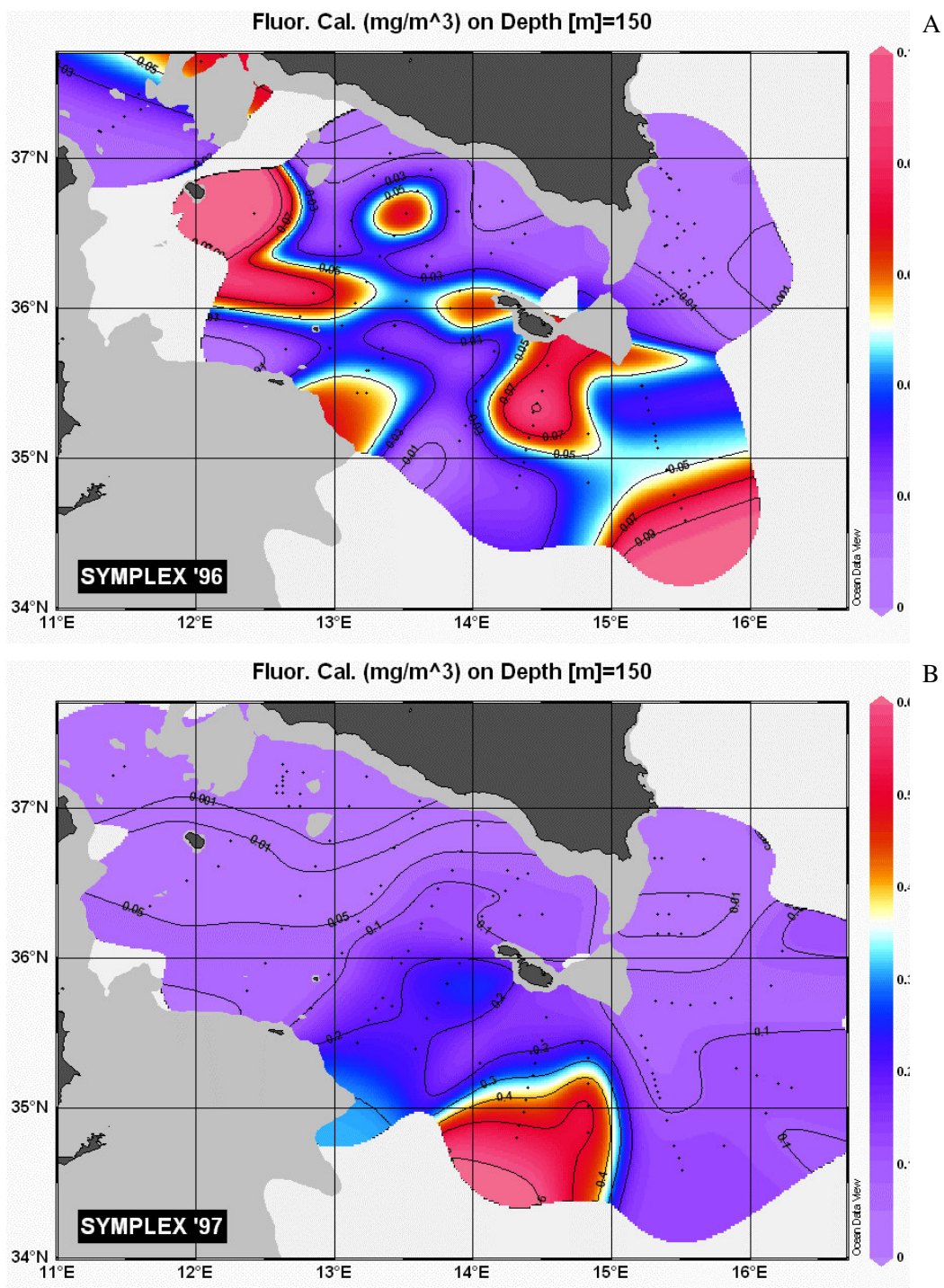


Fig. 3.13 (a,b) – Concentration of Chl-a (mg/m³) at depth 150 m, in the Channel of Sicily, from calibrated fluorometric profiles, during a) SY1 (Spring 1996), b) SY2 (Summer 1997)

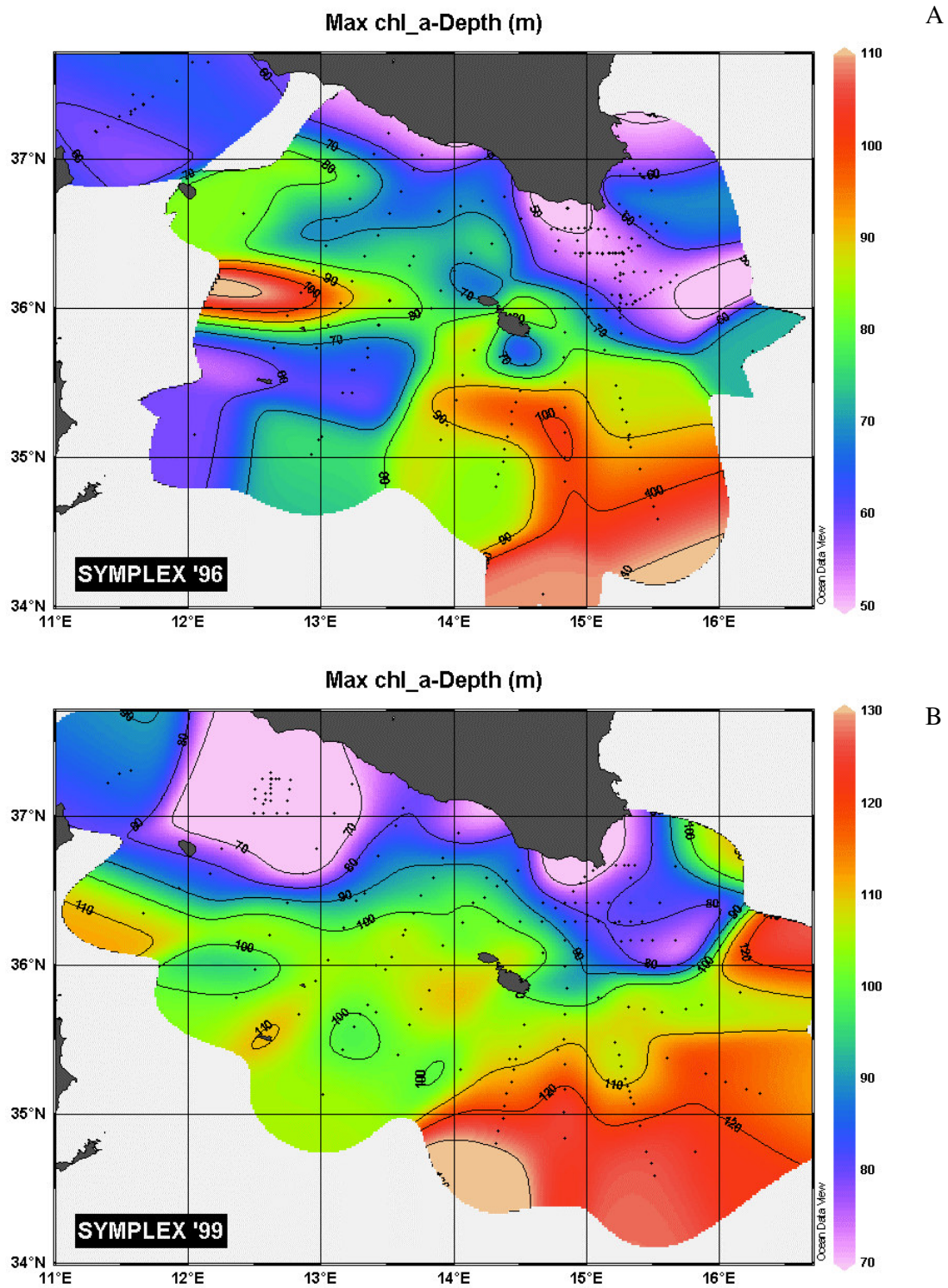


Fig. 3.14 (a,b) Depth distribution of maximum concentrations of Chl-a (mg/m^3), in the Channel of Sicily, during: a) SY1 (Spring 1996), b) SY2 (Summer 1997).

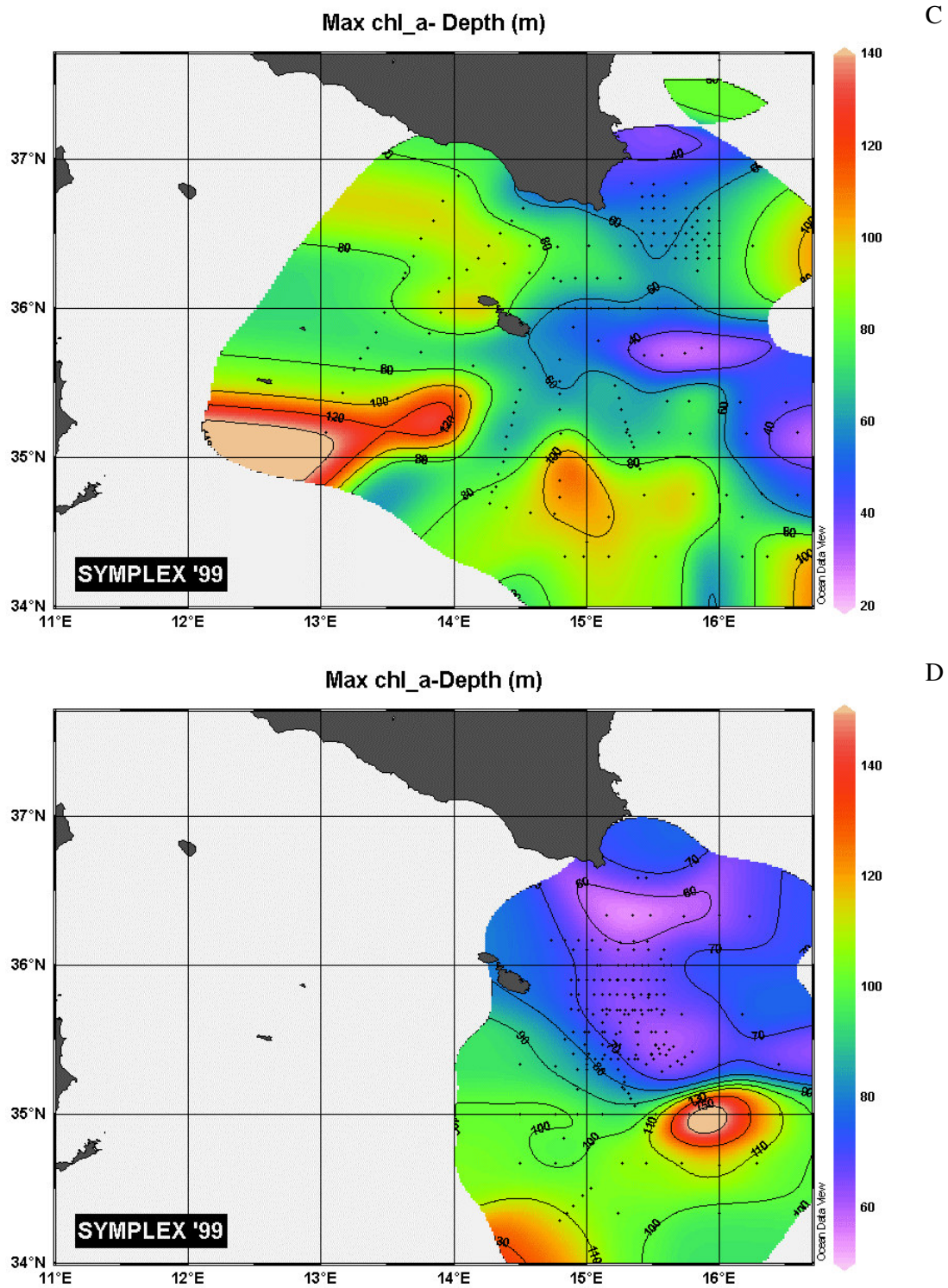


Fig. 3.14 (c, d) Depth distribution of maximum concentrations of Chl-a (mg/m^3), in the Channel of Sicily, during: c) SY3 Spring 1998), d) SY4 (Autumn 1999).

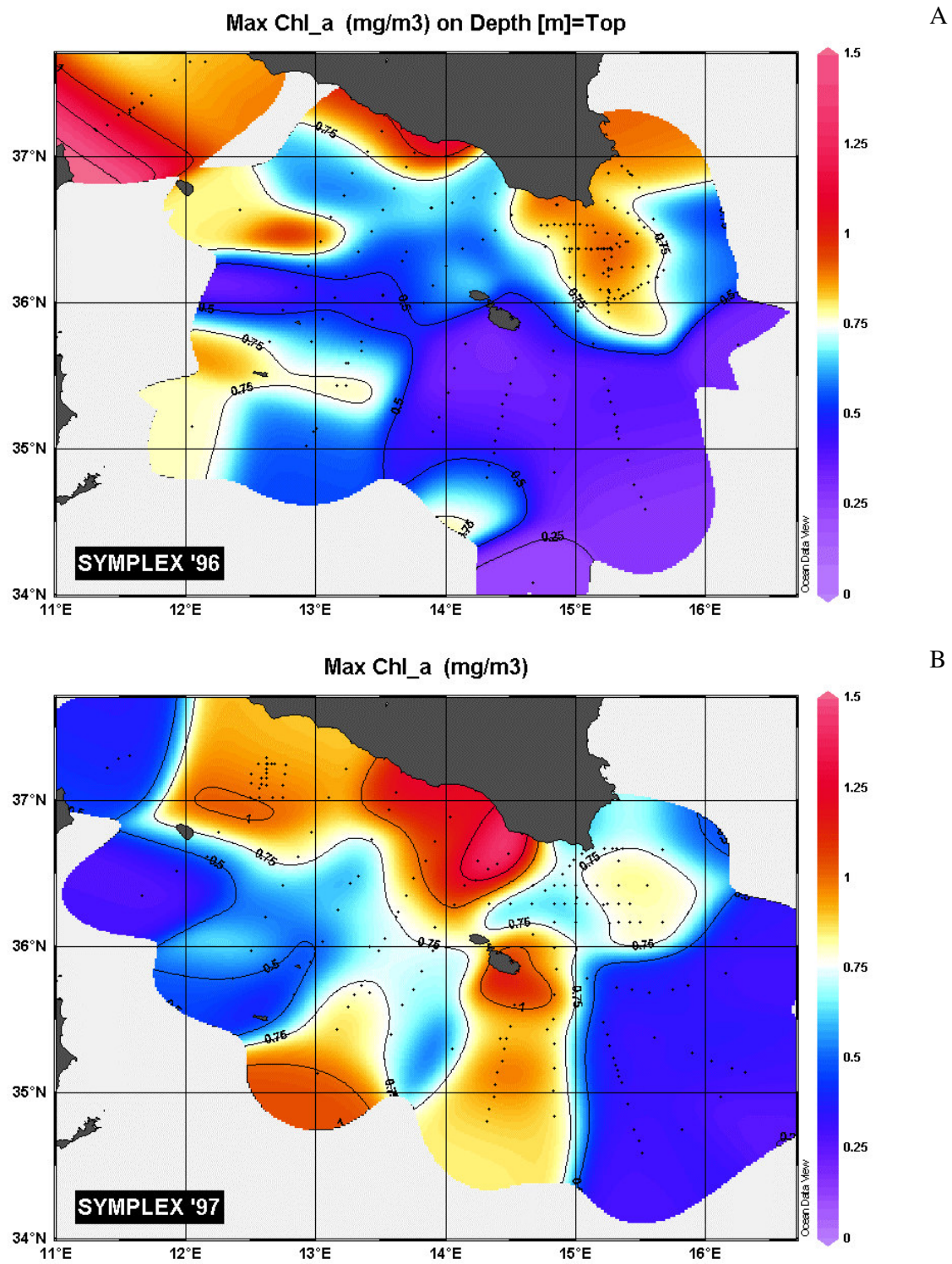


Fig. 3.15 (a, b) - Distribution of Chl-a (mg/m³) maximum concentration, in the Channel of Sicily, during: a) SY1 (Spring 1996), b) SY2 (Summer 1997).

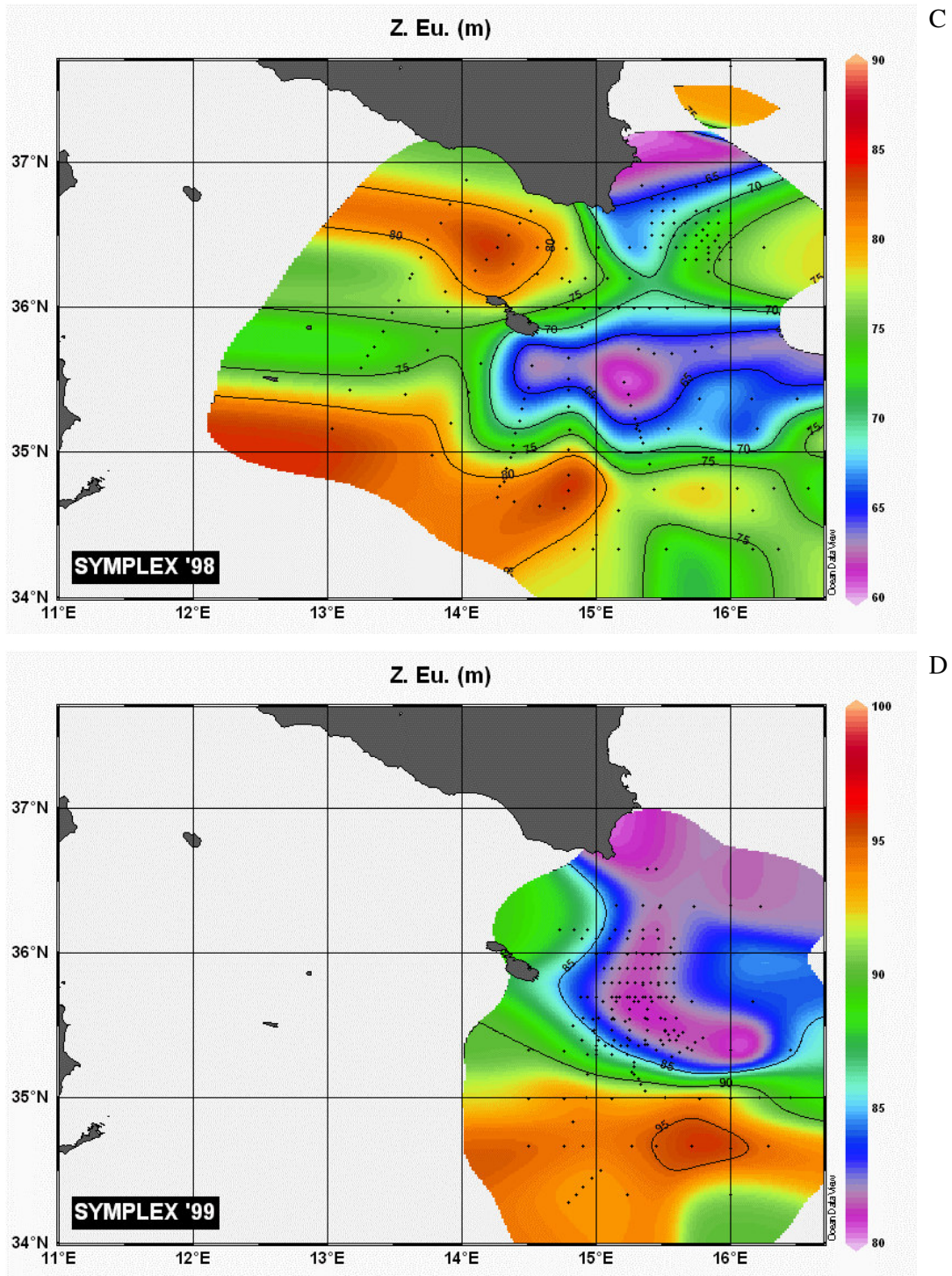


Fig. 3.16 (c, d): Depth of the Euphotic zone (Z_{eu} , m), in the Channel of Sicily, during: c) SY3 (Spring 1998), d) SY4 (Autumn 1999).

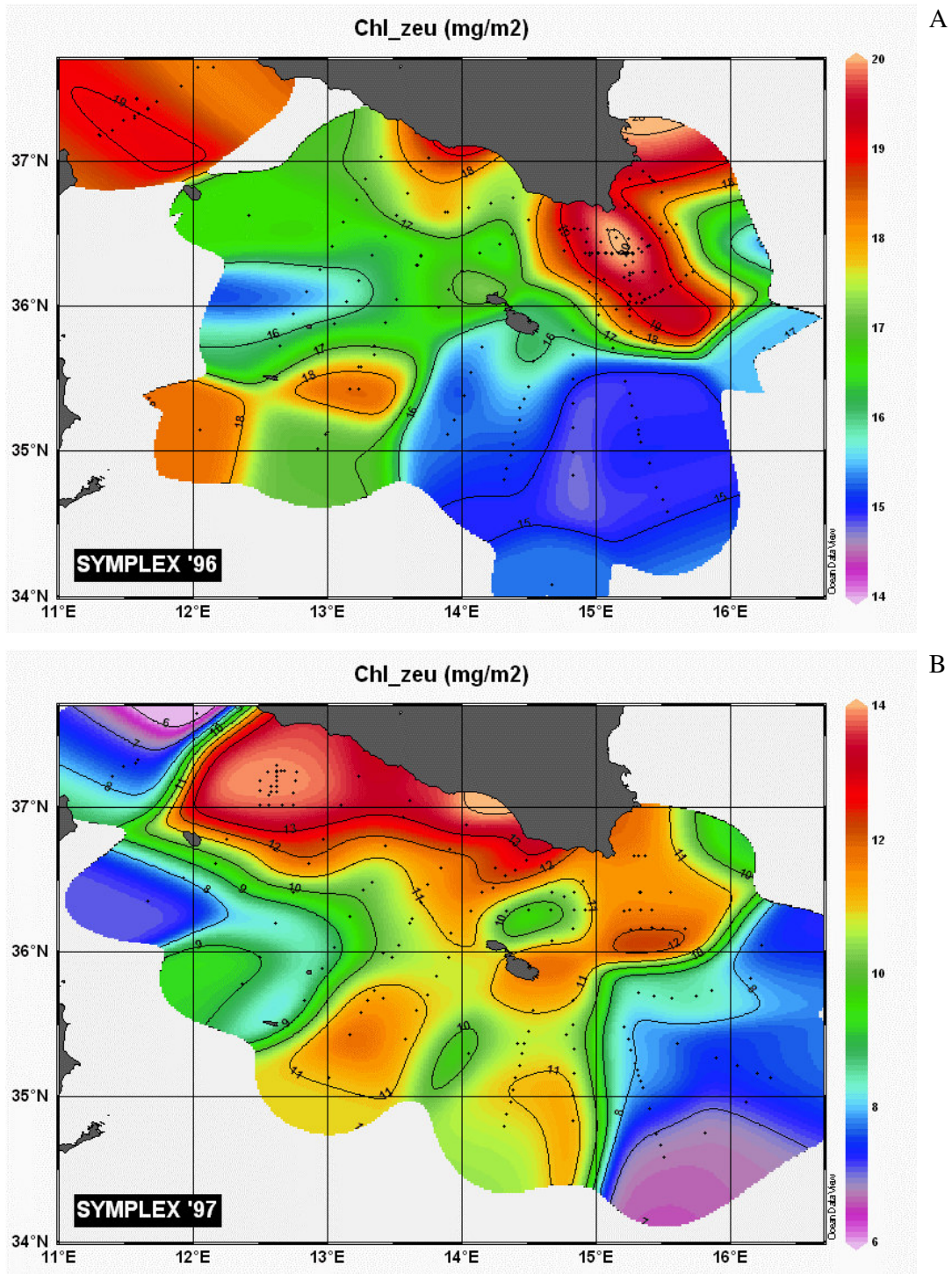


Fig. 3.17 (a,b) - Distribution of Chl-a concentration integrated over the Euphotic zone depth (Z_{eu} , m) in the Channel of Sicily, during: a) SY1 (Spring 1996), b) SY2 (Summer 1997).

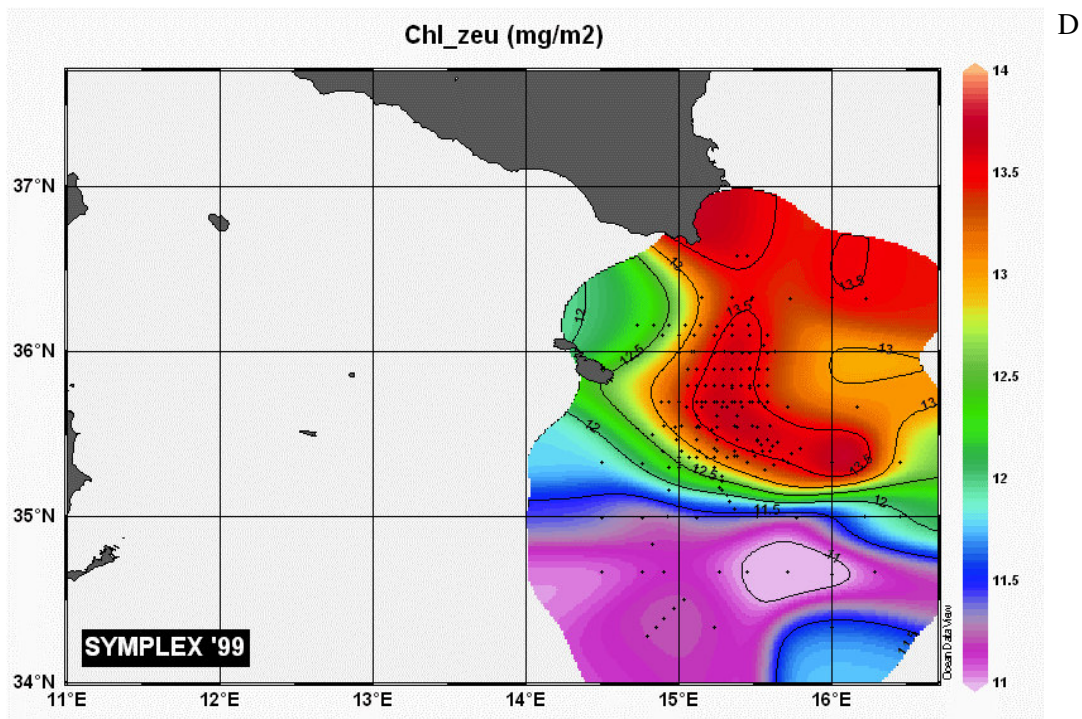
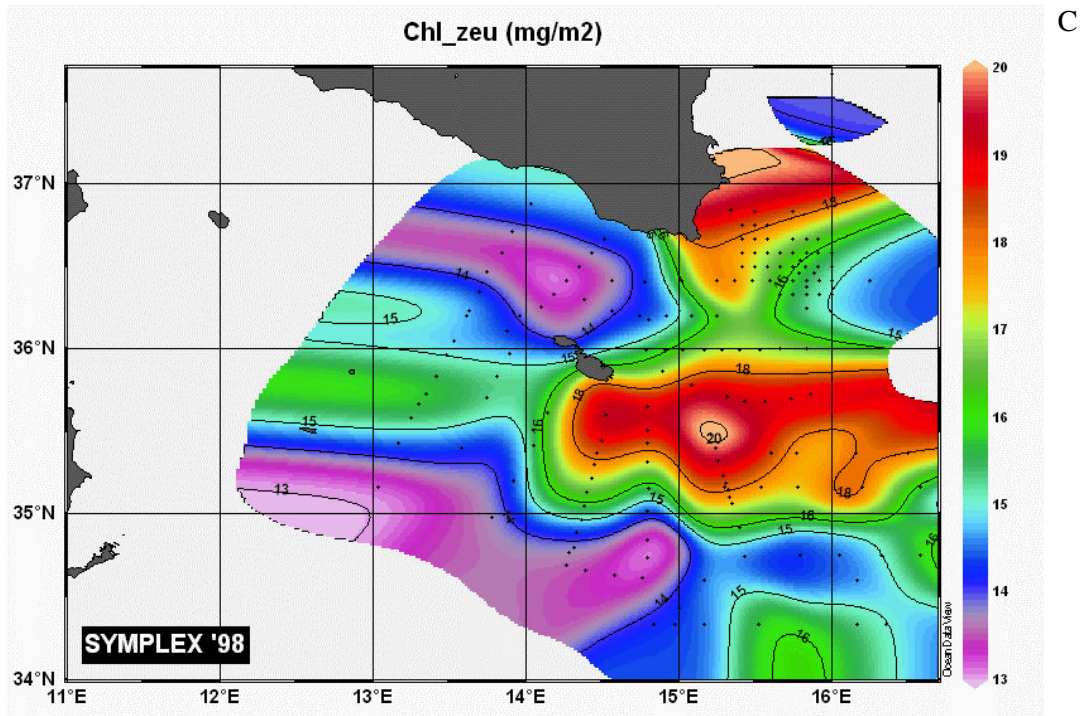


Fig. 3.17 (c,d) - Distribution of Chl-a concentration integrated over the Euphotic zone depth (Z_{eu} , m) in the Channel of Sicily, during: c) SY3 (Spring 1998), d) SY4 (Autumn 1999).

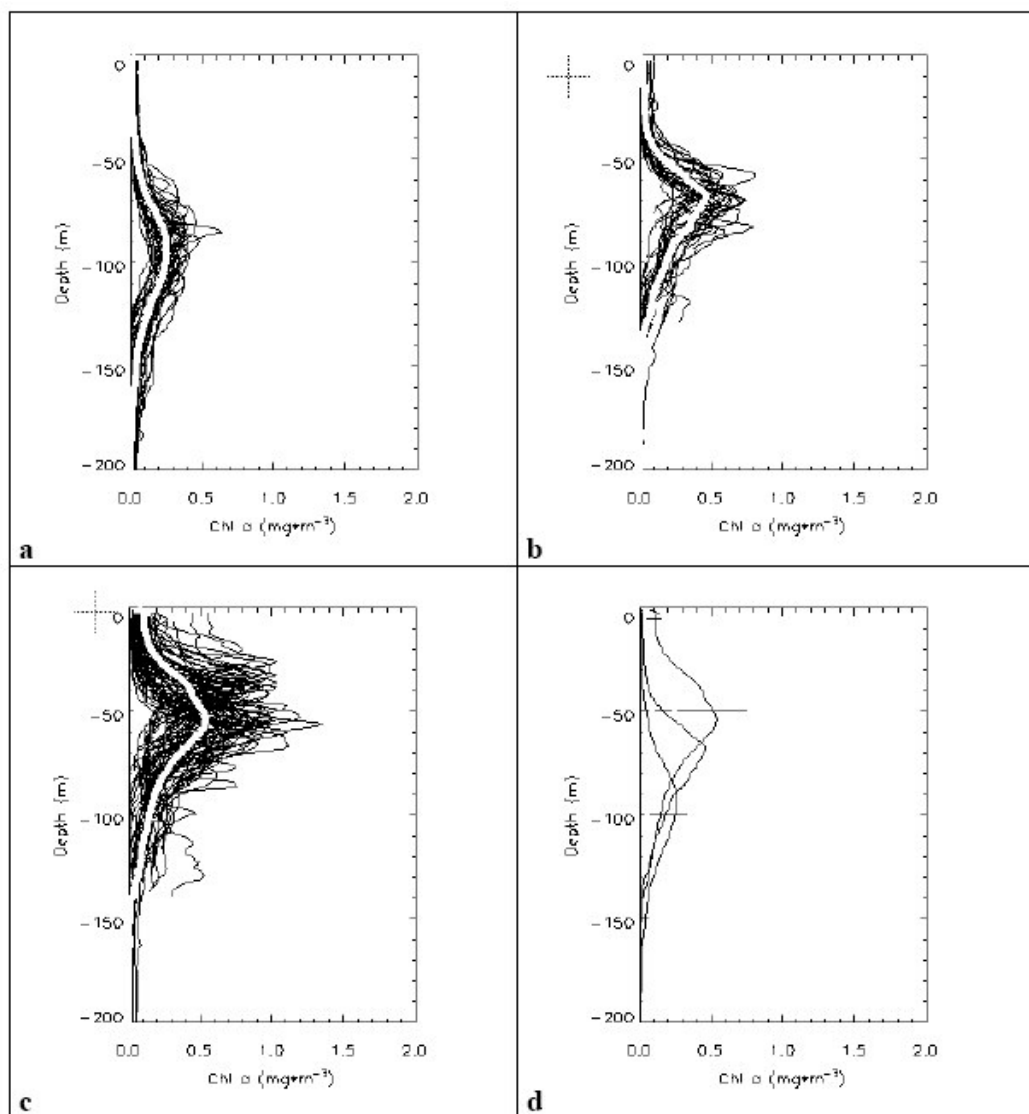


Fig. 3.18 - Chl-a profiles in the regions: SY1_0 (a); SY1_1 (b); SY1_2 (c). The average regional profile is in white in the plots a,b,c. In d): Three regional average profiles, with standard deviation bars at the depth 5, 50, 100, 150, and 195 m.

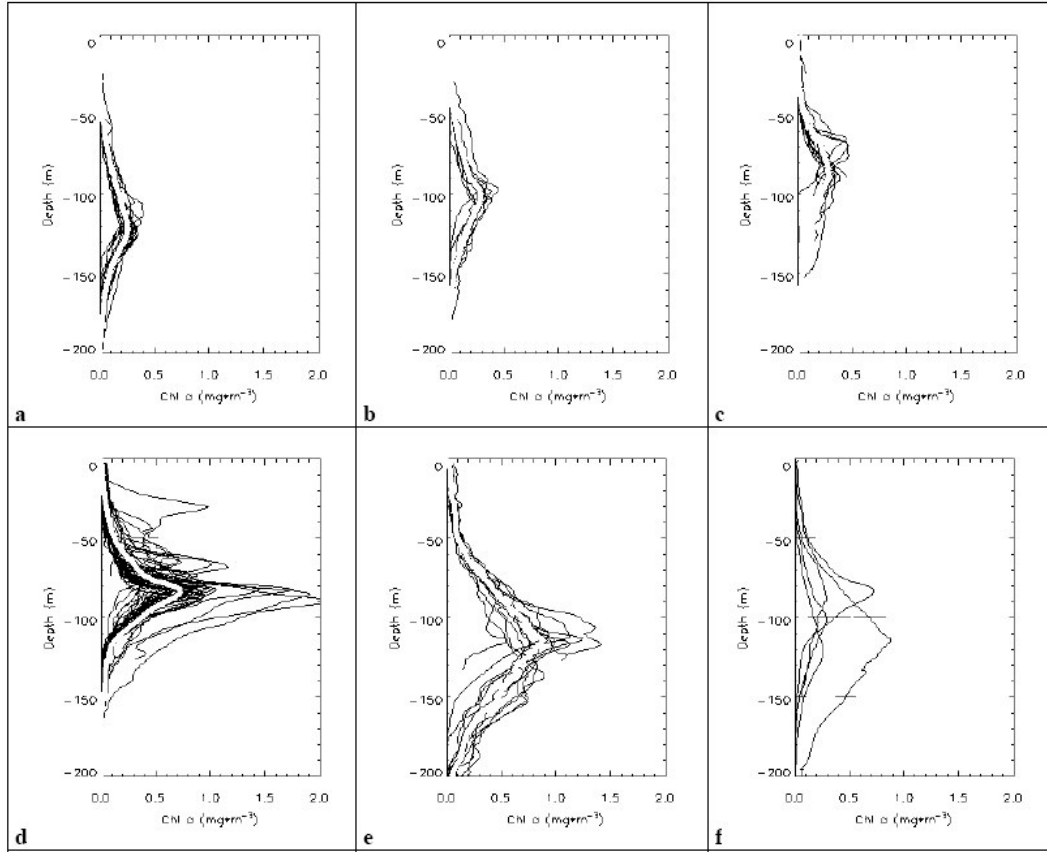


Fig. 3.19 - Chl-a profiles in the regions: SY2_0 (a), SY2_1 (b), SY2_2 (c), SY2_3 (d) e SY2_4 (e). The average regional profile is in white in the plots a-e. In f): five regional average profiles, with standard deviation bars at the depth 5, 50, 100, 150, and 195 m.

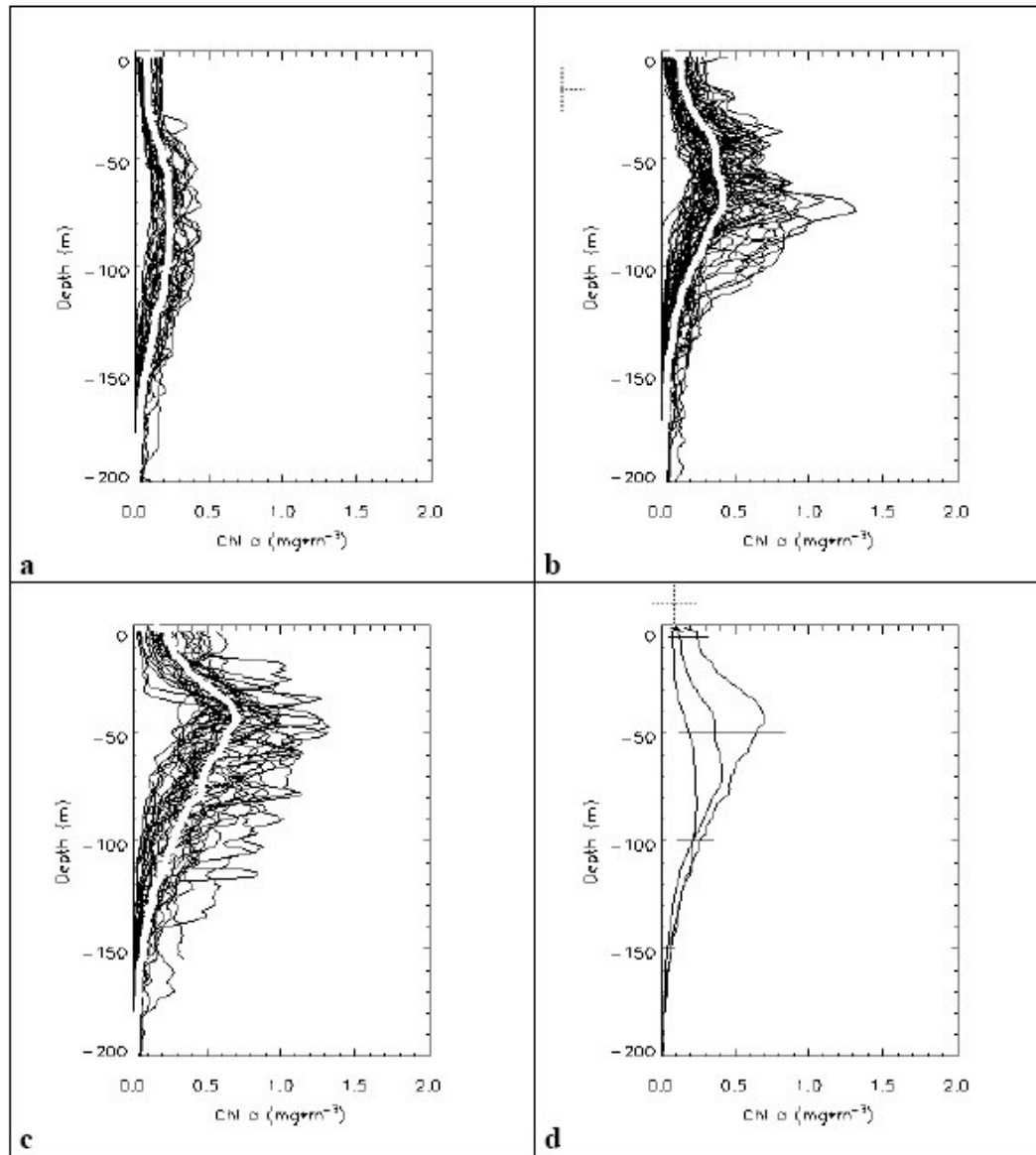


Fig. 3.20 - Chl-a profiles in the regions: SY3_0 (a), SY3_1 (b) eSY3_2 (c). The average regional profile is in white in the plots a-c. In d): three regional average profiles, with standard deviation bars at the depth 5, 50, 100, 150, and 195 m.

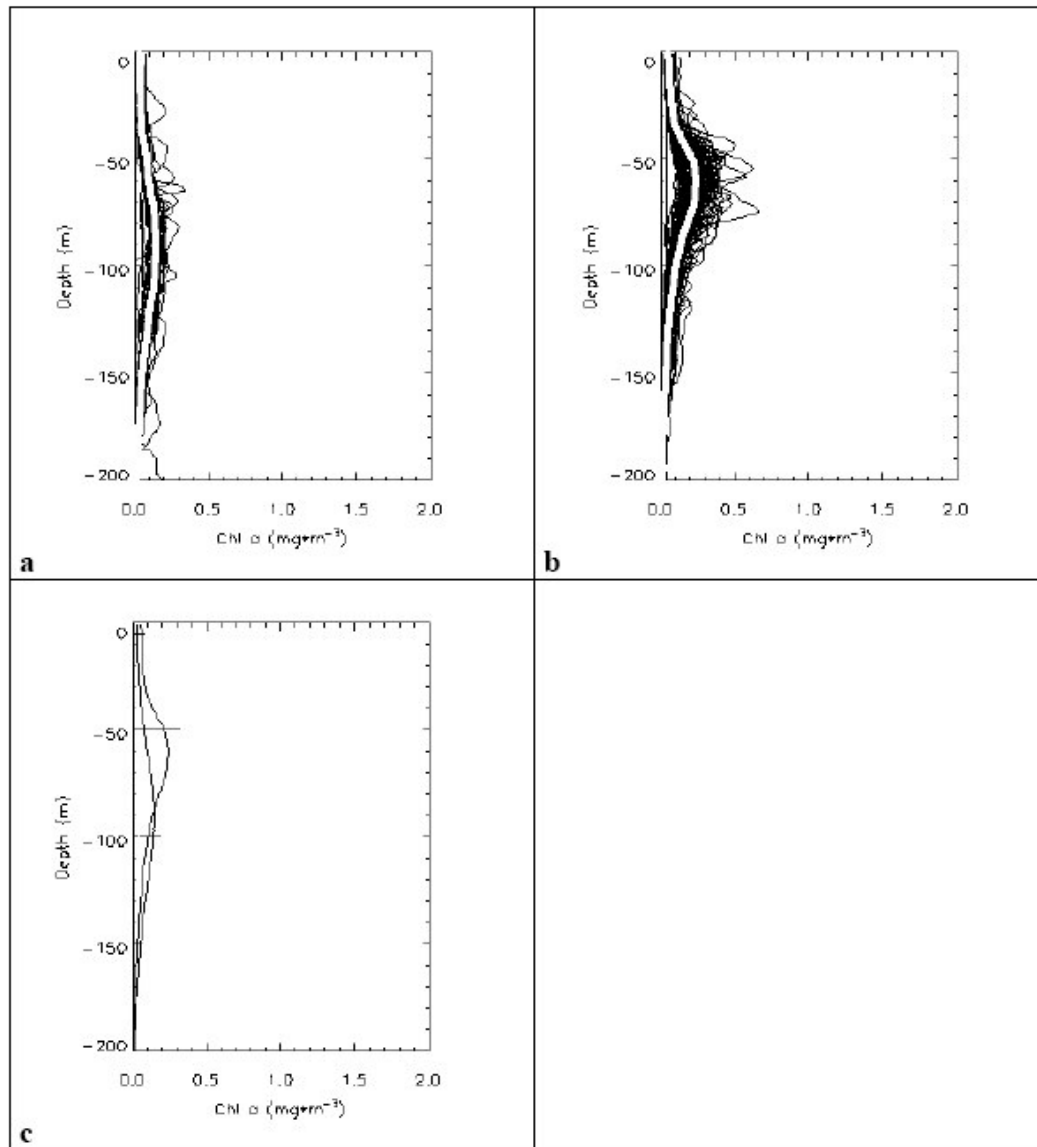


Fig. 3.21 (a,b,c) - Chl-a profiles in the regions: SY4_0 (a) e SY4_1 (b). The average regional profile is in white in the plots a-c. In c): two regional average profiles, with standard deviation bars at the depth 5, 50, 100, 150, and 195 m.

From each depicted “sub-region”, we have selected the stations where profiles of variable fluorescence (FRI), PAR irradiance, and chlorophyll-a were simultaneously collected and available for the application of the Phyto-VFP model of primary production. In the case of SY2 we could only retrieve 2 stations where all variables were simultaneously collected, despite 5 sub-regions were identified. In the case of SY3, Chl-a samples were not taken, but Chl-a biomass was evaluated from the general regression with fluorescence. We describe in tab. 4.5 the station numbers where we could apply the Phyto-VFP model, and the variables we could adopt in the calculations. These stations are then reported on Fig. 3.22.

	SY1_0	SY1_1	SY1_2	SY2_0	SY2_3	SY3_0	SY3_1	SY3_2	SY4_0	SY4_1
Station #	138	185	101	28	64	71	147	5	37	166

Tab. 3.5 - Stations numbers where we could apply the Phyto-VFP model, per campaign and per region.

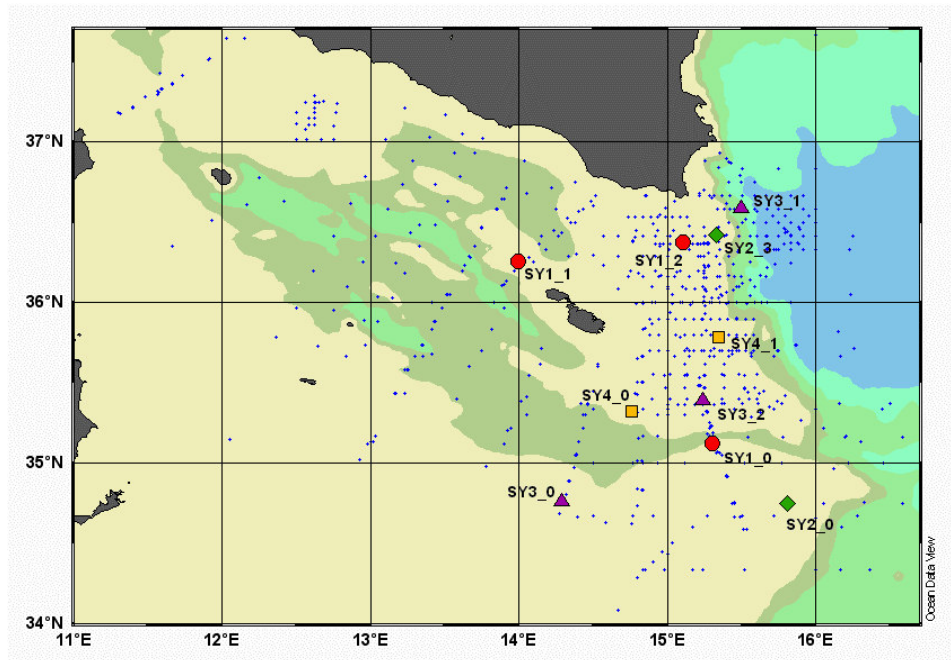


Fig. 3.22 Selected stations for the application of the Phyto-VFP model.

The results of the Phyto-VFP model show higher production rates at the end of winter (200-370 mg C m⁻² d⁻¹), with relatively sustained production rates in spring and autumn (70-170 mg C m⁻² d⁻¹), and minima (35-85 mg C m⁻² d⁻¹) during the summer months. Results are reported in tab. 3.6.

	April			July		March			October	
region	SY1_0	SY1_1	SY1_2	SY2_0	SY2_3	SY3_0	SY3_1	SY3_2	SY4_0	SY4_1
PP mg C m ⁻² d ⁻¹	104	169	72	34	84	197	368	369	72	137

Tab. 3.6 - Primary production estimates from Phyto-VFP model, in the Channel of Sicily, in stations from SYMPLEX 1996-1999 campaigns (SY1, SY2, SY3, SY4). Each station represents a sub-region, distinct on the basis of trophic conditions.

Seasonal variability of PP on an annual basis has been reconstructed by interpolation of average results per campaign (Fig. 3.23) and assuming they were representative of the season when they were collected. Because the stations chosen for the calculation represented a variety of trophic conditions, in each campaign, we assumed that the average values of PP for each campaign are representative of the Channel of Sicily average PP.

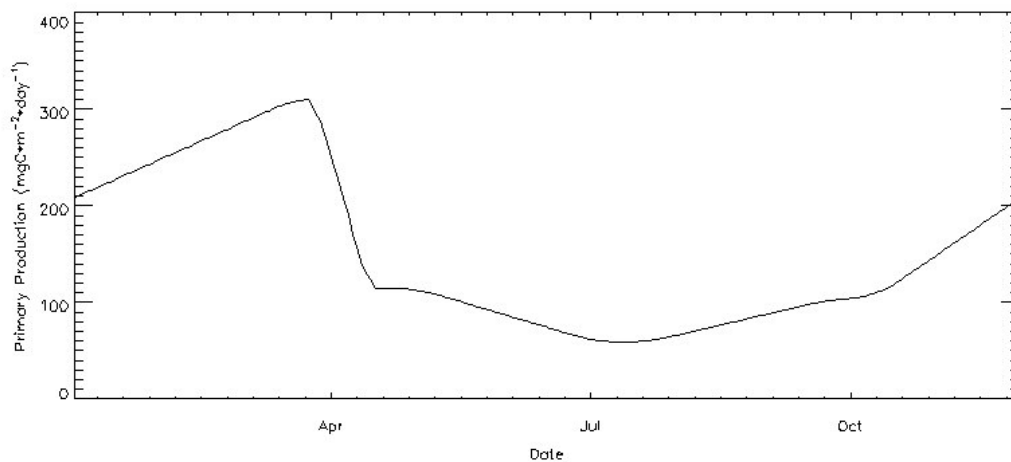


Fig. 3.23: Seasonal and annual primary production trends, according to estimates from Phyto-VFP model.

Results are coherent with expected seasonal variations in primary production rates, as described in Antoine et al. (1995), and Bricaud et al. (2002), for the Ionian basin above 35 deg. N. These authors adopted remote sensing applications for their estimates of PP, with results usually highly constrained by light availability. This might be the origin of the summer peak of PP they report, for the North Ionian basin as well as for the whole Mediterranean Sea, which does not appear in our results.

3.4 DISCUSSION

Comparisons with PP rates evaluated by other authors (Antoine et al., 1995; Bricaud et al., 2002) for the Ionian basin and the whole Mediterranean Sea confirm that our regional estimates are within the range of basin-scale estimates, and that also the annual trends of production are in agreement with the basin cycles (Bricaud et al., 2002).

In particular, PP integration over the whole year in the SYMPLEX area results in $54.35 \text{ g C m}^{-2} \text{ yr}^{-1}$, which is a typical value of oligotrophic areas. This results has the same magnitude as the ^{14}C estimates of PP by Boldrin et al. (2002), in a Ionian station off shore from the coast of Calabria ($61.8 \text{ g C m}^{-2} \text{ yr}^{-1}$). Bricaud et al. (2002) estimated a third of this value for the East Mediterranean, while Antoine et al. (1995) estimated half of the production in the Ionian Basin. Beside the differences in the techniques adopted by these authors (ocean color remote sensing), we want to remark that the latter estimates are for a much larger portion of marine environment, most of which is a open ocean. Mesoscale variability, and upwelling phenomena in the Channel of Sicily (Bohm et al., 1998) easily account for higher production rates in the blooming seasons (Videau, 1995; Estrada, 1985). In the Arcipelago Toscano, Innamorati et al. (1995) have reported annual PP rates of $104 \text{ g C m}^{-2} \text{ yr}^{-1}$, from the application of the bio-optical model of Morel and Berthon (1989).

An appropriate validation of the model can only be achieved with a larger application of the model, and the simultaneous availability of traditional (^{14}C) estimates of PP. On such a dataset, we will be able to perform robust statistical analysis on the full sampling area. On the other hand, our approach to modeling of PP in the coastal environment has produced encouraging results, with an overall agreement with scientific literature.

CHAPTER 4.0

Validation of ocean color radiometric retrievals (MODIS-A), in the optically-complex waters of the lower Chesapeake Bay.

4.1 BACKGROUND

By virtue of their location, the coastal ocean and atmosphere are transition regions, which can be highly complex and variable (Chang et al. 2002). In addition to well-known climatic variability (run off, stratification, etc.), event-scale phenomenon can dramatically change these systems on scales of hours to days. Because they are relatively dynamic, coastal oceans are also highly productive, and can be turbid. Remote sensing of ocean color in these Case II environments (Morel and Prieur, 1977; Gordon and Morel, 1983) is therefore a non-linear, multivariate problem, which needs accordingly designed algorithms in order to accurately deliver products from the observations. (S. Sathyendranath, 2000). *In situ* observations are necessary to validate the observation products, and to the development of regional-specific bio-optical algorithms. In-water observations have been the standard measurement technique for ocean color products validation. However, thanks to improvements in instrumentation, and to the automation of measurements, which can be carried out under less than ideal conditions, and due to the absence of some relevant drawbacks of in water continuous observations such as fouling, above water optical monitoring stations are becoming a viable alternative and highly desirable method for confirmation of AOP observations, or for satellite products validation (Gould et al. 1999, Mobley 1999, Hooker et al. 2002).

Satellite spectral radiometers provide information on the water properties and its constituents from the detection of water-leaving radiance, L_w , which is typically a small portion (<10-12%) of the total radiance signal received by satellite sensors. In the Chesapeake Bay, as in many coastal areas, even the fundamental assumptions of global algorithms break down, such as the accuracy of atmospheric correction, and the validity of the “black pixel assumption” (zero water-leaving radiance in the near-infrared part of the spectrum) (Gould et al. 1999, Hu et al. 2000, Ruddick et al. 2000, Siegel et al.

2000). Coastal waters can be highly absorbing or highly scattering compared with Case I waters, due to significant contributions from terrestrial colored dissolved organic material (CDOM), and/or sediments from run off, or re-suspension. Erroneous correction of atmospheric effects compromise retrievals of water-leaving reflectance, particularly in the violet and blue, often yielding negative values, and badly correlating to *in situ* measurements, with larger errors at higher chlorophyll concentration (Gould et al. 1999, Siegel et al. 2000, Hooker et al 2002, Hooker and Morel 2003).

A comprehensive time-series of coastal ocean (in-water and above-water) and atmosphere observations is necessary to reach the goals of accurate retrieval of ocean color products from space. Noteworthy examples are from the observation tower in the Adriatic Sea (Zibordi et al., 2002a; Zibordi et al., 2002b, Berthon et al. 2002, Hooker et al. 2002), and from the Boussole project at Dyfamed (Antoine et al., 2007). There are surprisingly few bio-optical observations from local coastal waters near the mouth of the Chesapeake Bay in the literature, and even fewer from the atmosphere. A suite of atmospheric observations were initiated at the Chesapeake Light Tower (CLT), offshore and 25 km East of the mouth of the Chesapeake Bay, in mid-1999, with NASA Langley's Clouds and Earth's Radiant Energy System Ocean Validation Experiment (COVE). The COVE site has a Baseline Surface Radiation Network (BSRN), at the CLT, sanctioned by the World Meteorological Organization. There is also a Cimel sun photometer at the CLT, from the Aerosol Robotic Network (AERONET) (Zibordi et al., 2006; Holben et al., 1998). For the marine environment, the Chesapeake Bay Plume Study (Superflux 1980) and the Chesapeake Outflow Plume Experiments ("COPE") (Johnson et al. 2001) are exceptions.

4.2 METHODS

4.2.1 – Study Area

The Chesapeake Bay undergoes a seasonal cycle of freshwater discharge that peaks in winter-spring, with occasional sub-peaks in summer-fall. The Chesapeake Light Tower (CLT; -75.713° E, 36.903° N), is just 25 Km offshore and East of the mouth of the Chesapeake Bay (Fig. 4.1a). This platform lies within the 20 m isobath (Fig. 4.1b, detail), with a mean depth of ~ 11 m, and prevailing continental atmosphere. The forces of freshwater run-off and semidiurnal tidal mixing combine with shallow bathymetric features to create a primary frontal zone, from inside the estuary, along the Chesapeake Channel to Cape Henry, which extends to the coastal margins of Virginia and North Carolina [Harding, 1994; Mann and Lazier, 1996; Harding et al, 2005]. Historical records confirm that the Chesapeake Bay outflow plume frequently extends beyond the Chesapeake Light Tower during the wet seasons but rarely in the dry one [Arnone and Gallacher, 1996; Austin, 2002]. Thus, the presence of water types (Case I and II) at the CLT varies, depending upon season (wet vs. dry) and tide cycles, with the result of an optically complex environment.

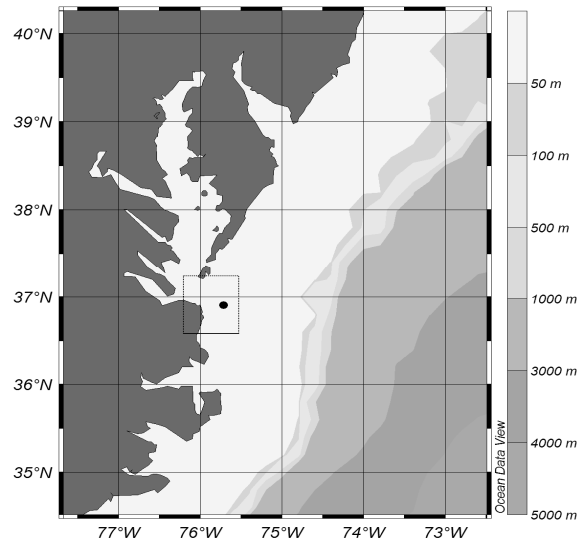


Fig. 4.1.a – Location of the study area, at the Chesapeake Bay Mouth, on the Eastern coast of the USA. The black dot represents the location of the Chesapeake Light Tower (CLT; Lon. -75.713° E, Lat. 36.903° N).

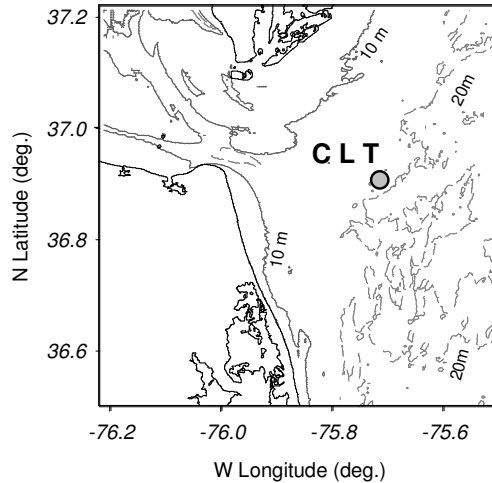


Fig. 4.1.b - The CLT area is expanded in this map, to show its bathymetric details, and the location of the Chesapeake Light Tower (CLT).s

4.2.2 - Observation Platforms

The radiometric and biological observations reported here were carried out during three years (2004-2006), from three different observation points: the Chesapeake Light Tower platform (CLT) (Fig. 4.2); the research vessel F.Slover operated by Old Dominion University; the “MODerate resolution Imaging Spectroradiometer” (MODIS) on NASA Earth Observing System (EOS) satellite Aqua.

In situ observations from the R/V Slover, encompassed above-water apparent optical properties (spectral remote sensing reflectance, $R_{rs\lambda}$; spectral water leaving radiance, $L_{w\lambda}$), as well as underwater inherent optical properties (spectral absorption coefficient, a_λ). Other measurements included phytoplankton pigments (chlorophaeopigments), and particulate suspended matter (PSM).



Fig. 4.2 – The ‘Chesapeake Light Tower’ (‘CLT’: -75.713° Lon. E; 36.903° Lat.N), photographed from the R/V F. Slover. This platform, which once served military purposes, is today maintained by the US Coast Guard. A suite of optical sensors is hosted on the very top of the tower, serving the Aerosol Robotic Network project, the CERES-OVE experiment, as well as our MODIS-A validation experiment.

4.2.3 Above-surface AOPs

Above-surface spectral radiance and irradiance, from which we calculated normalized water leaving radiance (nLw_λ) and remote sensing reflectance (Rrs_λ), are collected daily at the Chesapeake Light Tower (CLT), by Multi SAS sensors (Satlantic, Inc.) mounted on the tower, about 38 m above the sea surface (Fig. 4. 2). MultiSAS sensors recorded daily from July 2003 until March 2005, with interruptions due to instrument re-calibration and maintenance, or to problems related with all aspect of the

observatory maintenance (communications, software, power supply). The system acquires simultaneously the downwelling plane irradiance above the water ($E_d(0+)$), the sky radiance (L_{sky}), and the total radiance leaving the water surface (L_t), at $\sim 90^\circ$ azimuth angle and 40° nadir/zenith angle, in thirteen channels (380, 400, 412, 443, 490, 510, 532, 555, 665, 683, 700, 780, and 865 nm). The data reported here were acquired daily, for 45 minutes, around local solar noon, and within 2 hours from MoDIS-A passage overhead, as recommended by many authors (Hu et al, 2004; Bayley and Werdell, 2006). Only recordings within 2 standard deviations from the 30 min. set were eventually averaged. The upper limit of the variation coefficient (“cvar”) for these averages was 20%. Atmospheric correction of R_{rs} was made by assuming all photons at 780 nm entering the L_t sensor are scattered by the atmosphere and propagated through the whole visible bands (Mueller et al, 2002):

$$R_{rs} = [(R_{rs\lambda})_{raw} - (R_{rs\ 780})_{raw}] \quad (4.1)$$

Ground-based observations from the CLT were compared to images of normalized water leaving radiance (nLw), and R_{rs} , collected by the MODIS-A sensors aboard the NASA/EOS satellite. Daily scenes of ocean color of the Chesapeake Bay from MODIS-A (reprocessing 1.1 Jan. 2005) were obtained from the NASA “Ocean Color” web page: ‘<http://oceancolor.gsfc.nasa.gov/>’. Requested scenes were from daytime acquisitions (c.a 01:30 pm), in raw, level 1A format, 5min/1km granules, in the typical ocean color nominal wavelengths (nm): 412, 443, 488, 531, 551, 667, 678, 748, 869. L1A files were processed to Level 2 (geolocated, geophysical values) using the Multi-Sensor level 1 to 2 processing code (MSL12), distributed with the SeaWiFS Data Analysis System (SeaDAS), version 5.0, through IDL routines, and remapped at 1km spatial resolution at Nadir, on an equirectangular grid covering the entire Chesapeake Bay. L2 images were masked with standard OBPG L2 flags, corrected for atmospheric effect using the 765/865 model selection, with NIR and BRDF correction on, and ‘out of band - F_o (F_{0ob})’ correction on (Thuillier et al., 2003). For comparison with our in situ data (CLT), we averaged the values of a selected 5x5 pixel box, centered on the CLT coordinates, where minimum number of valid pixel=npix/2, variation coefficient < 0.20 (Morel and Gentili, 1996; Thuillier et al., 2003; Bailey and Werdell, 2006; Volpe et al., 2007) .

4.2.4 - *In situ* Chlorophyll-a and phaeopigments

Pigment concentrations reported in this study are relative to surface (ca. 2 m) waters from the CLT location, sampled with Niskin bottles. Three replicates of 100-400 ml seawater were filtered through 25mm GF/F filters (Whatman), directly on board, for both HPLC and fluorometric analyses. HPLC samples were preserved in liquid nitrogen and sent to Horn Point Laboratories for processing, according to the methods in Van Heukelem and Thomas (2001), and Hooker et al. (2005). Chlorophaeopigments for fluorometric dosage were extracted from the filters using acetone 90% (v./v.), at -20° C, for 24 hours [Parsons et al, 1984]. Samples were dosed through an analog Turner Designs fluorometer, calibrated with Chlorophyll-a standard (Sigma). A regression was drawn between values obtained from the two methods, as to allow all calculations in this work to be referred to HPLC total chlorophyll-a ($T_{Chl-a} = [\text{Monovinyl Chlorophyll-a} + \text{Divinyl Chlorophyll-a} + \text{Chlorophyllide-a}]$), also in the few cases these data were missing:

$$TChl-a = 0.96 Chl-a + 0.83 \quad (4.2)$$

$$r^2=0.89, n=21.$$

4.2.5 Absorption coefficients

Surface water samples were also collected to assess the spectral inherent optical properties of CLT waters. Measurements followed the recommendations of NASA's ocean optics protocols (Mitchell et al., 2002, and references therein). Absorption of total particles [$a_p(\lambda)$] and non-pigmented particles [$a_d(\lambda)$] was determined by the filter pad technique, before and after methanol extraction, using a Shimadzu UV2401 scanning spectrophotometer fitted with an integrating sphere, or a UV160 scanning spectrophotometer, using an opal glass diffuser [Kishino et al., 1985; Mitchell et al., 2002]. Phytoplankton absorption [$a_{ph}(\lambda)$] was calculated as [$a_p(\lambda) - a_d(\lambda)$]. CDOM absorption [$a_g(\lambda)$] was determined in a 10 cm quartz cuvette referenced to a Milli-Q pure water blank after water samples had been filtered through a 0.2 μm Nucleopore® membrane. Null corrections were made by subtracting the means at 790 to 800 nm and 690 to 700 nm for particulate and CDOM absorbance, respectively. The pure water

absorption spectrum [$a_w(\lambda)$] of Pope and Fry [1997] was used for all calculations performed here.

5.2.6 Diffuse attenuation coefficient (490) and optical penetration depth (OPD)

The diffuse attenuation coefficient for downwelling irradiance is calculated from MODIS-A observations as (O' Reilly et al, 2000):

$$K_{490} = K_{w490} + A [L_w \lambda_1 / L_w \lambda_2]^B \quad (4.3)$$

Where:

K_{w490} = diffuse attenuation coefficient for pure water = 0.016 m⁻¹

$\lambda_1 = 488(490)$; $\lambda_2 = 551(555)$

$A = 0.15645$; $B = -1.5401$

K_{490} (m⁻¹) indicates the turbidity of the water column – how visible light in the blue-green region of the spectrum penetrates within the water column. It is directly related to the presence of scattering particles in the water column. It is also a proxy of PAR penetration in the water column. For this reason, we calculated the optical penetration depth (OPD; m) from K_{490} , as $1/K_{490}$ (Bricaud et al., 1995).

4.2.7 Statistical Analysis

With the purpose to describe the empirical relationship between remotely sensed reflectance and *in situ* phytoplankton biomass, we regresses the log-transformed average values of *in situ* surface TChl-a with radiometric log-transformed max ratios of remote sensing reflectance acquired from the CLT, through a reduced major axis regression of *in situ* TChl-a measurements with contemporary ($\pm 1h$) Rrs observations. The statistical coefficients of the correlation were evaluated.

This relationship, if significant from a statistical point of view, would become the operational expression of a new empirical bio-optical algorithm (OC3CB later in the text) for the region of the mouth of the Chesapeake Bay.

For comparison, we have also applied different standard global chlorophyll-a algorithms to the radiometric observations of the CLT sensors:

1) The standard MODIS-A algorithm for Chl-a retrieval, OC3M (O'Reilly et al. 2000), the operational form of which is:

$$\text{TChl-a (mg/m}^3\text{)} = 10^{(0.283-2.753R+11.457R^2+0.659R^3-1.403R^4)} \quad (4.4)$$

where: $R = \log_{10}(\text{Rrs443} > \text{Rrs488} / \text{Rrs551})$

2) The OC4V4 algorithm (O'Reilly et al. 1998), the operational form of which is:

$$\text{TChl-a (mg/m}^3\text{)} = 10^{(0.368-3.067R+11.930R^2+0.649R^3-1.532R^4)} \quad (4.5)$$

where: $R = \log_{10}(\text{Rrs443} > \text{Rrs490} > \text{Rrs510} / \text{Rrs555})$

In order to evaluate the performance of the different algorithms, we have carried out analyses of their respective root mean square error (RMS), bias and percent difference, as in Volpe et al. (2007) (Tab. 4.1).

In addition, we have modeled the behaviour of a “pseudo” MODIS-A product, the water leaving radiance at 510 nm (Lw510). From the regression of CLT radiometric measurements of Lw at 510 nm against the average values of contemporary Lw acquisitions between Lw 490nm (band3: 490 ±10 nm) and Lw 532 (band 4: 532±10 nm), we obtained a strong correlation ($r^2 = 0.998$; see the “Results” section, below). We therefore regressed CLT radiometric measurements of Lw at 510 nm against the average values of MODIS-A Lw between Lw 490nm (band3: 490 ±10 nm) and Lw 532 (band 4: 532±10 nm).

RMS - Root mean square error It indicates the spread of data as compared to the best agreement.	$RMS = \sqrt{\frac{\sum_{i=1}^N (Alg_i - C_{Mi})^2}{N}}$	(4.6)
Bias - Mean bias error.	$BIAS = \frac{1}{N} \sum_{i=1}^N (Alg_i - C_{Mi})$	(4.7)
RPD - Relative percent difference It is the mean percentage difference between modeled and observed values. It is an estimate of the uncertainty of a modeled value as a function of the observed value.	$RPD = \frac{1}{N} \sum_{i=1}^N \left(\frac{Alg_i - C_{Mi}}{C_{Mi}} \right) \times 100$	(4.8)
APD - Absolute percent difference It is the difference between the model estimate and the measurement, weighted on the measured value. It has no direction (positive or negative).	$APD = \frac{1}{N} \sum_{i=1}^N \left \frac{Alg_i - C_{Mi}}{C_{Mi}} \right \times 100$	(4.9)

Tab. 4.1 – Summary of the statistical equations adopted in this analysis (after Volpe et al., 2007).

4.3 RESULTS

4.3.1 - Bio-optical and biogeochemical properties

TChl-a ranged from 0.7 to 6 mg/m³, CDOM concentration was between 0.13-0.28 1/m, and suspended matter loads were between 0.5-6 mg/l. The annual patterns of variations in the biogeochemical properties of seawater describe the typical dynamics of mid-latitude coastal environments, with two annual relative biomass peaks in spring (March-April) and autumn (October-November). The relative contribution of the various components of seawater to the absorption coefficient, at the CLT location, is summarized in the ternary plot of Fig. 4.3.

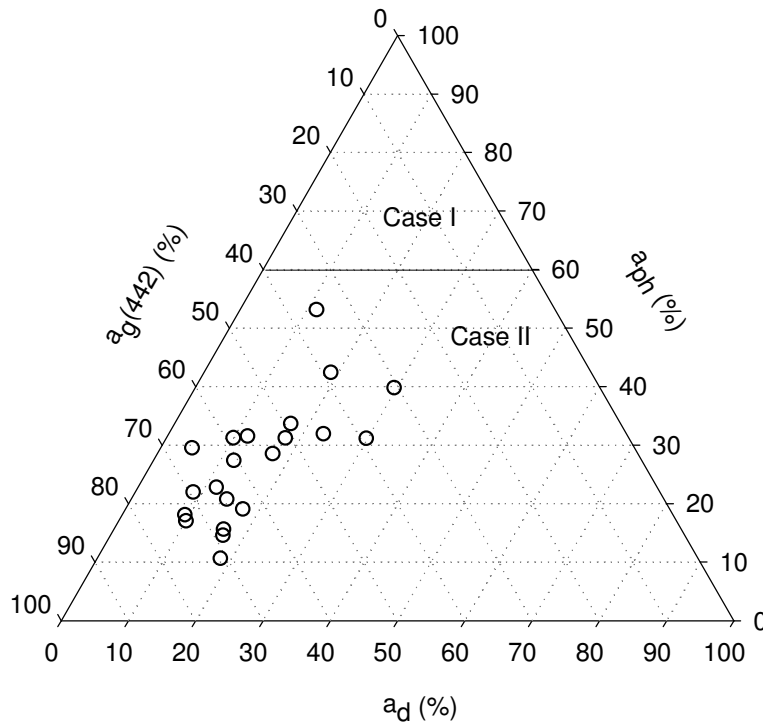


Fig. 4.3 - Biogeochemical properties of the seawater at the CLT site. The ternary plot illustrates the relative contribution of CDOM (a_g), detritus+minerals (a_d), and phytoplankton (a_{ph}), to total absorption, at 442 nm. Case I water is defined as the upper part of the triangle where $a_{ph}(442) > 60\%$ of $[(a_g + a_{ph} + a_d)(442)]$. The bottom part of the triangle represents Case II water. All samples ($n=21$) describe the CLT waters as a Case II environment.

The absorption coefficient appears largely dominated by the CDOM signal (a_g), while detritus+minerals (a_d), and phytoplankton (a_{ph}) usually only reach 30% in most cases, and do not co-vary with CDOM. In all cases, the water properties of this region clearly describe a Case II environment. The CLT area is exposed to the estuarine outflow of the Bay, which export significant amounts of dissolved and re-suspended particulate materials into coastal waters (Robertson and Thomas 1981, Gingerich and Oertel 1981). While the concentration of particulate inorganic materials diminishes rapidly in plume surface waters (Gingerich and Oertel 1981), as well as chlorophyll concentration, which is highest in the plume and usually declines seaward onto the shelf (Robertson and Thomas 1981), the concentration of soluble materials is rather conservative. These variations in the biogeochemical properties compare well with ocean color observations, either from the CLT optical station or from space observations. MODIS-A nLw ranges are between $-0.20 \div 2.65 \mu W/cm^2/nm/sr$. Maximum values are mostly recorded at 531nm, with greener peaks (551 nm) during the wet season, due to phytoplankton concentration. Maximum spectral values of nLw shift towards the blue (488 nm), during the summer (Julian days 150-280; June-October), when the output from the river is at the lowest (Fig 4.4, a-c). The dominance of the blue wavelengths in the nLw spectra in this period confirms the absence of freshwater contribution at the CLT location. In the summer, both TSM and TChl-a regularly present their minimum values, though locally formed CDOM is still a relevant contributor to light absorption (Fig. 4.5).

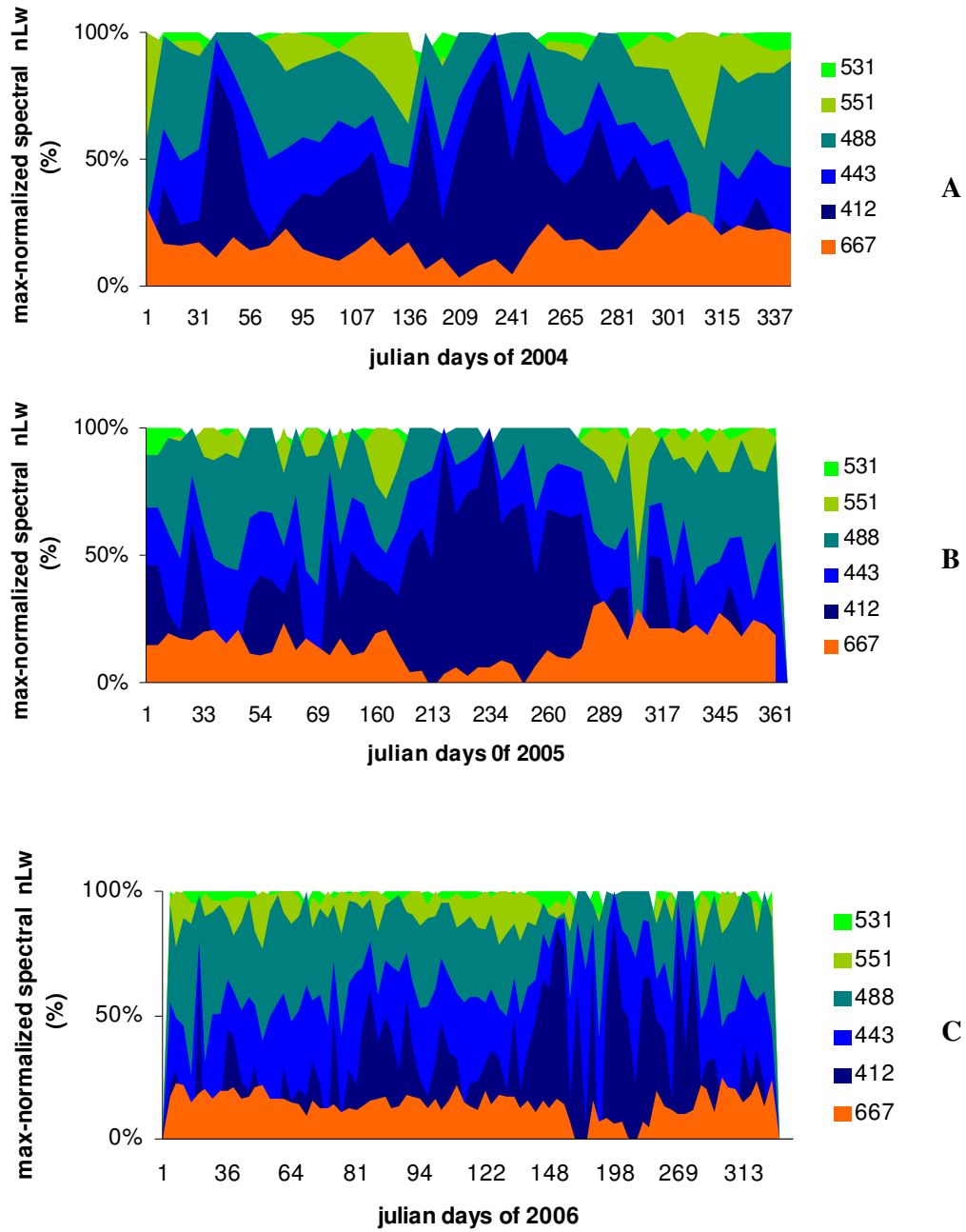


Figure 4.4 - Temporal patterns of spectral nLw maxima from MoDIS-A reveal variability in the dominant spectral bands around the CLT. During winter, spring and fall, nLw is dominated by green bands (532, 551 nm) but nLw is dominated by blue bands (488 nm) in summer. Each plot is relative to a different year: a) 2004, b) 2005, c) 2006.

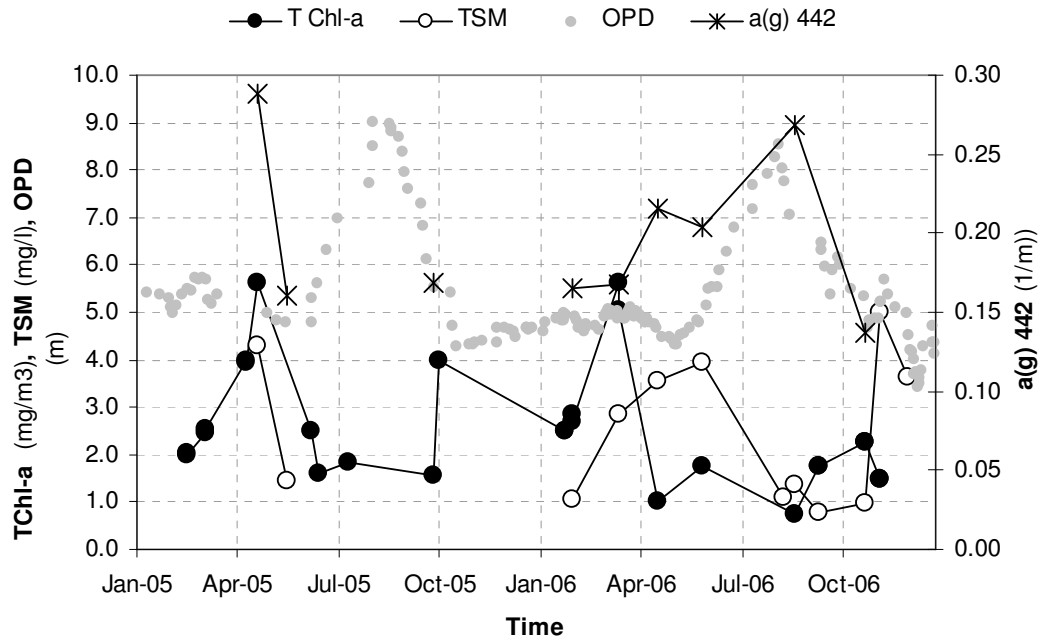


Fig. 4.5 – Year round distribution of biogeochemical properties in the CLT water: Chl-a concentration (mg/m^3) from HPLC analysis of *in situ* samples (T Chl-a); Total Suspended Matter concentration (mg/l), from *in situ* samples (TSM); concentration of CDOM ($1/\text{m}$), from absorption of filtered seawater samples, at 442 nm ($a_g 442$). On the secondary axis, we report the optical penetration depth (OPD, m), calculated from MODIS-A product K490 as $1/K490$, to show the impact of the biogeochemical cycles on water transparency, i.e. the effect of water absorption components on light penetration in the water column).

Accordingly, the optical penetration depth of downwelling irradiance at 490 nm, derived from MODIS-A product K490, is almost twice as deep in the summer than in the rest of the year, reaching c.a 10 m (Fig. 4. 5). This can raise concern as for the bottom-reflection contribution to the nLw signal captured from the tower's, or the satellite's, sensors, because the bottom depth at the CLT location is only around 11 meters, on average. Though calculation of bottom reflectance contribution to nLw in this area might deserve a deeper investigation, for our scope we have considered that the contribution of bottom reflectance to nLw is negligible, because the waters appeared optically shallow in less than 2% of retrieved satellite images ($n=200$), over three years (Fig. 4.6).

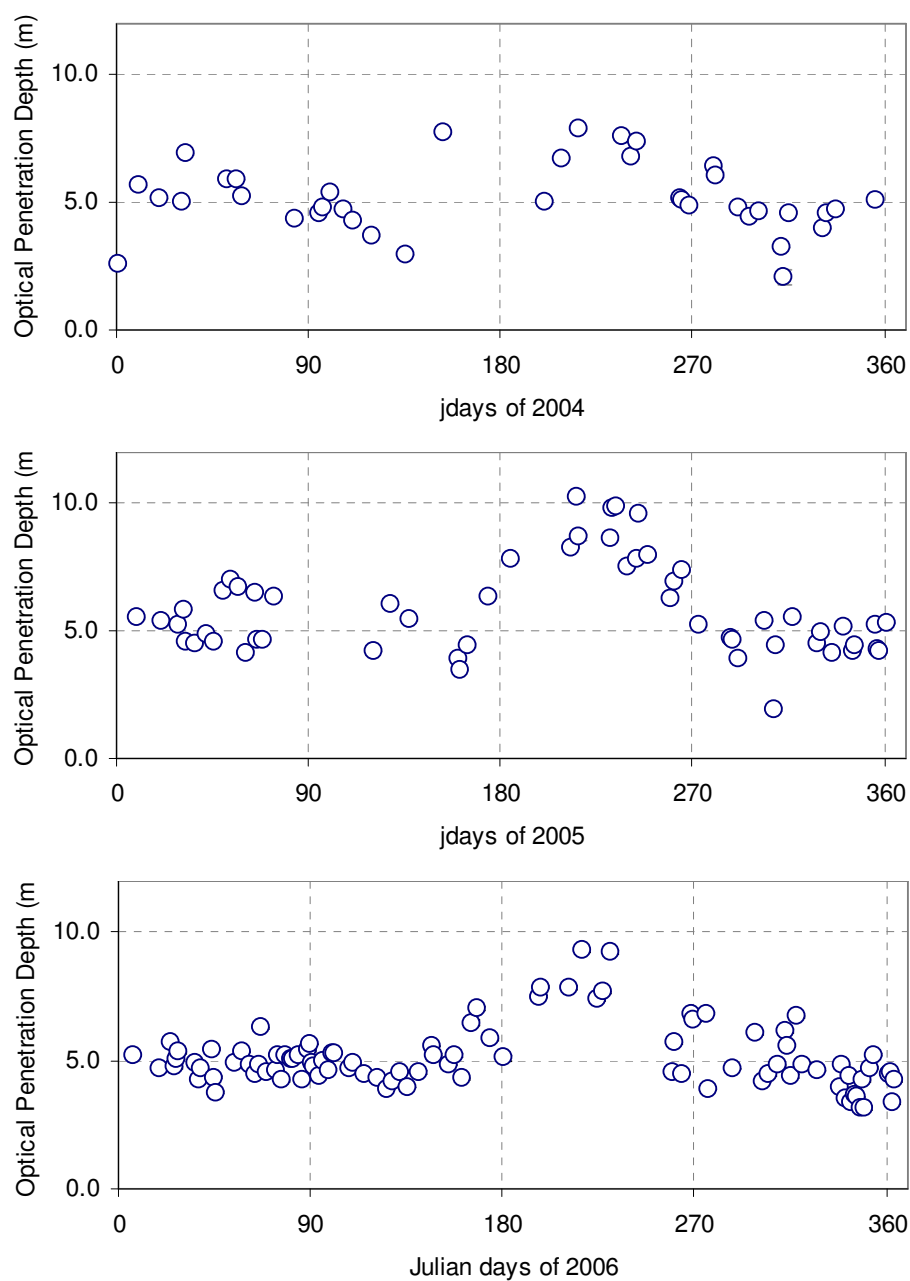


Fig. 4.6 - Optical penetration depth (OPD, m) of downwelling irradiance at 490 nm, at the CLT location, in the period a) 2004, b) 2005, c) 2006. OPD is derived from the MODIS-A product 'K490', as $1/K_{490}$, and it is almost twice as deep in the summer than in the rest of the year, reaching c.a 10 m.

4.3.2 - Correlation of tower optical sensors with MODIS-A sensors

We used the optical data recorded at the CLT to validate MODIS-A optical retrievals. Rrs values at the CLT are in the range $0.000 \div 0.023 \mu\text{W}/\text{cm}^2/\text{nm}/\text{sr}$ for daily acquisitions over a three year period (2004-2006). Reduced major axis regressions between simultaneous acquisitions from the two systems, in analogue bands, for each band separately show that nLw signals acquired from MODIS-A sensors are consistently lower than measured by CLT sensors (Fig. 4.7). A summary of the statistical analyses of these relationships is reported in tab. 4. 2 .

Wavelength (nm)	n	APD	Median ratio	RMS	r^2	m	q
ALL BANDS	318	30.449	0.774	0.255	0.89	0.778	0.001
412	51	49.367	0.682	0.275	0.204	0.711	-0.006
443	51	25.668	0.835	0.210	0.510	0.710	0.097
488	51	26.964	0.798	0.266	0.850	0.727	0.091
532	51	22.443	0.817	0.259	0.922	0.838	-0.026
551	51	33.447	0.749	0.353	0.929	0.757	-0.002
667	51	38.055	0.724	0.089	0.676	0.714	0.004

Tab. 4.2 - Summary of the match-up statistics between the acquisitions of spectral nLw ($\mu\text{W}/\text{cm}^2/\text{nm}/\text{sr}$) from MODIS-A and from the optical sensors at the CLT locations, during a three year period (2004-2006). Slope, intercept and r^2 are calculated from reduced major-axis regressions. NIR correction is on. Pixel coverage is 100% (n=25). APD = Median absolute percent difference; Median ratio = Median of the ratios of satellite observations over local detections; RMS = root mean square error (spread of data as compared to the best agreement); r^2 = coefficient of determination; m = regression slope; q = regression intercept.

From the diagrams in Fig. 4. 7, it is evident that the slope of the MODIS-A vs. MSAS relationship is lower than the unity, in all bands, with a median ratio of the all-bands relationship of 0.774 (n= 318). Regression coefficients are larger than, or equal to, 0.850 ($r^2 \geq 0.850$) only in the blue-green bands, 443, 490 and 551 nm, while the retrievals in the blue bands appear the most unreliable ($r^2 \leq 0.510$), especially at 412nm ($r^2 = 0.204$). It's worth noting that the central bands, 443-555nm, which are the best correlated to ground signals, are also the bands implemented in the bio-optical algorithms for retrieval of biomass from space.

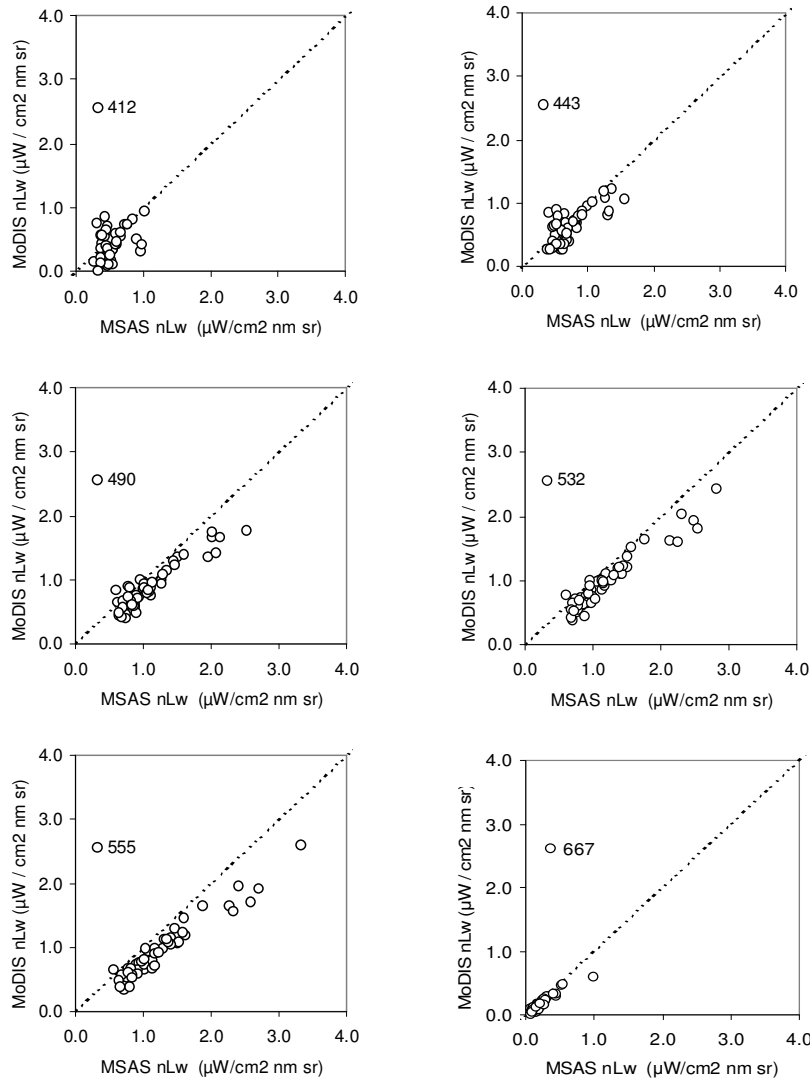


Fig. 4.7 - Diagrams of spectral nLw signals acquired from MODIS-A vs. simultaneous (± 1 h) acquisitions at the CLT location, during a three year period (2004-2006). NIR correction is on. Pixel coverage for MODIS-A images is 100% (25pixels on 5x5 1-Km resolution box centered over the CLT). Number of samples (n) in each diagram is 51.

4.3.3 - Correlation of tower optical sensors with Chl-a concentration

Results from the analyses of the biogeochemical properties of the waters surrounding the CLT compelled us to develop a regional algorithm for this optically complex case II environment. Since we could validate the retrievals of nLw from MODIS-A against the CLT sensors, and CLT observations were less influenced by

atmospheric effects, we have used CLT acquisitions of Rrs to develop a new bio-optical algorithm to retrieve Chl-a from ocean color, with the intention to extend its applications to satellite retrievals. The concentration of chlorophyll-a from HPLC analysis (TChl-a, mg/m³) was regressed against contemporarily acquired Rrs data from the tower optical sensors. In detail, we regressed the ratio of maximum Rrs between the bands at 443, 490, and 510 nm to Rrs at 555nm [(Rrs443>Rrs490> Rrs510)/Rrs555] (Fig. 4.8). We found that:

$$\text{TChl-a} = 10^{(-2.940(R) + 0.263)} \quad (4.10)$$

$$\text{where: } R = [(Rrs443 > Rrs490 > Rrs510) / Rrs555] \quad (4.11)$$

For a sample size (n) = 26, the correlation coefficient (r²) was 0.697 (Tab. 4.2).

algorithm	r ²	m	q	RMS
OC3B	0.697	1.00	0.003	0.110
OC3M	0.608	0.789	0.013	0.157
OC4v4	0.708	0.924	0.079	0.159

Tab.4.3 - Evaluation of different bio-optical algorithms to retrieve chlorophyll-a concentration from remote sensing reflectance ratios. In the first line, the validation is referred to estimates of TChl-a from the OC3B to a subset of TChl-a data from the CLT. In the second row, the validation is referred to estimates of TChl-a from OC3M (the standard MODIS-A algorithm; O'Reilly et al., 2000) against *in situ* measurements. OC4V4 (O'Reilly et al., 1998) is the evaluation of the Tchl-a product estimated by the standard SeaWiFS algorithm against *in situ* TChl-a, at the CLT.

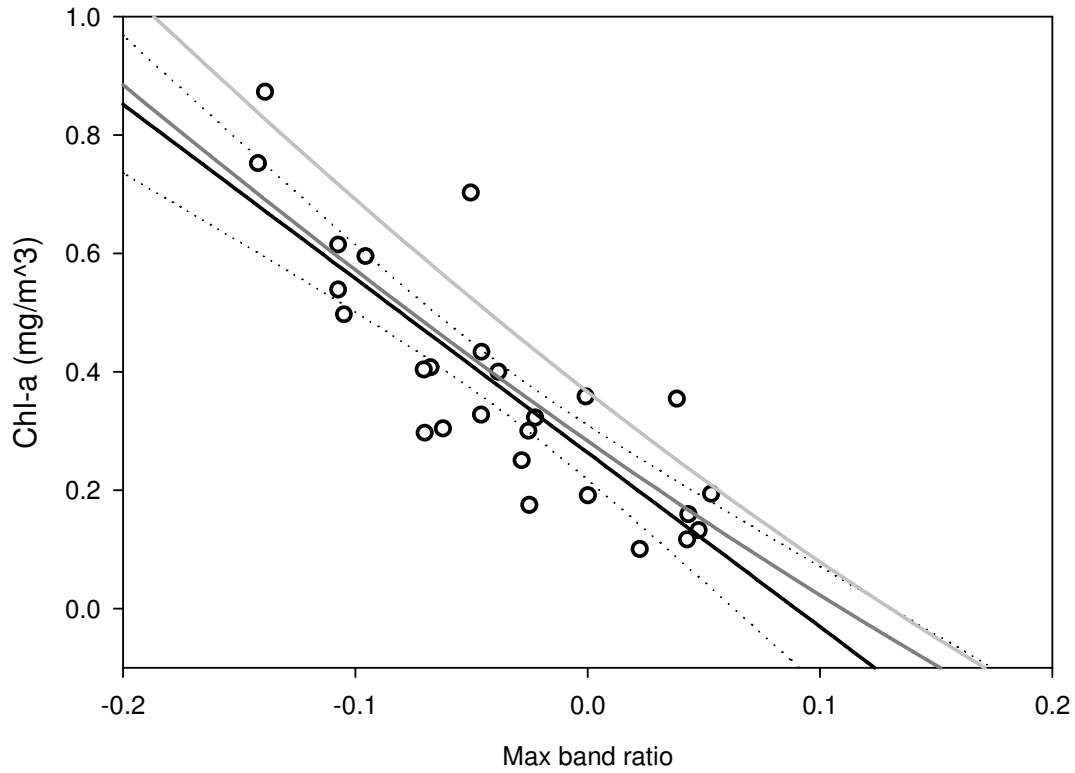


Fig. 4.8 – *In situ* Chl-a concentration at the CLT location is regressed against a maximum band ratio of remote sensing reflectance (R_{rs}), as observed by the CLT optical sensors. The open circles represent our observations, the black thick line is the regression line, the dotted lines are 90% confidence intervals. Both variables are log-transformed. $R^2 = 0.697$, $n = 26$. Chlorophyll-a concentration is from HPLC analysis. R_{rs} data are corrected for NIR contamination at 780nm. The maximum band ratio, in this case, is the ratio of maximum R_{rs} between the bands at 443 and 510 nm to R_{rs} at 555nm $[(R_{rs443} > R_{rs490})/R_{rs555}]$. We have estimated Chl-a concentration from the same maximum band ratio according to the standard MODIS-A algorithm for Chl-a (OC3M, O'Reilly et al., 2000). The result is the dark grey line, which is very close to the regression line of our observed data. We have also plotted (light-gray line) the result of the application of the OC4v4 algorithm (O'Reilly et al. 1998) to the same range of observed R_{rs} values. In this case the maximum band ratio is extended over the 443-510 nm region, and is $[(R_{rs443} > R_{rs490} > R_{rs510})/R_{rs555}]$.

We have used the standard MODIS-A algorithm for Chl-a (OC3M, O'Reilly et al., 2000) with our radiometric observations. The function of this two-band max-Rrs-ratio algorithm lies very close to the regression line of our observed data (Fig. 4.8), though r^2 is only 0.61 (Tab. 4.3), and its predictions of Chl-a show a larger RMS than our empirical regression (Tab. 4.3). We have also plotted the result of the application of the OC4v4 algorithm (O'Reilly et al. 1998) to the same range of observed Rrs values. In this case the maximum band ratio (R) is again expanded over the 443-510 nm region, and is $[(Rrs_{443} > Rrs_{490} > Rrs_{510}) / Rrs_{555}]$. This algorithm was developed for SeaWiFS sensors, which have a band in the channel 510nm, absent on MODIS-A sensors. The application of the OC4V4 algorithm to our Rrs data produces a line that fits the higher Chl-a values, but is distant from accuracy on the lower values of TChl-a (Fig. 4.8). The correlation coefficient is rather elevated, 0.708, but RMS is comparable to OC3M (Tab.4.3).

4.3.4 - Using MODIS-A images for coastal monitoring

Radiometric measurements at the CLT correlated reliably with in situ chlorophyll-a concentration, allowing the development of a regional algorithm (OC3CB), based on a three-band max ratio, like OC4v4. Estimates of biomass from OC3CB compare closely with global OC3M estimates. Nonetheless, OC3B algorithm performs better than OC3M. This is not surprising because this algorithm is based on a three max-Rrs band ratio, covering the 443-510nm range. This is of great relevance in coastal application of bio-optics, because at 510 nm the relevance of CDOM in the signal is extremely attenuated, and we can attribute most of the signal at 510nm to Chlorophylla-.

A three-max-band-ratio algorithm like OC4v4 is not suitable to MODIS-A retrievals of ocean color, because MODIS-A radiometry is not sensitive in the 510 nm range. We can only use a two max-band ratio (i.e. OC3M) when we adopt MODIS-A sensors to retrieve Chl-a. To allow the use of OC3CB or OC4v4 with MODIS-A, therefore allowing the improvement of Chl-a retrieval from this platform in coastal waters, we have studied the feasibility to make create a “ghost band” among MODIS-A sensors, at 510nm wavelength. For the purpose, from the measurements of the optical

sensors on the CLT, we have averaged the values of Lw between 490 and 532, and regressed it against simultaneous acquisitions of Lw510 (Fig. 4. 9). The regression shows a slope close to unity, an intercept close to zero and a coefficient of determination near to unity too. We have therefore modeled the measurements at 510 nm as the average of two adjacent bands, 490 and 532 nm, which are both available either from the CLT sensors or from MODIS-A sensors.

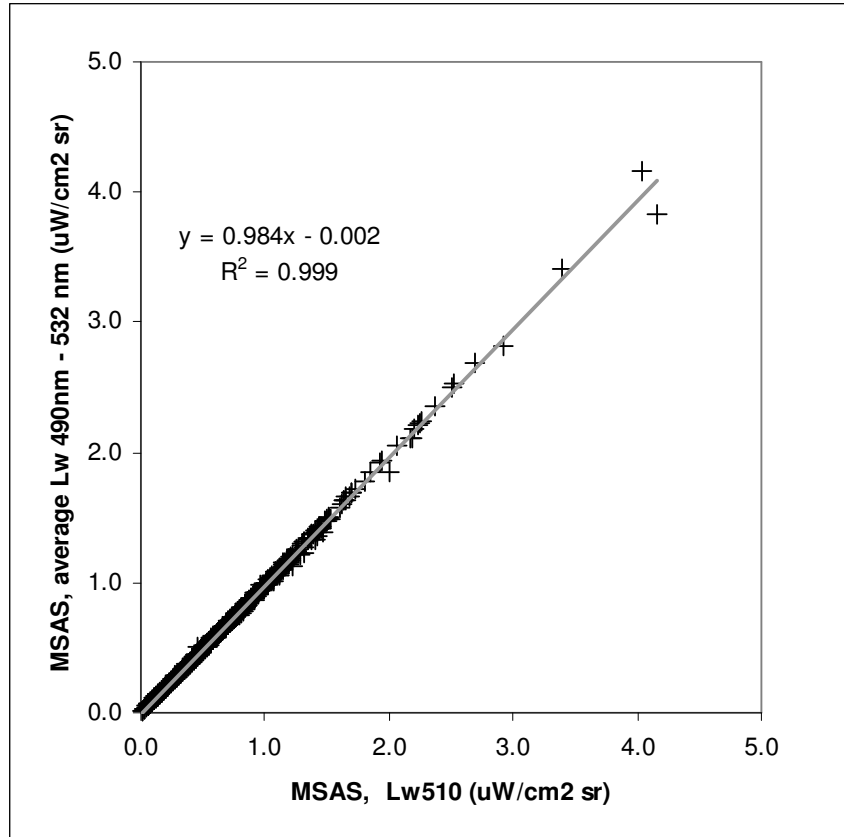


Fig. 4.9 - Use of CLT optical measurements of Lw to assess the feasibility to retrieve a reliable ‘ghost band’ at 510 nm from MODIS-A sensors. From the measurements of the optical sensors on the CLT, we have averaged the values of Lw between 490 and 532, and regressed it against simultaneous acquisitions of Lw510.

We have validated the retrieval of MODIS-A “ghost” product - nLw510, obtained from the average values of MODIS-A nLw between the bands 490 and 532, with simultaneous acquisition of nLw510 from the CLT sensors. The analysis for the

2004 time series shows a determination coefficient $r^2=0.886$, on a population of 16 quality controlled samples (Fig. 4.10)

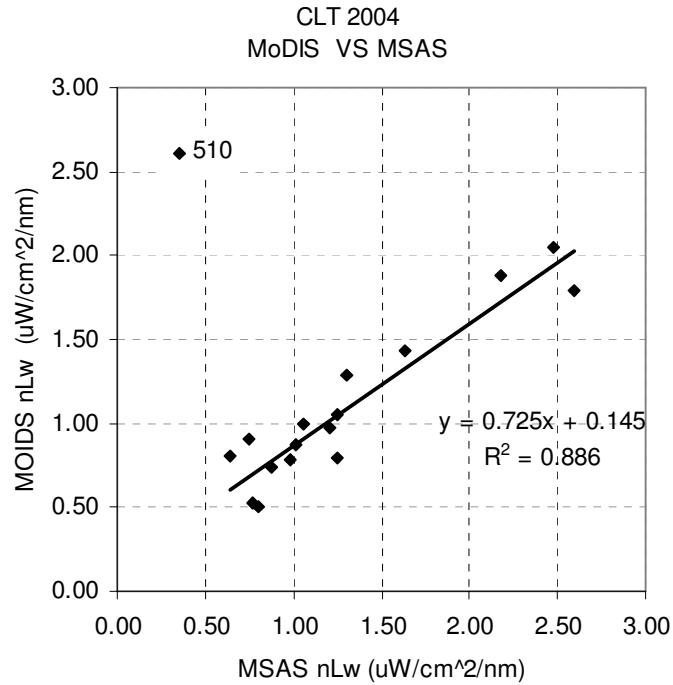


Fig. 4.10 - Validation of MODIS-A 'ghost band' from the regression of average MODIS-A values of nLw between the bands 490 and 532 with simultaneous acquisition of nLw510 ($\mu\text{W}/\text{cm}^2/\text{nm}/\text{sr}$) from the CLT sensors. We only show the analysis for the 2004 time series. $N=16$, $R^2=0.886$.

With these reassurances, we have applied the three-band max-Rrs-ratio algorithms OC3CB and OC4v4 to MODIS-A images and obtained the images in Fig. 4.11. The effect is a less saturated image (Fig. 4.11.a) with the use of our regional algorithm, in comparison to OC4v4 (Fig. 4.11.b). Both algorithm have a vocation for coastal applications, but OC3CB appears more suitable to the area of the Chesapeake Bay, with a clearer signal that allows distinctions of hydrodynamic features and detail of trophic sub-regions.

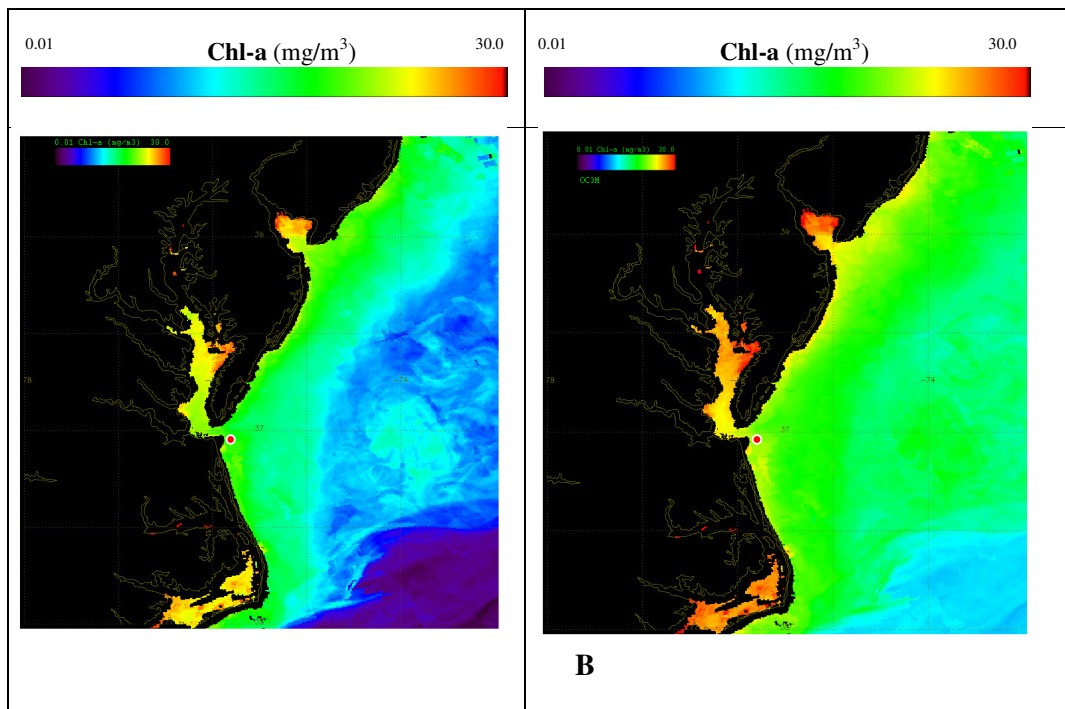


Fig. 4.11 – MODIS-A spatial distribution of Chlorophyll-a concentration (July 2006), A) with application of regional (CLT) 3-band max reflectance ratio, B) with application of global OC4v4 algorithm (O'Reilly, 1998). Red circle symbol: the Chesapeake Light Tower (CLT).

4.4 DISCUSSION

Three years of monthly bio-optical observations of the waters around the CLT location clearly indicate that these waters belong to a Case II environment where the concentration of phytoplankton does not co-vary with other components of light absorption (dissolved organic matter, sediments). Satellite radiometric observations confirm a rather shallow (3-5 m) light penetration depth, which deepens only during the summer months, to c.a 10m. As shown by the results of in situ sampling activity, the concentration of phytoplankton and suspended matter decreases in that period. Nonetheless, these waters belong to a case II environment in all occasions, as per Morel's optical classification methods (1977). In fact, CDOM absorption remains at high levels in the summer months, probably due to local formation from the degradation of biomass after the spring bloom. Historical records agree that riverine outflow seldom reaches the Chesapeake Light Tower (CLT) in summer dry season [Arnone and Gallacher, 1996]. Temporal patterns of color dominance from MODIS-A nLw spectra further confirm the absence of a riverine plume in the summer months around the CLT. During winter, spring and fall, the spectral nLw peaks are in green bands (532, 551 nm) but they shift towards the blue bands (488 nm) in the summer.

Radiometric observations from CLT sensors are well correlated with satellite observations (MODIS-A). In the match-up analysis presented here, we have used a quality-assured pixel choice for our correlations: all the pixels in each 5x5 box are valid, and variability in the average is <20%. Nonetheless, we have found (data not shown) that exclusion of satellite data where the number of valid pixels is <25 (n=57), and variability of the averages is >20%, did not improve either the regression parameters or the correlation coefficients (single-band and all-band analyses). We are therefore in agreement with the methods illustrated by Bailey and Werdell (2006), which indicate that the pixel-choice criteria can be relaxed in the coastal environment.

Instead, we have proved that, in this optically complex environment, subtraction of the signal at 780 nm to the whole spectrum (NIR correction) of local (CLT) radiometric acquisitions surely improves the correlations with satellite (MODIS-A) data. The signal in the NIR band may derive either from atmospheric contamination

(aerosols), or from the backscatter generated by sediment loads suspended in the water, or by bottom reflection.

We conducted a parallel analysis (data not shown) of the relationship of Rrs at 780nm, as acquired from different sources (a field spectrophotometer, a Satlantic in-water profiler, the CLT multi-spectral sensors), with the content of particulate suspended particulate material (PSM, mg/l). The Rrs780 signal is not related to PSM concentration. Therefore, sediment backscattering does not appear to influence the radiometric signal emerging from the water.

It seems plausible to attribute the interference at 780 nm, which propagates through the whole spectrum of above-water radiometric retrievals, to the atmosphere. On the other hand, the relationship between in-water (profiling radiometric probes) and above-water (CLT) radiometric acquisitions, separated by 48m of air (between the sea surface and the CIT sensors' position), does not show an offset. Therefore, and by exclusion, the largest part of the contamination can be attributed to higher-atmosphere effects, which do not influence tower observations, but might have an effect on satellite's view.

It is also possible that bottom reflectance contributes to the NIR signal through backscatter. This would especially happen during the summer months. In fact, the first optical depth is around 10 m, in the summer, at the CLT, and the bottom is only 1-2% deeper than that, and sandy (highly scattering). Ocean swell and tide effects could additively contribute to thinning the depth of the water, at least occasionally, allowing sunlight to reach the bottom and emerge back again from the surface.

Finally, a further hypothesis that explains why NIR correction of CLT radiometric acquisitions improves correlation with satellite data is found in a possible compensation for over-correction of the atmospheric effects by the algorithms applied on MODIS-A images. In fact, the satellite's spectral nLw signal is consistently lower than CLT retrievals, with regression slopes of the 'MODIS-A vs CLT' relationship that are lower than 1 in all bands, and are even farther from unity in the blue bands, where atmospheric disturbance is largely corrected by atmospheric algorithms, often leading to over-correction problems (Bailey and Werdell, 2006).

Radiometric measurements at the CLT correlated reliably with chlorophyll-a concentration, allowing the development of a regional algorithm (OC3CB), based on a three-band max Rrs ratio. On the other hand, the estimates of biomass from OC3CB compare closely with global OC3M estimates. The application of the global algorithm OC4v4, developed for coastal applications (O'Reilly, 2000), to the CLT radiometric measurements produced overestimates of chlorophyll-a, when compared to *in situ* actual measurements.

The original development of a semi-empirical model to calculate the signal retrieved from MODIS-A radiometers in a 'ghost-band' at 510 nm is an original finding of this work and we do not have reference to compare it to. On the other hand, our modeling has been validated two folds: first with CLT data (regression of estimated nLw510 against measured nLw510 produced: $r^2 = 0.998$, $n=51$) ; then with a regression between MODIS-A ghost product (ghost-Lw510) and CLT measured nLw510 ($r^2 = 0.886$, $n=16$). This finding opens to the application of three-band max Rrs ratios ($Rrs_{443} > 490 > 510 / Rrs_{555}$) with MODIS-A retrievals, with large potential for coastal applications.

Chapter 5.0

CONCLUSIONS

This study contributes to relevant open questions in marine ecosystem research, on the improvement of monitoring marine biomass and estimating primary production, especially in productive coastal regions of the world.

As a response to the lack of consideration to the photoacclimation process in primary production models (Bruyant et al., 2005; Geider et al. 1998; Zonneveld, 1997), the study defines the ranges of the variations of photosynthetic performance parameters as influenced by light conditions in the environment. Our study also defines that said variations are periodic, and we can exploit such periodicity towards improved predictability of photosynthetic performance in the natural environment. We have refined a bio-optical model of primary production by implementing this combination of information.

Skeletonema costatum was found to be a suitable organism to develop a model of primary production, especially for predictions in coastal environments. As demonstrated with our experiments, the photoacclimation characteristics of this diatom are in the range of an average phytoplankton organism, with predictable trends in the photosynthetic and accessory pigment content per cell (Kirk, 1994). In addition, this organism is a cosmopolitan species of phytoplankton, and a relevant component of coastal marine systems, including the Mediterranean, where it can give almost monospecific blooms, in springtime (Hulburt and Guillard, 1968; Lenzi Grillini and Lazzara, 1980). *Skeletonema costatum* can thus be adopted as a significant example of the effects of photoacclimation on marine phytoplankton production. This study also represents a step forward in the investigation of the role of taxonomy in the modeling of primary

production, which is indicated by various authors as a necessity (Juneau et Harrison, 2005; Sosik H., 1989).

The approach we adopted is also useful in contributing to the few studies on photosynthesis performed through variable fluorescence techniques. Comparative studies between fluorometer and traditional PE measurements should be urgently performed to further evaluate how these techniques compare, and where their differences lie. Traditional techniques appear more reliable, with easy-to-compare results, but optical techniques are much faster and non invasive. The positive comparisons of analogous parameters between the two methods (like E_k , αg , and growth rates), emerging from our discussion in chapter two, strongly support the adopted fluorometric approach, and encourage further studies.

Our Phyto-VFP bio-optical model introduces significant novelty in the calculation of how primary production varies according to time and spatial location of phytoplankton in the water column, accounting for the effect of photo-acclimation, which plays a relevant role in this respect. Photo-acclimation can be reached quickly in highly reproductive pico-phytoplankton, which can duplicate faster than once a day in blooming conditions, the number of generations required for these changes being around 3-4 (Arrigo, 2003). Larger phytoplankton cells, like diatoms, will undergo new generations more slowly. On the other hand, stratification and high-level nutrients can persist long in coastal environment, where blooms containing this taxon are more likely to occur. As a consequence, phytoplankton cells have enough time to adjust their photo-physiology to the existing conditions, adopting similar behaviors to those described by our experiments.

The application of the Phyto-VFP model to the coastal environment of the Channel of Sicily has produced relatively reliable results, both on the intensity of the process, as well as for its temporal dynamics. A larger effort is necessary to obtain a full model validation, but results are promising. In particular, the sub-regionalization of our extensive case-I-waters dataset, on the basis of the trophic levels (Chl-a concentration) described, was essential to express average estimates of primary production for the whole basin, in both a reliable and timely fashion. We are convinced that this approach should be pursued further to obtain more accurate estimates of primary production in

environments that appear homogeneous (Case I) from the sole application of the traditional optical classification Case I /II.

The case study at the Chesapeake Bay mouth confirms that the sole optical classification between Case I/II is not sufficient to appropriately describe a marine environment.

Conversely from the Channel of Sicily, the CLT waters clearly belong to a Case II environment, with transparency correlated mainly to variation of CDOM concentration, moderate sediment loads, and mesotrophic condition. Also, the water-leaving radiances (L_w) measured at this site in the near-infrared (NIR) are greater than zero, as expected in turbid coastal environments, and it was demonstrated that it is useful to correct for them over the whole.

Given these conditions, we were convinced of the necessity to develop a regional algorithm for this area (S. Sathyendranath, 2000). On the contrary, despite the presence of a purely Case II environment, chlorophyll-a concentration could be retrieved from radiometric acquisitions at the CLT adopting a global (and non regional) algorithm, like 'OC3M'. Uncertainties were found within acceptable limits, as per the assessment of the statistical analysis.

We attribute this phenomenon to the scarcity of documentation on the optical properties of Case II waters. We do not know, for example, how differences in the taxonomic composition of phytoplankton populations contribute to differences in absorption among different regions characterized by Case II waters (X. Pan, 2007). Similarly to the Channel of Sicily case, a sub-regionalization of this area, based both on optical and biogeochemical properties, would help in the definition of the ecological value of this environment, and allow a distinction of the waters just around the CLT from the waters closer to the coast and to the mouth of the Chesapeake Bay.

On the other hand, the regional algorithm we developed (OC3CB) performs largely better than the global algorithm OC4v4, and has the potential to result in a useful application for the monitoring of the coastal waters of Virginia, in the Chesapeake Bay area.

Another relevant finding of the CLT studies was the original development of a semi-empirical model to calculate the signal retrieved from MODIS-A radiometers in a

‘ghost-band’ at 510 nm. Said band is derived with a simple computation from adjacent MODIS-A bands (490, 532nm). This model has been validated accurately in the study, and its application opens to the adoption of max-band Rrs ratios based on three wavelengths (443, 490, 510 nm), such as OC4v4, which have a stronger vocation for coastal environments, when using MODIS-A data.

For the results we obtained, we believe that CLT site has proven an extraordinary validation platform for satellite products. The use of oceanic observatories that can feed in real time their calibrated retrievals to broader-observation systems (i.e. satellites) could improve the accuracy of remote sensing products further. For said reasons, we believe such observation systems have a great potential for “coastal zone management” applications.

Besides the specific results we have obtained on the various aspects of our studies, this work wants to highlight the necessity of refining the criteria of the optical classification of water bodies. The use of bio-optical techniques remains the most advanced method to obtain quasi-synoptic observations of the ecological systems, but the issues related to the accuracy of biomass and primary production retrievals are not solved yet. The refinement of models of biomass and primary production, as well as the definition of sub-regionalization criteria in the context of the Case I/II classification, could significantly improve the representation of the natural variability of the marine ecological systems in coastal environments.

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