**Identification of dominant Phytoplankton Functional Types in the Mediterranean
Sea based on a regionalized remote sensing approach.**

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Keywords: Ocean colour; Phytoplankton Functional Types; PHYSAT; Mediterranean
Sea.

26

27 **Abstract**

28 During the last decade, the analysis of the ocean colour satellite imagery has allowed
29 determining the dominant phytoplankton groups in surface waters through the
30 development of bio-optical models aimed at identifying the main phytoplankton
31 functional types (PFTs) or size classes from space. One of these bio-optical model is
32 PHYSAT, which is a global method applied for oceanic Case I water and used to
33 identify in satellite pixels specific dominant phytoplankton groups, such as
34 nanoeukaryotes, *Prochlorococcus*, *Synechococcus*, diatoms, Phaeocystis-like and
35 coccolithophores. Here, we present a regionalised version of the PHYSAT method that
36 has been specifically developed for the Mediterranean Sea due to the peculiarities of
37 phytoplankton assemblages and succession than can be found in the basin and its
38 particular optical properties. The updated version of the method, the so called
39 PHYSAT-Med, has been validated successfully with large *in situ* datasets available for
40 this oceanic region, mainly for nanoeukaryotes, *Prochlorococcus*, *Synechococcus* and
41 diatoms. PHYSAT-Med allows to include a much higher number of pixels for the
42 Mediterranean than PHYSAT does, through the use of a new Look-Up-Table created
43 specifically for this oceanic region. Results provided by PHYSAT-Med showed the
44 dominance of *Synechococcus* versus prochlorophytes throughout the year at the basin
45 level, although nanoeukaryotes were more abundant during winter months. In addition,
46 PHYSAT-Med data identified a rise in the eukaryote biomass (mainly diatoms) during
47 the spring period (March to April), especially in the Ligurian and Adriatic seas.
48 PHYSAT-Med represents a useful tool for the spatio-temporal monitoring of different
49 dominant Phytoplankton Functional Types in Mediterranean surface waters at a high
50 resolution.

1.Introduction

The Mediterranean Sea is the largest semi-enclosed sea on Earth and is considered one of the most complex marine environments where much remains to be known with regard to circulation dynamics, biogeochemistry and biological activity (Tanhua et al., 2013). The Mediterranean Sea presents a deficit hydrological balance, as evaporation exceeds the supply of fresh water from streams and precipitation. This deficit is partially compensated by the inflow of Atlantic waters through the Strait of Gibraltar, which penetrates into the basin as a surface current that is less salty and less dense than the deeper counter current of the Mediterranean outflow. This export of intermediate depth water to the Atlantic directly influences the oceanographic conditions in the North Atlantic (Peliz et al., 2009) and its biogeochemical inventories (Flecha et al., 2012, Huertas et al., 2012). The Mediterranean is sensitive to climatic changes and hence monitoring the evolution of its dynamics and biogeochemistry is essential not only for the basin itself but also for the Atlantic Ocean.

Hydrological differences along the basin cause the presence of an increasing oligotrophy gradient from west to east in the Mediterranean, which can be evidenced by both satellite data and *in situ* measurements. A decreasing chlorophyll-a (Chla) gradient from north to south has been also described, with the exception of a high Chla region detected along the Algerian coast (see review by Siokou-Frangou et al., 2010). Overall, low chlorophyll concentrations are present over large areas in the Mediterranean although local phytoplankton blooms that can be regularly found in the Liguro-Provencal region, Alborán Sea and the Catalan-North Balearic front (Ignatiades et al., 2009). The nutrients and chlorophyll-a pools rank the basin as oligotrophic to ultraoligotrophic (Krom et al., 1991; Antoine et al., 1995).

In oligotrophic waters, phytoplankton community is mainly composed by picoplankton and ultraplankton (Brunet et al., 2006; Dandonneau et al., 2006; Li et al., 1983; The Mermex Group, 2011). In the Mediterranean, on the other hand, phytoplankton community reveals a considerable diversity variability over spatial and temporal scales (Siokou-Frangou et al., 2010) and large dissimilarities in phytoplankton species composition and other microorganisms across the basins have been highlighted. The picture emerging from many studies shows the dominance of the picoplankton as the fingerprint of the Mediterranean Sea and its overriding oligotrophy but local physical structures that allow the formation of phytoplankton blooms cause the coexistence of more microalgal groups (Siokou-Frangou et al., 2010). An extensive amount of information on the phytoplankton community structure along the Mediterranean coastline is available. On the contrary, longitudinal data based on large-scale investigations in open ocean waters are scarce in the literature (Ignatiades et al., 2009).

This lack of measurements can be partly overcome by using new tools, such as remote sensing techniques. During the last decade and based on different approaches, several algorithms are now able to detect phytoplankton functional types (PFTs) or size classes from space (Aiken et al., 2009; Alvain et al., 2005, 2008; Brewin et al., 2010; Ciotti and Bricaud, 2006; Hirata et al., 2009; Raitsos et al., 2008; Sathyendranath et al., 2004; Uitz et al., 2006). Platt et al (2006) concluded that the detection of different phytoplankton groups from remote sensing images was a major challenge in ocean optics.

The PFTs are groups of species that play specific roles in the marine biogeochemical cycles and trophic flows (see Le Quéré et al., 2005; Nair et al., 2008, Rudorff and Kampel, 2012). One of the methods that enables to detect PFTs from space

is the so called PHYSAT (Alvain et al., 2005; 2008), which was specifically developed to identify the dominant phytoplankton groups from ocean colour measurements. Briefly, PHYSAT is a global model applied for oceanic Case I water and is designed to detect satellite pixels in which the dominant groups are nanoeukaryotes (and separately *Phaeocystis*-like and coccolithophores), two types of picoplankton (*Prochlorococcus* and *Synechococcus*-like cyanobacteria) and diatoms (Alvain et al., 2008). PHYSAT is based on the analysis of normalized water-leaving radiance (nLw, Table 1) measurements anomalies, computed after removal the impact chlorophyll *a* variations. Specific nLw spectra anomalies (in terms of shapes and amplitudes) have been empirically associated to the presence of dominant phytoplankton groups, based on *in situ* biomarkers pigments observations. (Alvain et al., 2005; 2008; 2012; Ben Mustapha et al., 2014). Alternative methods have been also applied to detect distinct phytoplankton groups, for instance, diatoms (Sathyendranath et al., 2004), the cyanobacteria *Synechococcus* (Morel, 1997), the N₂-fixing cyanobacteria *Trichodesmium* (Subramaniam et al., 1999; 2001), *Phaeocystis globosa* (Astoreca et al., 2009; Lubac et al., 2008) and *coccolithophores* (Ackleson et al., 1994; Brown & Podestá, 1997; Brown & Yoder, 1994; Cokacar et al., 2001; Gordon et al., 2001; Iglesias-Rodríguez et al., 2002; Kopelevich et al., 2013; Moore et al., 2012; Smyth et al., 2002; Tyrell et al., 1999). Nevertheless, the PHYSAT method allows to distinguish several groups of phytoplankton simultaneously and identify the dominant PFT at each particular pixel and at each point in time. This method has been successfully validated and used in recent years (Alvain et al., 2005; 2006; 2008; 2012; 2013; Arnold et al., 2010; Belviso et al., 2012; Ben Mustapha et al., 2014; Bopp et al., 2005; Demarcq et al., 2012; De Monte et al., 2013; D'Ovidio et al., 2010; Gorgues et al., 2010; Hashioka et al., 2013; Masotti et al., 2010; Masotti et al., 2011). Results were satisfactory for

nanoeukaryotes (82%) and a decrease in the percentage of successful retrieval was observed for diatoms (73%), *Synechococcus* (57%) and *Prochlorococcus* (61%) (Alvain et al., 2012).

However, due to the specific character of phytoplankton assemblages in the Mediterranean Sea and their associated bio-optical relationships that can be affected by continental inputs such as rivers discharge and desert dust events (Alvain et al., 2006; Bricaud et al., 2002; Claustre et al., 2002; Loisel et al., 2011), it is necessary to adapt the PHYSAT method and evaluate its derived results in this specific ocean region. In fact, Santoleri et al. (2008) pointed out that the difference in bio-optical characteristics at the regional scale in Mediterranean Sea is due to ecological reason such as the presence of specific phytoplankton groups. Moreover, the presence the aerosols due to anthropogenic atmospheric emissions from continental Europe and Saharan dust makes it difficult to apply standard remote sensing procedures for the atmospheric correction (Moulin et al., 1997). In fact, the presence and abundance of aerosols is one of the factors determining the different optical properties of the Mediterranean with respect to the global ocean (Claustre et al., 2002). Therefore, during the last years, many regional algorithms have been developed for the Mediterranean Sea, such as DORMA-SeaWiFS (D'Ortenzio et al., 2002), BRIC-SeaWiFS (Bricaud et al., 2002), MedOC4-SeaWiFS (Volpe et al., 2007), MedOC3-MODIS (Santoleri et al., 2008) and MedOC4ME-MERIS (Santoleri et al., 2008) since standard algorithms overestimate low Chl a and conversely, underestimate high concentrations.

In this work, the previous version of PHYSAT (Alvain et al., 2005; 2008) has been modified in order to estimate the most frequent phytoplankton groups in the Mediterranean Sea. The updated version (hereafter PHYSAT-Med) has been validated using *in situ* measurements collected in different cruises conducted throughout the

entire basin. Therefore, the main objectives of this study were: (i) to adapt the original PHYSAT method to the Mediterranean Sea for MODIS satellite images datasets; (ii) to validate the new PHYSAT-Med method with *in situ* measurements and (iii) to evaluate the spatio-temporal patterns of the PFTs in the Mediterranean Sea for MODIS era.

2. Material and Methods

2.1. PHYSAT Method

The PHYSAT method is based on the identification of specific signatures in the normalized water leaving radiance (nLw, Table 1) spectra measured by an ocean colour sensor (Alvain et al., 2005; 2008). This approach considers the analysis of the second order variation in nLw measurements after removal the impact of chlorophyll *a* variation. The first version (PHYSAT-v2005; Alvain et al., 2005) established the relationship between SeaWiFS-nLw measurements anomalies and four phytoplankton groups, such as diatoms, nanoeukaryotes, *Synechococcus* and *Prochlorococcus* for a wide range of water types (Alvain et al., 2005) such as the North Atlantic and the Equatorial and South Pacific. The second version of PHYSAT (PHYSAT-v2008, Alvain et al., 2008) included additional phytoplankton groups as “*Phaeocystis*-like” and *coccolithophorids*. The latest version used the new specific water leaving radiance (nLw^{ref} , see Table 1) model that considered all the SeaWiFS data available for all the geographical areas during the same year, including diatoms data from Southern Ocean that had been excluded in the former versions (PHYSAT-v2005). This modification led to slight changes in the criteria used to identify the four initial phytoplankton groups. Alvain et al. (2008) presented a new Look-Up Table (LUT) with the characteristics of specific radiance anomalies (Ra, see Table 1) spectra for six phytoplankton groups that could be detected with the PHYSAT-v2008 method. Although PHYSAT is empirically

based on radiance anomalies, recently Alvain et al. (2012) have provided a theoretical explanation to understand the derived results, showing that each phytoplankton group is generally associated with a specific bio-optical environment, represented by different values of particulate scattering (b_p), absorption by the coloured dissolved organic matter (a_{cdom}) and the absorption by the phytoplankton (a_{phy}). In addition, PHYSAT has been successfully applied to other ocean colour sensors, such as Coastal Zone Color Scanner - CZCS (Masotti et al., 2009) and Ocean Colour and Temperature Sensor - OCTS (Masotti et al., 2009; 2011). In this work, we have incorporated the PHYSAT-v2008 version and adapted to the Mediterranean Sea using the MODIS dataset. Figure 1 displays a schematic diagram of the steps followed to adapt PHYSAT-Med.

2.1.1. Step 1: Satellite data

All Level 3 MODIS daily products (reprocessing version R2013.0) at 4-km resolution acquired from NASA Oceancolor website (<http://oceancolor.gsfc.nasa.gov>) during more than a decade (since July 2002 to May 2013) were downloaded (Fig. 1, step 1) and remapped on an equi-rectangular grid on the Mediterranean area (-6 to 36° E and 30 to 46° N, Fig. 2). The Black Sea was masked, as its dynamics follow an independent regime and was considered distinct from the rest of the basin (Oguz et al., 2004). These products were chlorophyll-a concentration (Chl a), estimated by the global standard algorithm OC3M-Chl a (O'Reilly et al., 1998; 2000), aerosol optical thickness at 869 nm (AOT), diffuse attenuation coefficient at 490 nm (K_{490}) and remote sensing reflectance ($R_{rs}(\lambda)$) at 412, 443, 469, 488, 531, 547 and 555 nm.

Since the global chlorophyll algorithm generally leads to a significant overestimation in the Mediterranean Sea (Bricaud et al., 2002; Claustre et al., 2002; D'Ortenzio et al., 2002; Volpe et al., 2007), the regional MedOC3-Chl a algorithm for

MODIS images (Santoleri et al., 2008) was considered to estimate the chlorophyll *a* concentration in the Mediterranean Sea. This MedOC3-Chl*a* algorithm is an adaptation of the Mediterranean bio-optical algorithm (MedOC4) developed initially by Volpe et al. (2007) for SeaWiFS images. Therefore, daily images of chlorophyll *a* concentration using MedOC3-Chl*a* algorithm (Fig. 1, step 2):

$$MedOC3 - Chla = 10^{(0.380 - 3.688R + 1.036R^2 + 1.616R^3 - 1.328R^4)} \quad (1)$$

$$\text{where } R = \log_{10} \left(\frac{Rrs_{443} > Rrs_{488}}{Rrs_{555}} \right) \quad (2)$$

This bio-optical algorithm is based, like OC3M, on fourth-power polynomial regression between log-transformed Chl*a* and log-transformed maximum band ratio (MBR). It is known that using multiple Rrs ratios decreases the noise-to-signal ratio and thereby enhancing the algorithm's performance (O'Reilly et al., 1998). MedOC3 algorithm was calibrated on a representative open-water bio-optical dataset collected in the Mediterranean Sea, and is declared to match the requirements of unbiased satellite chlorophyll *a* estimation (Santoleri et al., 2008).

At the second step, the remote sensing reflectance (Rrs) was converted to nLw using the nominal band solar irradiance (F_o , in $\text{mW cm}^{-2} \mu\text{m}^{-1}$) for any specific spectral band (λ) for MODIS sensor (Fig. 1).

$$nLw_{(\lambda)} = Rrs_{(\lambda)} * F_{o(\lambda)} \quad (3)$$

Following PHYSAT methodology (Alvain et al., 2005; 2008), a two new Look-Up Table (Fig. 1, step 3) of $nLw^{\text{ref}}(\lambda, \text{Chl-a})$ was empirically generated for the Mediterranean Sea from a large dataset of MODIS Chl*a* and nLw pixels for all daily images comprised within the study period (July 2002 to May 2013). Turbid pixels (defined as $nLw_{555} > 1.3 \text{ mW cm}^{-2} \text{ mm}^{-1} \text{ sr}^{-1}$, Nezlin and DiGiacomo, 2005) and pixels with AOT higher than 0.15 (Alvain et al., 2005) were excluded in order to minimize the

impact of high suspended matter loads as well as atmospheric correction error and clouds boundaries respectively. Briefly, nLw^{ref} is calculated from nLw data and the associated $Chla$ computed from the OC3M- $Chla$ (Fig. 3a) and MedOC3 (Fig. 3b) algorithms within the chlorophyll a range between 0.01 and 10 $mg\ m^{-3}$ (41 narrow intervals). Note that this latter threshold is higher than that of the original PHYSAT method (3 $mg\ m^{-3}$; Alvain et al., 2005) in order to maximize the incorporation of all values obtained by the *in situ* measurements, as maximum chlorophyll a estimations around 8 $mg\ m^{-3}$ can be found in the Alboran Sea (Arin et al., 2002). In addition, a Look-Up Table at global scale for MODIS sensor is also displayed in Fig. 3c (data obtained from PHYSAT website, <http://log.univ-littoral.fr/Physat>).

Once the new LUT for the Mediterranean Sea was calculated using regional MedOC3- $Chla$ algorithm (Fig. 3b), the radiance anomalies ($Ra(\lambda)$, Fig. 1, step 4) were computed for all daily MODIS wavelengths analysed using the following equation (eq. 4) for all wavelengths available (412, 443, 469, 488, 531, 547 and 555 nm), where $Ra(\lambda)$ is an adimensional unit parameter and by definition, this parameter is independent of the chlorophyll a level (being by extension independent of the biomass) and represents hence the second order variation of $nLw(\lambda)$.

$$Ra_{(\lambda)} = \frac{nLw_{(\lambda)}}{nLw_{(\lambda)}^{ref}} \quad (4)$$

The radiance anomaly represents the second order variation of the normalized water-leaving radiances after removal the first order effect of the $Chla$ variation (Alvain et al., 2005).

The new LUT (Fig. 3b) can be assumed to include both the mean ocean colour signal and potential differences between the MODIS sensor and the previously used SeaWiFS sensor, allowing to use with confidence classical thresholds on anomalies (see Table 5 in Alvain et al., 2005 and Table 1 in Alvain et al., 2008). The shape and amplitude of the R_a spectra mainly depend on the bio-optical environment, represented by different values of the b_p , a_{cdom} and a_{phy} , as it has been proven by the sensitivity study of R_a made by Alvain et al., (2012). These analyses showed that for a given chlorophyll concentration, the particle scattering variability explains the largest part of the remotely sensed R_a spectral variability, especially when focusing on R_a magnitude changes. However, variations in colored dissolved organic matter and phytoplankton absorption coefficients can also have a large impact on R_a with specific spectral signatures.

2.1.2. Spectral characterization of phytoplankton groups

As noted above, Alvain et al. (2005) established the thresholds of R_a for each of the four phytoplankton groups analysed in PHYSAT-v2005 (*see Table 5 in Alvain et al., 2005*). This table was subsequently updated with two more phytoplankton groups and some thresholds were hence modified (*see Table 1 in Alvain et al., 2008*). Recently, Ben Mustapha et al. (2014) have provided the spectral signatures of R_a corresponding to the four PHYSAT-SOM labelled classes. In our study, we have established new characteristics of acceptable R_a spectra (Table 2) for MODIS wavelengths using the linear interpolation between SeaWiFS and MODIS wavelengths and the R_a thresholds described by Alvain et al., (2008). This can be done considering the relatively flat signals used in PHYSAT and the very close wavelengths of the two sensors.

The new thresholds described in Table 2 were used to process daily images to calculate daily PFTs maps (Fig. 1, step 5). For a spectra to be associated with one

group, all criteria must be fulfilled. These thresholds (Table 2) have been fixed in order to avoid any overlapping. This labellisation step consists in relating the Ra spectral patterns to *in situ* pigment inventories assigning each to a particular phytoplankton group (labelling procedure). Pixels with nLw values that were not classified for any phytoplankton groups summarized in Table 2, based on available *in situ* information for the calibration step of PHYSAT, were catalogued as “*unidentified (unid.)*”. These unidentified pixels are due to the fact that Ra spectra of these pixels have not been labelled although it is worthy to note that the number of unidentified pixels could diminish when more additional *in situ* information is available. These situations could lead to no-classification or misclassification of the radiometric signal in terms of phytoplankton groups. However, most of these cases lead to no-classification or unidentification rather than to a misclassification. The remaining misclassification cases lead to wrong identification and explain the wrong identifications found during the previous PHYSAT validation exercise (Alvain et al. 2012).

From this image database (near to 4000 daily images), 10-days and monthly maps of dominant phytoplankton group detection were obtained by calculating at each geographical pixel, the phytoplankton group present during more days during the integration period (10-days or monthly respectively), including the “unidentified” pixels. Map of detection frequencies over each month, between 0 (group never detected) and 1 (all valid pixels were associated with the group), were also calculated to each group from the entire dataset.

2.2. *In situ* validation

High-Performance Liquid Chromatography (HPLC) allows the quantification of a suite of accessory pigments (carotenoids and chlorophylls) in phytoplankters. Accordingly, the chemotaxonomic correspondence of HPLC-determined pigments can

be used to study the phytoplankton community composition (e.g. Claustre and Marty, 1995; Gieskes et al., 1988; Goericke and Repeta, 1993; Mackey et al., 1996) as these pigments are specific of individual phytoplanktonic taxa or groups (Jeffrey and Vesk, 1997; Mackey et al., 1996; Vidussi et al., 2001; Uitz et al., 2006). However, some pigments may covary with others or be redundant, which makes data interpretation and visualization tedious.

In this study, more than 3000 HPLC measurements collected in the Mediterranean Sea have been analysed (Table 3). The data source is composed by data from DYFAMED Time Series obtained from MAREDAT global database of HPLC (Peloquin et al., 2013), the BOUSSOLE project (i.e. Antoine et al., 2006; Organelli et al., 2013) and the SESAME Project database (Ras et al., 2011). Fig. 2 displays the spatial location of the *in situ* HPLC measurements used in this study that covers a wide range of water types in the Mediterranean Sea. All these HPLC analysis were carried out by the members of the SAPIGH analytical platform from the *Laboratoire d'Océanographie de Villefranche (LOV)*, following the protocol described in Marty et al., (2002) for DYFAMED Time Series, Vidussi et al., (2001) and Antoine et al., (2006) for BOUSSOLE project and Ras et al., (2008) for SESAME cruises.

Here, we only considered samples limited to the first optical depth, which reduces the number of available pigment inventories to 1382 samples. The first optical depth is the depth at which 90% of the light originates for remote sensing purposes (Trees et al., 2000) and was calculated from K_{490} daily MODIS images according to the following equation:

$$\text{Optical depth} = 1/K_{490} \quad (5)$$

where daily K_{490} images were downloaded from the oceancolor website. When daily K_{490} value for the matchup HPLC *in situ* data was missing, a climatology values for optical depth were included.

2.2.1. Identification of phytoplankton groups in HPLC measurements.

Many pigments are specific of individual phytoplanktonic taxa or groups (Jeffrey and Vesk, 1997). A review of taxonomic pigments can be found in Jeffrey (1997). They can thus be used as biomarkers of phytoplankton groups (e.g., Gieskes et al., 1988), and with some cautions, assigned to different size classes, such as microphytoplankton, nanophytoplankton and picophytoplankton (Vidussi et al., 2001). For instance, divinyl chlorophyll-a is the typical marker of prochlorophytes (Claustre and Marty, 1995; Goericke and Repeta, 1992; Vidussi et al., 2001), whereas Chl*a* is the universal descriptor of the rest of phytoplankton taxa. Fucoxanthin is the principal marker of diatoms (Jeffrey, 1980) even though it is also present in prymnesiophytes and chrysophytes (Jeffrey and Vesk, 1997). 19'-hexanoyloxyfucoxanthin (19'HF) concentration was mainly related to prymnesiophytes (Wright and Jeffrey, 1987; Latasa et al., 1992) and peridinin, which appears only in traces, is present in small dinoflagellates that can be heterotrophic (Jeffrey and Hallegraeff, 1987). This pigment has not been found in significant amounts in oceanic natural samples of mixed populations containing dinoflagellates, although part of them should have been presumably autotrophic (Latasa et al., 1992). Alloxanthin is another pigment typical of the cryptomonads and is also present in the ciliate *Mesodinium rubrum* (Latasa et al., 1992; Gieskes and Kraay, 1983), but the occurrence of these organisms in NW Mediterranean Sea were too low and erratic to be used in a ratio (Latasa et al., 1992). Finally, zeaxanthin is associated with cyanobacteria (Guillard et al., 1985) although it is

also present in prochlorophytes (Chisholm et al., 1992; Goericke and Repeta, 1992) and also a marker of protection in green algae (Brunet et al., 2006).

As indicated above, we have used the method described by Alvain et al. (2005) to associate dominant phytoplankton groups with *in situ* HPLC pigment inventory. Pigments ratios (P_{rel}) were defined as:

$$P_{rel} = P / (Chla + d - Chla) \quad (5)$$

where P is the measured pigment concentration in the seawater, Chla and d-Chla are the concentration of chlorophyll-a and divinyl Chla respectively. Alvain et al. (2005, see Table 4) established a threshold of relative pigments concentration to a specific phytoplankton group based on an extended analysis of previous literature. This approach based on biomarker pigment ratio thresholds was used recently at global scale (PHYSAT-SOM, Ben Mustapha et al., 2014). The added-value of the PHYSAT approach is that any phytoplankton group could be added to the method as more *in situ* observation of dominant phytoplankton groups and pigments data are made available allowing comparing the Ra spectra for these waters and include these new spectral characteristics in Table 2.

3. Results

In this study, phytoplankton *in situ* measurements available for the entire Mediterranean Sea (Fig. 2) from different sources and databases (see Table 3) have been considered, thereby including oligotrophic areas in the middle and eastern part of the basin and local eutrophic regions originated either by upwelling processes in the Alboran Sea or by the winter convergence in the Ligurian Sea. Fig. 4 shows the monthly climatology of the most frequent phytoplankton groups estimated by PHYSAT-Med method in Mediterranean Sea during the study period in the first optical depth. The monthly climatological distributions show that *Synechococcus* is the most

dominant phytoplankton group at a basin level during spring-summer months, whereas nanoeukaryotes are more abundant during autumn-winter seasons. In coastal areas proliferate other groups, as it is the case of diatoms with a dominant presence in the northern Adriatic Sea, Ligurian Sea and Gulf of Lion, overall during the spring season. The analysis of the monthly distribution of phytoplankton groups reveals a high spatial and temporal variability (see Supplementary Material). It is evident that *Synechococcus* and nanoeukaryotes are the most abundant PFT in the basin over the year, although other phytoplankters can be identified in different sub-basins. For instance, during the spring season, diatom blooms are clearly distinguishable in several areas of the Mediterranean, such as the Catalan Sea, the Adriatic Sea, the Ligurian Sea and the Ionian Sea. Moreover, coccolithophorids occur over the coastline associated to the vicinity of the large river mouths, such as the Nile, Ebro, Rhone, etc.

Fig. 5 shows the map of detection frequencies (mean and standard deviation) for four PTF groups considering the unidentified pixels: nanoeukaryotes, *Prochlorococcus*, *Synechococcus* and diatoms. *Synechococcus* is the most frequent PFT in the basin (Fig. 5e), mainly in the eastern half, being twice or three fold higher than the presence of *Prochlorococcus* (Fig. 5c). However diatoms dominated in the northern Adriatic Sea and Gulf of Lions (Fig. 5g) and nanoeukaryotes presented a high frequency along the coastal fringe and the Aegean Sea (Fig. 5a). The standard deviation maps show where the variability of different PFTs is higher (Fig. 5b, d, f and h). It can be argued that unidentified pixels may represent a high percentage, but they can potentially decrease if more *in situ* data are included to improve the labellisation step.

These spatial patterns of PFTs are in concordance with the distribution of the main taxonomic pigments estimated from the *in situ* HPLC dataset (Table 3). These data can be then used to visually compare the maps generated by the PHYSAT-Med

method. Fig. 6 shows surface pigments ratios of divinyl Chl a , zeaxanthin, fucoxanthin and 19'HF in the Mediterranean Sea over different seasons (spring, summer and autumn). The pigment ratio of divinyl Chl a , which is indicative of the presence of *Prochlorococcus* (i.e. Claustre and Marty, 1995; Goericke and Repeta, 1992; Vidussi et al., 2001), exhibits low values in surface water in the entire basin, especially in summer. This finding is consistent with the distribution of *Prochlorococcus* estimated by the PHYSAT-Med method, where only a few pixels denoted this phytoplankton group (Figs. 6m, n and o). In contrast, zeaxanthin, which is diagnostic mainly for *Synechococcus* (Chisholm et al., 1992; Claustre and Marty, 1995; Goericke and Repeta, 1992; Guillard et al., 1985; Marty et al., 2002; Marty and Chiavérini, 2002; Vidussi et al., 2001), was found to represent the highest pigment ratio concentration throughout the year, mainly during summer, coinciding with the PHYSAT-Med outputs for the Mediterranean Sea (Figs. 4, 5 and 6). Furthermore, the *in situ* HPLC results indicated that fucoxanthin, which fundamentally marks the presence of diatoms (Jeffrey, 1980), presented higher values during spring and autumn and particularly in coastal areas (Alborán, Aegenan and north of the Adriatic seas), in concordance with the PHYSAT-Med distribution (Fig. 6m, n and o). Finally, maximum distributions of pigment ratio of 19'-HF (Fig. 6j, k and l) were found during spring cruises, particularly in coastal areas, coinciding with the distribution of nanoeukaryotes given by PHYSAT-Med (Fig. 6m, n and o).

In order to compare the results obtained with the two approaches considered here to estimate the pigments distribution over the Mediterranean Sea, we found 29 match-ups between pigment inventories (using thresholds of Table 4 in Alvain et al. 2005) and associated with simultaneous high quality satellite phytoplankton groups given by PHYSAT-Med (AOT_{865} lower than 0.15 and nLw_{555} lower than 1.3 mW cm^{-2}

mm⁻¹ sr⁻¹). These validation data sets contain 16 water samples dominated by *Synechococcus* and 13 by nanoeukaryotes (Fig. 7). A synthetic view of this validation exercise is provided in Table 4 by showing, for each of these two groups, the percentage of valid and wrong identifications. Table 4 shows that 74.07% of the pigment inventories corresponding to nanoeukaryotes are associated with the same phytoplankton group in the PHYSAT-Med daily images. Moreover, *Synechococcus* are correctly identified in 60.71% of the *in situ* pigment inventories. In this validation exercise, the rest of the groups were discarded, as no pigment inventories in matchup were associated to them.

The temporal variability of PFTs (*Prochlorococcus*, *Synechococcus*, diatoms and nanoeukaryotes) in the Ligurian Sea (Fig. 2) was also analysed and compared with HPLC measurements (pigment ratios) provided for the same sub-basin during certain periods of time at the first optical depth. Fig. 8 shows oscillations of the four PFTs during the year, with peaks and minima appearing at different times of the year depending on the group. *Prochlorococcus* exhibited maxima at the end of the stratification period (normally in October) over several years (Fig. 8a). The maxima found by the PHYSAT-Med model were in close agreement with the maxima in the concentrations of the pigment ratio for divinyl Chl-a in the first optical depth provided by HPLC, which is indicative of prochlorophytes (Claustre and Marty, 1995; Goericke and Repeta, 1992; Vidussi et al., 2001), although this pigment ratio value is normally lower than 0.40. This pattern coincides with the results reported by Vaultot et al (1990) and Marty et al., (2002) estimated by flow cytometry and HPLC analysis respectively. However, during the stratification period in this region, between July and October (Marty et al., 2002), the phytoplankton group identified by PHYSAT-Med as the most abundant was *Synechococcus* (Fig. 8b). This feature also matches the highest values for

zeaxanthin measured by HPLC during this period in the Ligurian Sea (Fig. 8b). As indicated above, zeaxanthin is associated with cyanobacteria (Guillard et al., 1985) and is normally used in this area to estimate *Synechococcus* concentration (Marty et al., 2002; Vidussi et al., 2001), particularly in surface waters, where it is more abundant (Lasternas et al., 2010). The visual concordance between the results obtained with both approaches also applies to diatoms, as the annual peaks registered by PHYSAT-Med every year during the spring bloom closely resemble the maxima of fucoxanthin given by HPLC in the first optical depth (Fig. 8c). Finally, the nanoeukaryotes present maxima during winter season, normally around January, although the maxima pigment ratio of 19'-HF is achieved during spring-summer seasons (Fig. 8d), which can be due to the heterogeneity of this phytoplankton group in terms of species composition. Therefore, the broad coincidence between both temporal patterns (Fig. 7, HPLC Pigment ratio vs PHYSAT-Med outputs) confirms in general terms that the regionalized version of PHYSAT (PHYSAT-Med) is in relatively good agreement with the results obtained by long-term monitoring programs for phytoplankton distribution, at least in the Ligurian Sea area, where the largest database of HPLC measurements is available (Table 3).

Once it has been established that PHYSAT-Med works properly in the Mediterranean Sea, the temporal and spatial variability of PFTs in different Mediterranean sub-basins have been also analysed through this approach, and represented by the Hovmöller diagrams (Figs. 9 and 10). The transects selected to perform the analysis were quite similar to those used by D'Ortenzio and Ribera d'Alcalá (2009), who considered one zonal (Fig. 9) and three meridional transects (Fig. 10) that encompass the most representative areas of the chosen sub-basins (see Fig. 2 for location of the transects).

The West-East distribution of PFTs during the study period shows differences mainly between the two sub-basins. Nanoeukaryotes appear in the whole Mediterranean whereas in the Levantine basin *Synechococcus* is the most abundant group in surface waters, especially during summer (Fig. 9). This pattern can be observed every year between 2002 and 2013. On the other hand, diatoms are in general less frequent and are identified during spring, prior to the proliferation of *Synechococcus*. After the stratification period, during the autumn months, some pixels mark the prevalence of *Prochlorococcus* over the whole zonal transect whereas in winter, a large number of unidentified pixels was found, particularly in the western part of the transect. This temporal variability is also detected in the three meridional transects (Fig. 10). In all cases, the dominant group was *Synechococcus*, mostly during summer. Before the maximum, over spring, a diatom bloom can be identified in both the central and eastern transects whereas in the western transect, nanoeukaryotes emerge during winter, when many undetermined pixels were again found in the three transects. As noted previously, although unidentified pixels may represent a high percentage, this updated version of PHYSAT can still be very useful to monitor the spatio-temporal variability of some PFTs in Mediterranean Sea, as the approach can be potentially improved when more *in situ* measurements are incorporated.

4. Discussion and Conclusion

The Mediterranean Sea is generally characterized by an oligotrophy regime, with a clear decrease in nutrients and chlorophyll concentration being observed from west to east (Krom et al., 2010). However, local physical structures promoting the inputs of nutrients from the deep layer to surface water allow the formation of some productive areas that present their own dynamics within the general circulation scheme (Siokou-Frangou et al., 2010). The presence of convergences zones immersed in an oligotrophic

environment and the particular biogeochemical characteristics of the Mediterranean are reflected in the composition of the phytoplankton community, whose diversity has been reported in previous studies (Siokou-Frangou et al., 2010). Given the complexity to perform large *in situ* studies at a basin scale, the use of novel remote tools that facilitate the monitoring the oceanic biodiversity from space (De Monte et al., 2013) represents a powerful and useful alternative approach that helps to understand phytoplankton dynamics in the Mediterranean basin.

Here, we present an updated version of the PHYSAT method (Alvain et al., 2005, 2008) that has been specifically developed for the Mediterranean Sea and for the MODIS sensors. This version, the so called PHYSAT-Med, has been compared with large *in situ* datasets (Tables 3 and 4, Figs. 6, 7 and 8) available for the basin. In addition, the spatio-temporal variability of the phytoplankton groups estimated by PHYSAT-Med was consistent with the previous knowledge on the phytoplankton distribution patterns in the Mediterranean Sea (Claustre and Marty, 1995; Marty et al., 2002; Marty and Chiaverini, 2002; Marty and Chiavérini 2010; Siokou-Frangou et al., 2010; Vidussi et al., 2000, 2001; Vaulot et al., 1990). The concordance between the results obtained with remote data and previous *in situ* measurements was particularly relevant in the Ligurian Sea region (Fig. 8), where the largest database of HPLC measurements is available (Table 3).

Our results show that the temporal variability of the four phytoplankton groups discriminated by PHYSAT-Med in the Mediterranean Sea, *Synechococcus*, *Prochlorococcus*, diatoms and nanoeukaryotes can be reproduced (Fig. 8, 9 and 10). This enables studies of multi-scale processes, such as seasonal species' successions, interannual anomalies or decadal trends. In fact, PHYSAT has been already used successfully to analyse large-scale shifts in phytoplankton groups at a global scale (Ben

Mustapha et al., 2014; Masotti et al., 2010), in the Equatorial Pacific (Masotti et al., 2011) and in high latitudes (Alvain et al., 2013; Hashioka et al., 2013), seasonal distribution of PFTs at a global scale (Alvain et al., 2008) and mesoscale patches of dominant phytoplankton groups at the confluence of the Brazil and Malvinas currents (D'Ovidio et al., 2010).

According to our study, PHYSAT-Med can be considered the first model for estimating the dynamics of the four PFTs analysed in the Mediterranean Sea. Sathyendranath et al. (2004) proposed an algorithm to discriminate diatoms from other types of phytoplankton groups in the Northwest Atlantic, and Subramaniam et al. (2001) developed an alternative method able to detect *Trichodesmium* blooms through SeaWiFS imagery in the South Atlantic Bight. Brown and Yoder (1994) proposed an algorithm to identify *coccolithophorids* blooms in the global ocean, which was later used by Iglesias-Rodríguez et al., (2002) who included the Mediterranean Sea, although no specific study was dedicated to the Mediterranean Sea in spite of its particular optical and ecological character (Bricaud et al., 2002; Claustre et al., 2002; Loisel et al., 2011). Recently, Uitz et al (2012) proposed a phytoplankton class-specific bio-optical model to calculate primary production in the Mediterranean Sea, but none of these models provides the spatio-temporal distribution of different phytoplankton groups in the basin.

Furthermore, the PHYSAT-Med allowed us to include a high number of pixels in the Mediterranean by using a new LUT (Fig. 3b), which was specifically created for the basin using the regional chlorophyll *a* algorithm MedOC3-Chl *a* (Santoleri et al., 2008). In fact, the number of pixels identified by PHYSAT-Med (Fig. 11a) is three-fold higher than that detected by the global version of PHYSAT (Alvain et al., 2005; 2008), being also in agreement with *in situ* HPLC measurements at the Ligurian sub-basin (i.e.

diatoms, Fig. 11b). Recently, Ben Mustapha et al. (2014) applied modified PHYSAT approach (PHYSAT-SOM) at global scale, which provides a much higher number of pixels (twice as high) than the previous PHYSAT version.

The comparison exercise performed here shows consistent results for the Ligurian Sea region. In this sub-basin, the seasonal succession of hydrological conditions induces production regimes varying from mesotrophy in spring to oligotrophy in summer and fall (Marty et al., 2002). The winter convective-mixing period that extends from the beginning of December to the end of March (Marty et al., 2002) allows the replenishment of nutrient in the surface layers, which supports phytoplankton new production (Marty and Chiavérini, 2010). According to the PHYSAT-Med outputs (Fig. 12), nanoeukaryotes were the dominant phytoplankton group during the winter months, whereas diatoms achieved a maximum during the spring bloom in this area (Fig. 8c and Fig. 12), coinciding with the maximum in chlorophyll *a* concentration measured *in situ* (Marty and Chiavérini, 2010; Marty et al., 2002). Diatoms are known to be opportunistic organisms (Fogg, 1991) that take advantage of the newly available nutrients after the winter mixing. The seasonal variations in diatoms obtained by PHYSAT-Med (Fig. 8c) were obvious and markedly repetitive from year to year, in agreement with previous results (Marty et al., 2002). Moreover, diatoms also occur in winter-spring in other sub-basins, such as the Adriatic and Ionian seas (Fig. 4 and Supplementary Material). Therefore, the geographic pattern obtained by satellite data was consistent with the previous knowledge of phytoplankton communities organization in these regions (Caroppo et al., 2006; Socal et al., 1999).

After decay of the spring bloom in the Ligurian Sea, the warming of surface waters causes a rapid stabilization of the surface layer, which increases in depth from June to October (Marty and Chiavérini, 2010). During the stratification period,

Synechococcus was found to be the most abundant group by PHYSAT-Med (Figs. 4, 8 and 11), coinciding with the maxima concentration of zeaxanthin (*Synechococcus*) measured in the surface layers by Marty and Chiavérini (2002). This period also corresponds to a shift from a phytoplankton population dominated by diatoms (Fig. 8, Fig. 10 and Fig. 12) towards an increase in cyanobacteria (*Synechococcus*). Marty et al. (2002) established that the maximum contribution of cyanobacteria-characteristic pigment (zeaxanthin) occurs in summer and in surface waters, which is also the period of phosphate limitation. During the winter mixing, nutrient limitation is essentially due to nitrate depletion in surface waters (where most of the productivity proceeds). Marty and Chiavérini (2002) suggested that this increase could be due to the better efficiency for cyanobacteria to grow under nutrient limited conditions, either by atmospheric nitrogen fixation or by utilization of atmospheric deposition of nutrient-aerosols or rain, which are relevant fertilization processes in the Mediterranean nutrient cycling (Huertas et al., 2012). Our data confirm that maxima in *Synechococcus* distribution are achieved during the stratification period (Fig. 12), which is unequivocally detected during this season every year (Fig. 8b). Zeaxanthin, a pigment that likely acts as a photo-protector (Paerl et al., 1983), is particularly abundant in the surface layer during the nutrient-depleted stratification period. The abundance of cyanobacteria-associated zeaxanthin in the upper layers can be attributed to a photosynthetic adaptation resulting from changes in their phycobiliprotein concentrations at high irradiances (Kana and Glibert, 1987). However, it is also consistent with their potential capability to fix atmospheric nitrogen, as already suggested for *Synechococcus* (Mitsui et al., 1986; Sachs and Repeta, 1999). The presence of *Synechococcus* is particularly important in the Eastern Mediterranean during 2003 and the rest of years analysed (Supplementary Material). Its distribution, specifically restricted to surface layers, is consistent with data reported in other

Mediterranean sub-basins, such as the Tyrrhenian Sea (Decembrini et al. 2009), Ionian Sea (Brunet et al., 2006; Casotti et al., 2003), Ligurian Sea (Bustillos-Guzman et al., 1995), Adriatic Sea (Santic et al., 2011) and in surface coastal water of the northwestern Mediterranean Sea (Sommaruga et al., 2005).

On the contrary, *Synechococcus* abundance markedly decreases during the destratification period that begins in November, when mixing starts and triggers nutrients inputs to the surface layer. High divinyl Chl-a, which is the marker for *Prochlorococcus* (i.e. Goericke and Repeta, 1992), concentrations have been measured from summer to winter but remain undetectable the rest of the year (Marty et al., 2002). This pattern is corroborated by data plotted in Fig. 8a, where the divinyl Chl-a in the first optical depth peaks between October and December, coinciding with *Prochlorococcus* maxima given by PHYSAT-Med (Fig. 8a). In any case, our data indicates that *Synechococcus* is more abundant than *Prochlorococcus* at any time of the year, in agreement with the study carried out by Schauer et al., (2003). This finding would be also coherent with the oligotrophic character of the Eastern Mediterranean, at least during the destratification period. In addition, our data indicate that prochlorophytes (Fig. 4 and Supplementary Material) are generally less abundant in the northwestern Mediterranean Sea than in the south-eastern, in agreement with previous observations (Barlow et al., 1997; Vidussi et al., 2000). Nevertheless, it should be pointed out that *Prochlorococcus* mostly thrives in deeper layers, particularly at the deep chlorophyll maximum (Lasternas et al., 2010; Mella-Flores et al., 2010) and hence, it would not be detect by PHYSAT-Me, which is suitable for identification of PFTs in the surface layer (first optical depth).

Our study then evidences that PHYSAT-Med data matches *in situ* picoplankton abundance and dynamics in the Mediterranean, at least in surface and for

Prochlorococcus and *Synechococcus*. This concordance can be extended in the case of nanoeukaryotes. Thus, Vidussi et al. (2001) found that nanophytoplankton always prevailed over picoplankton in the Ionian Sea during winter months and Uitz et al. (2012) proposed that primary production was dominated by nanophytoplankton throughout the year in the entire basin. The distribution of nanoeukaryotes given by PHYSAT-Med (i.e. year 2004, Supplementary Material) is also in agreement with such observations, with this pattern being particularly evident in the Aegean Sea. In contrast, Vidussi et al., (2001) also noted that picoplankton was dominant in the Levantine basin, which was confirmed by our satellite data (i.e. year 2004 in Supplementary Material). PHYAST-Med may have, however, certain limitations to detect other phytoplankton groups. This is the case of diatoms, which may present two spectral behaviours in terms of shape and amplitude (Ben Mustapha et al., 2014) and therefore its distribution in the Mediterranean Sea would be underestimated by the current approach.

This constrain can be also applied to coccolithophorids. PHYSAT-Med generated maps (Fig. 4 and Supplementary Material) indicated that coccolithophorids were mainly located along the Mediterranean coastline, particularly in the vicinity of large rivers mouths, such as Nile, Ebro, Rhone and Adriatic Sea. However, it can be argued that the observed patches could be well due to the presence of terrestrial matter and/or a specific phytoplankton group not yet sampled. In addition, coccolithophorids occurrence has been described globally at the Mediterranean (Coll et al., 2010; Cros and Fortuño, 2002; Oviedo et al., 2014) whereas PHYSAT-Med outputs only marked a few pixels where this group was clearly represented (Fig. 4 and supplementary material). Such disparity could be related to the use of a mask for turbid pixels that were defined by $nLw_{555} > 1.3 \text{ mW cm}^{-2} \text{ mm}^{-1} \text{ sr}^{-1}$ (Nezlin and DiGiacomo 2005), leading to an overestimation of this group. It has been reported that suspended sediments having a

calcareous composition can also mimic coccolithophorids blooms (Brown and Podestá, 1997). All these factors highlight that the identification of coccolithophores by remote sensing is not straight forward (Nair et al., 2008), even though in the literature there are several algorithms already in use to identify this phytoplankton group from space, which are generally based on retrieval of the backscattering coefficient and the estimation of the calcite concentration via empirical relationship (Ackleson et al., 1994; Brown & Podestá, 1997; Brown & Yoder, 1994; Cokacar et al., 2001; Gordon et al., 2001; Iglesias-Rodríguez et al., 2002; Kopelevich et al., 2013; Moore et al., 2012; Smyth et al., 2002; Tyrell et al., 1999).

Similarly, even though PHYSAT-Med can be also applied to detect “*Phaeocystis*-like” group, no signals were found in our study. Since *Phaeocystis* is a genus with a worldwide distribution (Schoemann et al., 2005), including the Mediterranean Sea (Zingone et al., 1999), it is then clear that is possible that more *in situ* information will allow to support others anomalies labellisation in the future. In fact, *Phaeocystis*-like groups were initially included in formulation of PHYSAT-v2008 method (Alvain et al., 2008) based on coherent specific spatial structures associated to this group available in the literature, but a more powerful validation against *in situ* observations is needed to mark the presence of this group from space, at least in the Mediterranean. This would be case of other specific phytoplankton groups occurring in the Mediterranean Sea could be well added to PHYSAT-Med in the future, when extensive and accurate *in situ* data about on their abundance allow calibration and validation of the method. For instance, dinoflagellates occur widely across the Mediterranean Sea (Gómez 2003; Coll et al., 2010) but *in situ* measurements have not been taken into account in the present first attempt to compute a PHYSAT-Med version.

Recent studies have also shown that well marked mixed situation could be theoretically detected by the PHYSAT approach (Alvain et al., 2012). As indicated above, the shape and amplitude of the R_a spectra mainly depend on the bio-optical environment, represented by different values of b_p , a_{cdom} and a_{phy} . As shown by those authors, the a_{cdom} strongly controls the spectral shape of R_a in the blue part of the spectrum. For low a_{cdom} values, the R_a spectra decrease from the blue to red wavelengths, while they increase with the wavelengths for high a_{cdom} values. For a_{phy} , the highest R_a sensitivity is found at 443 nm. The b_p has a restricted effect on the spectral shape but represents the main factor affecting the R_a absolute values at all the wavelengths.

In conclusion, PHYSAT-Med provides us a regionalized algorithm to estimate dominant phytoplankton groups (nanoeukaryotes, *Prochlorococcus*, *Synechococcus*, diatoms, coccolithoprorids and *Phaeocystis*-like) in the first optical depth of the Mediterranean Sea. The comparison exercise performed here using *in situ* HPLC datasets and previous measurements shows a reasonable visual agreement for four groups, *Prochlorococcus*, *Synechococcus*, nanoeukaryotes and diatoms. Our study evidences the dominance of *Synechococcus* over prochlorophytes throughout the year, whereas nanoeukaryotes were more abundant during winter months. However, diatoms seemed to augment during the spring period (March to April), especially in the Ligurian and Adriatic seas. It is clear that data analysis through PHYSAT-Med provides hints only on the most abundant phytoplankton types and in the surface layer but it could be still considered a useful tool for monitoring the dynamics and maintenance of planktonic biodiversity in the Mediterranean. Satellite information on the phytoplankton groups distribution can be used for a better estimate of Chl *a* concentration (Alvain et al., 2006) in the Mediterranean Sea where standard bio-optical algorithms display a

considerable bias (D’Ortenzio et al., 2002; Bosc et al., 2004; Bricaud et al., 2002; Morel and Gentili, 2009; Volpe et al., 2007, Santoleri et al., 2008). PHYSAT-Med could potentially be also used to verify PFTs in ecological models, as the lack of *in situ* phytoplankton groups data is regarded as a major problem in phytoplankton ecosystem modelling (Anderson, 2005). Our approach should be then understood as the first version of an algorithm susceptible to be completed when more *in situ* measurements allow validation for other phytoplankton groups that, at the moment, could not be included in the method formulation.

Acknowledgements.

This research was supported by the PERSEUS European Project and PR11-RNM-7722 Andalucía Regional Project. The authors acknowledge the NASA/GSFC/DAAC for providing access to L3 MODIS products. *In situ* HPLC dataset was obtained from SESAME EU Project, BOUSSOLE Project and MAREDAT data. We thank J. Ras and the members of the SAPIGH analytical platform from the *Laboratoire d’Océanographie de Villefranche* for the HPLC analysis. GN was supported by the program “Salvador Madariaga, Ministerio de Educación, Cultura y Deporte, Programa Nacional de Movilidad de Recursos Humanos del Plan Nacional de I-D+i 2008-2011, prorrogado por Acuerdo de Consejo de Ministros de 7 de octubre de 2011”.

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 1114

1115 **Table Captions.**

1116 **Table 1.** Acronym definitions.

1117

1118 **Table 2.** Characteristics of acceptable Ra spectra for each phytoplankton groups.

1119

1120 **Table 3.** Cruises, Location, Sampling Period and Season, Dataset and number of HPLC
1121 samples (in brackets number in first optical depth).

1122

1123 **Table 4.** Validation results for the PHYSAT-Med method. For each group, the
1124 percentage of valid (bold) and wrong (italic) identifications are shown.

1125

1126 **Figure captions.**

1127 **Figure 1.** Schematic view of steps followed to adapt PHYSAT-Med.

1128

1129 **Figure 2.** Map of *in situ* sampling stations (see Table 3). Blue and red lines indicate the
1130 position of the four transects used to extract PFT data. Green area shows the Ligurian
1131 Sea region (Bricaud et al., 2002).

1132

1133 **Figure 3.** MODIS reference model ($nLw^{ref}(\lambda, Chla)$) of the normalized water-
1134 leaving radiance (nLw) as a function of wavelength for various Chla concentrations
1135 (colour scale, in $mg\ m^{-3}$) for the Mediterranean Sea (excluding Black Sea) during the
1136 study period (July 2002 – May 2013) using a) standard algorithm for chlorophyll-a
1137 (OC3M- Chla), b) regional algorithm (MedOC3-Chla). c) $nLw^{ref}(\lambda)$ at global scale for
1138 MODIS sensor (data obtained from PHYSAT website, <http://log.univ-littoral.fr/Physat>).

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1140

Figure 4. Monthly climatology of the dominant phytoplankton group detected by PHYSAT-Med during the study period (July 2002 – May 2013).

Figure 5. Map of detection frequencies (0 = group never detected, 1 = all valid pixels associated with the group) during the study period for different phytoplankton groups. Left and right columns represent the mean and standard deviation, respectively. Colour scale is different for each group.

Figure 6. Pigment ratio from *in situ* HPLC data (Table 2) averaged over the upper 35 meters depth: (a, b and c) divinyl chl-a; (d, e and f) zeaxanthin; (g, h and i) fucoxanthin and (j, k and l) 19'HF. Plots m, n and o display the map of the most frequent dominant phytoplankton group during March, July and October 2008, respectively.

Figure 7. Validation of PHYSAT-Med using HPLC *in situ* dataset (Table 2). Blue (nanoeukaryotes) and green (*Synechococcus*) symbols represent the location of the station where PHYSAT-Med works successfully.

Figure 8. Temporal percentage (green bars, left axis) of each phytoplankton group identified by PHYSAT-Med in the Ligurian Sea. Red and black dots (right axis) represent respectively the HPLC pigments ratio for SODYF and BOUSSOLE dataset in the first optical depth. Note that in panel d), only *in situ* measurements with zeaxanthin pigment ratios >0.20 have been represented.

Figure 9. Høevmöeller diagram on the west-east transect for the phytoplankton groups (see Fig.1 for geographical position of the transect), which are calculated from the 10-days composite.

Figure 10. Høevmöeller diagram on the north-south transects of the phytoplankton groups (see Fig.1 for geographical position of the transect), which are calculated from the 10-days composite. (a) Levantine basin, (b) central Mediterranean and (c) western Mediterranean.

Figure 11. (a). Monthly time series of the percentage of pixels of PFT identified by PHYSAT-Med (green bars) and PHYSAT (cyan bars). (b). Temporal percentage (cyan bars, left axis) of diatom group identified by PHYSAT (Alvain et al., 2005; 2008) in the Ligurian Sea. Red and black symbols (right axis) represent the fucoxanthin pigment ratio for SODYF and BOUSSOLE datasets respectively in the first optical depth. PHYSAT Global data can be downloaded from the website: <http://log.univ-littoral.fr/Physat>

Figure 12. Monthly climatological mean of the seasonal cycle of the percentage of each phytoplankton group in the Ligurian Sea during 2003-2012 period.

Table 1. Acronyms information

Acronym	Name	Units	Explanation
Chl <i>a</i>	Chlorophyll <i>a</i> concentration	mg m ⁻³	Chlorophyll <i>a</i> concentration
OC3M-Chl <i>a</i>	Chlorophyll <i>a</i> concentration	mg m ⁻³	Chl <i>a</i> estimated by standard OC3M algorithm for MODIS images
MedOC3-Chl <i>a</i>	Chlorophyll <i>a</i> concentration	mg m ⁻³	Chl <i>a</i> concentration estimated by regional MedOC3 algorithm for MODIS images
K490	Diffuse Attenuation Coefficient at 490 nm	m ⁻¹	The diffuse attenuation coefficient in water indicates how strongly light intensity at a specified wavelength is attenuated within the water column.
nLw	Normalized water-leaving radiance	mW cm ⁻² μm ⁻¹	The upwelling radiance just above the sea surface, in the absence of an atmosphere, and with the sun directly overhead
nLw ^{ref}	Specific water-leaving radiance	mW cm ⁻² μm ⁻¹	Represents the average nLw spectrum for a given value of Chl <i>a</i> . This reference is used to remove the first order effect of Chl <i>a</i> on nLw(λ) measurements.
LUT	Look-Up Table		Look-Up Table of nLw ^{ref} for a given λ and Chl <i>a</i> concentration
Rrs	Remote sensing reflectance	adimensional	Upwelling radiance emerging from the ocean divided by the downwelling irradiance reaching the water surface
□o	Mean solar irradiance	mW cm ⁻² μm ⁻¹	Mean solar irradiance to convert nLw into Rrs
Ra	Radiance anomalies	adimensional	Represents the second order variation of the nLw after removal the first order effect of the Chl <i>a</i> variation. Ra is independent of the Chl <i>a</i> levels and represents the second order variation of nLw(λ)

Table 2. Characteristics of acceptable Ra spectra for each phytoplankton groups.

MODIS Wavelengths(nm)	412	443	469	488	531	547	555	Additional citreria
Phytoplankton groups								
Nanoecaryotes min.	0.4	0.55	0.5777	0.5979	0.6	0.6	0.6	nLw*412<nLw*443
Nanoecaryotes max.	0.8	0.9	0.9277	0.9479	1	1	1	nLw*443<nLw*488
Prochlorococcus min.	0.8	0.9	0.9	0.9	0.9	0.9	0.9	
Prochlorococcus max.	1	1	1	1	1	1	1	
Synechococcus min.	1	1	1	1	1	1	1	
Synechococcus max.	1.2	1.2	1.1723	1.1521	1.15	1.15	1.15	
Diatoms min.	1.2	1.2	1.1723	1.1521	1.15	1.15	1.15	nLw*412>nLw*488
Diatoms max.	2.2	1.8	1.6340	1.5128	1.4	1.4	1.4	nLw*555<nLw*488
Phaeocystis min.	1.3	1.4	1.4	1.4	1.4	1.4	1.4	nLw*412<nLw*443
Phaeocystis max.	1.5	1.6	1.6	1.6	1.6	1.6	1.6	nLw*443<nLw*488 nLw*531>nLw*555
Coccolithporids min.	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
Coccolithporids max.	6	6	6	6	6	6	6	

Table 3. Cruises, Location, Sampling Period and Season, Dataset and number of HPLC samples (in brackets number in first optical depth).

Cruises	Location	Period	Season	Dataset	n
SODYFT	Ligurian Sea	02/25/2002 – 12/19/2005	All	DYFAMED ¹	451(160)
BOUSSOLE	Ligurian Sea	07/22/2001 – 11/10/2007	All	BOUSSOLE ²	1877(1113)
SESAME_IT1_BOT	Adriatic Sea	02/16/2008 – 02/25/2008	Spring	SESAME ³	34 (21)
SESAME_IT2_BOT	Ionian Sea	03/02/2008 – 03/08/2008	Spring	SESAME ³	47(16)
SESAME_IT3_BOT	Ionian Sea	03/17/2008 – 03/18/2008	Spring	SESAME ³	44(16)
SESAME_IT4_BOT	Western Basin	03/20/2008 – 04/05/2008	Spring	SESAME ³	58(24)
SESAME_IT5_BOT	Ionian Sea	09/16/2008 – 09/26/2008	Autumn	SESAME ³	45(20)
SESAME_IT6_BOT	Ionian Sea	09/20/2008 – 09/23/2008	Autumn	SESAME ³	28(11)
SESAME_IT7_BOT	Adriatic Sea	10/09/2008 – 10/13/2008	Autumn	SESAME ³	34(21)
SESAME_SP1_BOT	Alborán Sea	04/08/2008 – 04/11/2008	Spring	SESAME ³	15(5)
SESAME_SP2_BOT	Alborán Sea	09/20/2008 – 09/27/2008	Autumn	SESAME ³	62(39)
SESAME_Turkey_BOT	Levantine Basin	03/20/2008 – 04/26/2008	Spring	SESAME ³	78(34)
SESAME_Turkey_BOT	Levantine Basin	09/19/2008 – 10/19/2008	Autumn	SESAME ³	22(10)
SESAME-IS-1_BOT	Levantine Basin	04/08/2008 – 04/09/2008	Spring	SESAME ³	20(6)
SESAME-IS-2_BOT	Levantine Basin	09/07/2008 – 09/12/2008	Autumn	SESAME ³	55(14)
SES_UNLUATA_gr1	Levantine Basin Aegean Sea	03/27/2008 – 04/06/2008	Spring	SESAME ³	26(12)
SES_UNLUATA_gr2	Levantine Basin Aegean Sea	08/29/2008 – 09/07/2008	Autumn	SESAME ³	32(12)
BOUM_bot	Mediterranean Sea	06/21/2008 – 07/18/2008	Summer	SESAME ³	299(64)

Data sources: ¹ <http://doi.pangaea.de/10.1594/PANGAEA.793246>; ² <http://www.obs-vlfr.fr/Boussole/html/home/home.php>

³ http://isramar.ocean.org.il/perseus_data/CastMap.aspx

Table 4. Validation results for the PHYSAT-Med method. For each group, the percentage of valid (**bold**) and wrong (*italic*) identifications are shown.

PHYSAT-MED / in situ	Nanoecaryotes	Synechococcus
Nanoecaryotes	74.07	<i>39.29</i>
<i>Synechococcus</i>	<i>25.93</i>	60.71

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