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► To cite this version:

Gonzalo Luis Pérez, Martí Galí, Sarah Jeanne Royer, Marina Gerea, Eva Ortega-Retuerta, et al.. Variability of phytoplankton light absorption in stratified waters of the NW Mediterranean Sea: The interplay between pigment composition and the packaging effect. *Deep-sea research. Part A, Oceanographic research papers*, 2021, 169, pp.103460. 10.1016/j.dsr.2020.103460 . hal-03095064

HAL Id: hal-03095064

<https://hal.science/hal-03095064v1>

Submitted on 4 Jan 2021

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Variability of phytoplankton light absorption in stratified waters of the NW Mediterranean Sea: the interplay between pigment composition and the packaging effect

By Perez et al.

HIGHLIGHTS:

- Changes in pigment composition, ruled by PPCs, explained 50% of a^*_{ph} vertical variation
- The remaining spatiotemporal variation of a^*_{ph} was attributed to packaging effect
- Variation in c_p^* (660) was coincident with vertical and seasonal differences in irradiance.
- Changes in intracellular pigment content explained differences in packaging effect
- Photoacclimation shaped the vertical and inter-cruise variability of a^*_{ph}

1 Variability of phytoplankton light absorption in stratified waters of the NW 2 Mediterranean Sea: the interplay between pigment composition and the 3 packaging effect

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16
17 **Keywords:** phytoplankton absorption, pigment composition, packaging effect, community size
18 structure, photoacclimation, North-Western Mediterranean Sea

19 20 Abstract

21 The Variability of chlorophyll-specific phytoplankton light absorption [$a^*_{ph}(\lambda)$] was examined
22 over depth and time in stratified offshore waters of the North-Western Mediterranean Sea. Coherent
23 water patches were tracked with Lagrangian drifters during two oceanographic cruises in September
24 (late summer) and May (post-spring bloom phase). By simultaneously analysing the phytoplankton
25 absorption and pigment measurements, we explicitly separated the impact of pigment composition
26 from that of pigment packaging on $a^*_{ph}(\lambda)$. We further partitioned the packaging effect by comparing
27 the variation of the packaging index [$Q_a^*(440)$], the phytoplankton community size structure (derived
28 from diagnostic pigment analysis), and the chlorophyll-specific beam attenuation by particles [c_p^*
29 (660)] as an optical index of phytoplankton photophysiology. In the ensemble of cruises, around
30 50% of the $a^*_{ph}(440)$ vertical variation was explained by changes in the pigment composition (ruled
31 by the decrease of photoprotective pigments with depth). The remaining vertical and inter-cruise
32 variation of $a^*_{ph}(440)$ was attributed to the packaging effect. We found that differences in the
33 $c_p^*(660)$ index (most likely indicating changes in the intracellular pigment concentration due to
34 photoacclimation) mainly explained the observed variation in the packaging effect. Differences in c_p^*
35 (660) were coincident with either the vertical gradient of light availability or the lower mean daily
36 PAR irradiance in the euphotic layer of the September cruise. These explained the stronger
37 packaging and lower $a^*_{ph}(440)$ values observed with increasing depth in both cruises, and in
38 September relative to May. On the other hand, differences in the phytoplankton community size
39 structure did not explain the observed patterns in the packing effect. Our results highlight the
40 importance of phytoplankton short-term acclimation to the prevailing light conditions, determining
41 the vertical and temporal variability of $a^*_{ph}(\lambda)$. A better understanding of the $a^*_{ph}(\lambda)$ variability and
42 its main drivers are key to improve different bio-optical applications.

43

44 1.0 INTRODUCTION

45 The absorption and scattering coefficients of various optically active constituents determine
46 the ocean's optical properties (Preisendorfer, 1961). Among them, the *in vivo* phytoplankton spectral
47 absorption coefficient [$a_{ph}(\lambda)$, m^{-1}] is a critical component because it quantifies phytoplankton
48 potential light absorption, providing an optical signature of the autotrophic community in the ~380-
49 700 nm spectral region (Johnsen et al., 2011; Morel, 1978). Its chlorophyll-specific counterpart [a^*_{ph}
50 (λ), $m^2 mg^{-1}$], constitutes the link between phytoplankton biomass and light absorption. Altogether,
51 $a_{ph}(\lambda)$ and $a^*_{ph}(\lambda)$ are key to understand the quantitative regional and global significance of
52 phytoplankton to ocean ecology and biogeochemical cycling.

53 Characterization of $a_{ph}(\lambda)$, as well as the drivers of its natural variability, is essential for a
54 variety of research applications, including the study of light propagation and ocean thermal structure
55 (Chang and Dickey, 2004; Sathyendranath and Platt, 1988), algal physiology (Moore et al., 1995;
56 Morel et al., 1993; van Leeuwe et al., 2005) and modelling of primary production (PP) (Marra et al.,
57 2007; Morel et al., 1996). Furthermore, since $a_{ph}(\lambda)$ affects the ocean spectral reflectance, it is
58 important for ocean colour remote sensing applications such as the retrieval of *chlorophyll a* (*Chl*
59 *a*) (D'Ortenzio et al., 2002; O'Reilly et al., 1998; Siegel et al., 2013; Volpe et al., 2007), the estimation
60 of PP (Behrenfeld and Falkowski, 1997; Lee et al., 2015; Morel, 1991; Tilstone et al., 2015) and the
61 inference of phytoplankton size, functional types and taxonomic composition (Brewin et al., 2011;
62 Bricaud et al., 2012; Zeng et al., 2018).

63 The variability of $a_{ph}(\lambda)$ at different spatial and temporal scales has been widely studied over
64 the past 25 years in regions of the world's oceans. Studies have shown that the variability of $a_{ph}(\lambda)$
65 magnitude is mainly explained by differences in *Chl a* concentration (as a proxy of phytoplankton
66 biomass). In spite of the strong covariation between $a_{ph}(\lambda)$ and *Chl a*, $a^*_{ph}(\lambda)$ is generally far from
67 being constant because it is affected by pigment composition and by the pigment packaging effect
68 (Bricaud et al., 1999, 1995; Cleveland, 1995; Mitchell and Kiefer, 1988). This latter effect is defined
69 as the reduction in the absorption of pigmented particles relative to the absorption of the pigments
70 in solution. It is predicted by theory to depend on phytoplankton size and intracellular pigment
71 concentrations. As a consequence, the packaging effect increases with cell size and cellular pigment
72 content; as a consequence, higher values of $a^*_{ph}(\lambda)$ are expected for small cells with low intracellular
73 pigment concentrations (Kirk, 1976; Morel and Bricaud, 1981).

74 In natural waters, the effects of the different causes that contribute to the variability of $a^*_{ph}(\lambda)$
75 are intermingled and are commonly difficult to evaluate individually. Various methods have been
76 implemented to explicitly separate the influences of pigment composition and packaging effect (see,
77 e.g., Bricaud et al., 2004; Lohrenz et al., 2003; Stæhr et al., 2004; Stuart et al., 1998). Different
78 approaches have also been proposed to independently quantify the impact of the algal community
79 size structure on pigment packaging (Bricaud et al., 2004a; Ferreira et al., 2009). Diagnostic
80 accessory pigments analysis (DPA) has been used to estimate the relative proportions of the three
81 phytoplankton size classes (micro-, nano- and picophytoplankton) (Uitz et al., 2006; Vidussi et al.,
82 2001). From DPA, a size index (*S*) was derived to obtain a single indicator of the average size of

83 the phytoplankton population within the first optical depth (Bricaud et al., 2004). This has been
84 frequently used to relate changes in phytoplankton cell size with the variability of $a^*_{ph}(\lambda)$ (e.g.,
85 Bricaud et al., 2010; Nunes et al., 2019; Wang et al., 2014). Instead, also for surface layers, Ciotti
86 et al. (2002) proposed the use of the spectral shape of $a_{ph}(\lambda)$ to determine the cell size factor (S_f),
87 being this procedure also used to explain $a^*_{ph}(\lambda)$ variability (Ferreira et al., 2013, 2017; Kheireddine
88 et al., 2018a).

89 The relative contributions of the different factors affecting $a^*_{ph}(\lambda)$ depend on the spatial
90 dimension under analysis. In horizontal surveys across surface waters, the dominant source of a^*_{ph}
91 (λ) variability generally results from differences in the pigment packaging due to changes in the size
92 structure of phytoplankton populations (e.g., Bricaud et al., 2004; Ciotti et al., 2002; Ferreira et al.,
93 2013). In contrast, in the vertical dimension of oligotrophic stratified waters, the variability of $a^*_{ph}(\lambda)$
94 is mainly related to gradients of photoprotective carotenoids and changes in the intracellular pigment
95 concentration resulting from photoacclimation (e.g., Allali et al., 1997; Bouman et al., 2000; Bricaud
96 and Stramski, 1990).

97 Although several studies have invoked photoacclimatory changes in the cell pigment content
98 to explain part of the variability of $a^*_{ph}(\lambda)$, they have often not evaluated this effect directly (e.g.,
99 Organelli et al., 2011; Stuart et al., 1998; Wang et al., 2014). Changes in the pigment concentration
100 per cell can be evaluated by cytometry measurements, though this approach is commonly restricted
101 to the picophytoplankton fraction (Bricaud et al., 1999; Hickman et al., 2009; Lazzara et al., 1996;
102 Morel et al., 1993). For the micro- and nanophytoplankton fractions, this can be assessed by
103 combining pigment analysis and microscopic determination of cell numbers and dimensions
104 (Brunelle et al., 2012; Fujiki and Taguchi, 2002; Stæhr et al., 2004). However, this approach is
105 cumbersome and often difficult to operate routinely for systematic measurements in natural waters.
106 As a result, less is known about the interactive effects of changes in the intracellular pigment
107 concentration and cell size on pigment packaging and, therefore, on the $a^*_{ph}(\lambda)$ variability.

108 Here we present a comprehensive analysis of the phytoplankton light absorption variability,
109 across the spatial and temporal scales, in offshore stratified waters of North-Western Mediterranean
110 Sea (NWMS). The study was performed during two oceanographic cruises, right after (Sept-2011)
111 and before (May-2012) the maxima of summer stratification. We hypothesize that, given the
112 expected contrasting light exposure conditions (strong vertical light gradients and seasonal
113 variations of incident solar radiation), photoacclimation (due to changes in the intracellular pigment
114 content and/or in the proportion of accessory pigments per unit of *Chl a*) will contribute significantly
115 to the vertical and temporal differences in $a^*_{ph}(\lambda)$. The importance of photoacclimation, in turn, will
116 depend on the degree of variation of the phytoplankton community composition and the associated
117 changes in the size structure. Hence, we aim at investigating the impact of photoacclimation on a^*_{ph}
118 (λ) variability using a suite of optical and biological parameters. To do this, we separate the effect of
119 pigment composition from that of pigment packaging. In addition, we evaluate the use of the
120 chlorophyll-specific beam attenuation by particles at 660 nm [$c_p^*(660)$, $m^2 mg^{-1}$] as an optical index
121 of phytoplankton photoacclimation (see Behrenfeld and Boss, 2003; Mignot et al., 2014; Xing et al.,

122 2014). With the concurrent use of $c_p^*(660)$ and the analysis of the size structure of phytoplankton
 123 community (derived from DPA approach, light microscopy phytoplankton counts and flow
 124 cytometry), we evaluate the relative contributions of the two intrinsic factors that determine the
 125 packaging effect. This study may contribute to improve our comprehension of the $a^*_{ph}(\lambda)$ variability
 126 and its main drivers with implications in a variety of bio-optical applications, such as the primary
 127 production models and the remote sensing of *chlorophyll a* concentration.

128

129 2.0 MATERIAL AND METHODS

130 2.1. Study area and sampling

131 The study was carried out in offshore waters of the NWMS (the Balearic area, over a water
 132 column depth of nearly 2000 m) during two Lagrangian cruises located in the same oceanographic
 133 system. The first on 12 - 22 September 2011 (hereinafter referred to as Sept-2011) and the second
 134 on 22 - 24 May 2012 (hereinafter referred to as May-2012) (Fig. 1).

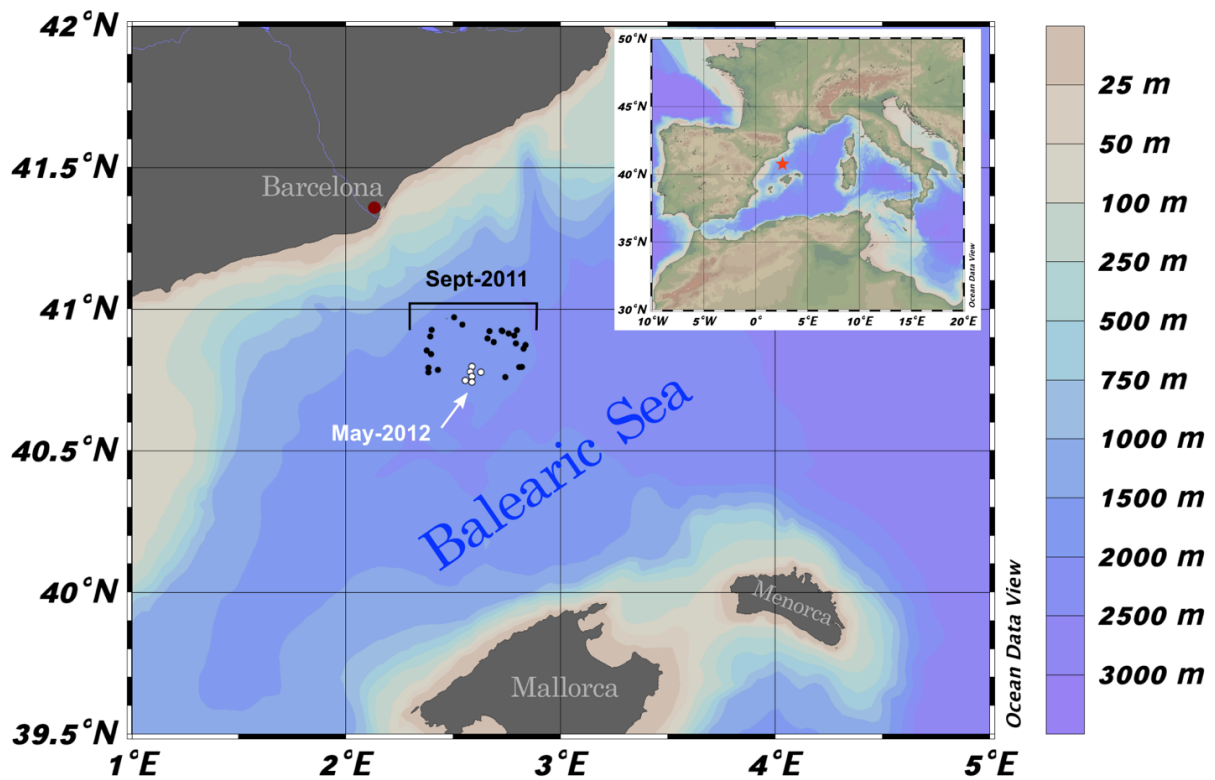


Fig. 1. Map showing the stations sampled in the two SUMMER cruises: Sept-2011 (black dots) and May-2012 (white dots).

135

136 The cruises were conducted in the framework of project SUMMER on board the RV 'García
 137 del Cid'. A CTD probe (Seabird 9/11 plus) equipped with a radiometer (QCP-2300, Biospherical),
 138 chlorophyll fluorometer (Seapoint) and C-Star transmissometer (Wet Labs, Inc.), provided depth
 139 profiles of temperature, salinity, downwelling irradiance integrated over the photosynthetically active
 140 radiation spectrum [E_d (PAR, Z)], *Chl a* fluorescence and beam transmission. The surface mixed
 141 layer (SML) was estimated from CTD temperature profiles and its depth (Z_{SML}) was defined by a >

142 0.2 °C deviation with respect to the temperature at 1 m (D'Ortenzio et al., 2005; de Boyer Montégut
 143 et al., 2004) (see Table 1 for a list of symbols, definitions and units) . The CTD system was mounted
 144 on a Niskin-bottle rosette used to collect water samples at several depths. In this study, samples of
 145 28 stations were analysed, 22 in the longer cruise (Sept-2011) and 6 in the shorter one (May-2012).
 146 In Sept-2011 four depths were sampled (3, 8 or 10, 30 m and the deep chlorophyll maximum, DCM)
 147 and 3 depths in May-2012 (3, 25 m and DCM). The DCM was detected from fluorescence profiles
 148 and its depth (Z_{DCM}) was generally located between 45 and 60 m. Data was analysed and presented
 149 separately for three different layers. Samples at 3, 8 and 10 m corresponded to the SML, samples
 150 at 25 and 30 m to the middle layer between the mixed layer depth and DCM (hereinafter Middle),
 151 and the remaining samples to the DCM.

Symbol	Units	Description
Z_{SML}	[m]	Depth of surface mixed layer (SML)
$T-Chl a$	[mg m ⁻³]	Total <i>chlorophyll a</i> concentration
$Fl-Chl a$	[mg m ⁻³]	Calibrated profiles of fluorescence
$C_p(660)$	[m ⁻¹]	Beam attenuation coefficient at 660 nm due to particles
$C_p^*(660)$	[m ² mg ⁻¹]	<i>T-Chl a</i> -specific beam attenuation coefficient
$E_d(PAR)$	[μmol quanta m ⁻² s ⁻¹]	Downward irradiance of photosynthetically active radiation (PAR)
$\bar{E}_d(PAR)$	[mol quanta m ⁻² d ⁻¹]	Daily integrated underwater downward irradiance of PAR
$K_d(PAR)$	[m ⁻¹]	Diffuse attenuation coefficient for PAR in the euphotic layer
$Z_{1\%}$	[m]	Depth of euphotic layer (1% criterion)
Z_{DCM}	[m]	Depth of deep <i>chlorophyll a</i> maximum (DCM)
PSC	[μg L ⁻¹]	Photosynthetic carotenoids concentration
PPC	[μg L ⁻¹]	Photoprotective carotenoids concentration
$T-APSP$	[μg L ⁻¹]	Total accessory photosynthetic pigments concentration
NPP index	[μg L ⁻¹]	Nonphotosynthetic pigment index
$a_{ph}(\lambda)$	[m ⁻¹]	Phytoplankton spectral absorption coefficient
$a_{ph}(440)$	[m ⁻¹]	Phytoplankton absorption coefficient at 440 nm (blue band)
$a_{ph}(675)$	[m ⁻¹]	Phytoplankton absorption coefficient at 675 nm (red band)
$a^*_{ph}(\lambda)$	[m ² mg ⁻¹]	<i>Chl a</i> -specific spectral phytoplankton absorption coefficient
$a^*_{ph}(440)$	[m ² mg ⁻¹]	<i>Chl a</i> -specific phytoplankton absorption coefficient at blue band
$a^*_{ph}(675)$	[m ² mg ⁻¹]	<i>Chl a</i> -specific phytoplankton absorption coefficient at red band
$a^*_{sol}(440)$	[m ² mg ⁻¹]	<i>Chl a</i> -specific total pigment absorption coefficient in solution at blue band
$Q_s(440)$	[d.l.]	Pigment packaging index at 440 nm
$a^*_{ph-ppc}(440)$	[m ² mg ⁻¹]	Photoprotective component of $a^*_{ph}(440)$
$a^*_{ph-psp}(440)$	[m ² mg ⁻¹]	Photosynthetic component of $a^*_{ph}(440)$
F_{micro}	[d. l.]	Microphytoplankton fraction of total biomass
F_{nano}	[d. l.]	Nanophytoplankton fraction of total biomass
F_{pico}	[d. l.]	Picophytoplankton fraction of total biomass
SI	[μm]	Size index of algal population based on Diagnostic Pigment Analysis (DPA)

152 **Table 1.** Symbols used in the present study and their significance

153 In addition to the CTD casts, vertical profiles of downwelling spectral irradiance $E_d(\lambda, Z)$ and
 154 $E_d(PAR, Z)$ were determined with a PRR-800 multichannel profiling radiometer (Biospherical).
 155 Profiles were carried out around noon and recorded from the surface down to 100 m. All the
 156 irradiance profiles (with PRR-800 radiometer and CTD casts) were carried out on calm days, during
 157 clear sky and constant light conditions. Incident solar radiation in air was simultaneously monitored
 158 on deck, with an Aanderaa Instruments automated meteorological station on board. A Solar
 159 Radiation Sensor 2770 provided global radiation in the wavelength range 0.3–2500 μm. This sensor
 160 was used to derive PAR irradiance above the sea surface following Baker and Frouin (1987). The

161 instantaneous quantum PAR irradiance above the sea surface [$E_d(\text{PAR})^{+0}$, $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$]
 162 was calculated from $W \text{ m}^{-2}$ by multiplying by 4.60 (Kirk, 2011). The daily PAR irradiance above the
 163 sea surface [$\bar{E}_d(\text{PAR})^{+0}$], in $\text{mol quanta m}^{-2} \text{d}^{-1}$, was obtained by integrating $E_d(\text{PAR})^{+0}$ over a day.

164 **2.2. Apparent optical property measurements: diffuse attenuation coefficients for**
 165 **downwelling irradiance and related variables**

166 Diffuse attenuation coefficients for broadband PAR [$K_d(\text{PAR})$], from both CTD and PRR-800
 167 E_d profiles, were determined as the slope of the linear regression between the logarithm of
 168 irradiance and depth, within the entire euphotic layer (from about 5 to 60 m). Prior to analysis, the
 169 data were carefully examined for irregularities (Hargreaves et al., 2007): near-surface noise caused
 170 by smooth waves and ripples was eliminated from $E_d(\text{PAR}, Z)$ profiles. The regression analyses
 171 were statistically significant ($p < 0.01$) and had R^2 above 0.98 for all evaluated casts. The depth of
 172 the euphotic layer ($Z_{1\%}$) was then calculated as $4.605/K_d(\text{PAR})$, being the depth at which $E_d(\text{PAR})$
 173 is reduced to 1% of its value just below the surface (Kirk, 2011). The average daily PAR irradiance
 174 for the surface mixed layer was computed in accordance with Babin et al. (1996). In addition, for the
 175 depths corresponding to the other two evaluated layers (i.e., Middle and DCM), the daily PAR
 176 irradiances were calculated from $K_d(\text{PAR})$ and $\bar{E}_d(\text{PAR}^{-0})$, in accordance with Kirk (2011) as
 177 follows:

178
$$\bar{E}_d(\text{PAR}, Z) = \bar{E}_d(\text{PAR}^{-0}) \cdot e^{(-k_d(\text{PAR}) \cdot Z)} \quad (1)$$

179 where $\bar{E}_d(\text{PAR}^{-0})$ is the daily downwelling PAR irradiance just beneath the air-water interface.

180 **2.3. Inherent optical properties and related variables**

181 Total particulate matter absorption coefficients [$a_p(\lambda)$] were determined by the quantitative
 182 filter technique (QFT), using the simple transmittance method (T-Mode) in a Lambda 800 (Perkin-
 183 Elmer) dual beam spectrophotometer (Mitchell, 1990; Mitchell and Kiefer, 1988; Trüper and Yentsch,
 184 1967). Water samples (1 to 2 L) were filtered on-board using 25 mm-diameter GF/F filters.
 185 Immediately after filtration, absorbance scans were measured from 300 to 750 nm at 1 nm intervals.
 186 Absorbances were checked to be lower than 0.4 (Cleveland and Weidemann, 1993). The QFT
 187 method was applied according to NASA's optics protocols for absorption coefficient measurements
 188 (Mitchell et al., 2002). Spectrophotometric scans were made against a blank clean filter wetted with
 189 filtered (0.2 μm) seawater. Absorption coefficients of non-algal particles [$a_{nap}(\lambda)$] were determined
 190 with the methanol extraction method (Kishino et al., 1985). Absorption coefficients of $a_p(\lambda)$ (first
 191 measurement) and $a_{nap}(\lambda)$ (measurement after extraction) were estimated according to the
 192 equation:

193
$$a_{p,nap}(\lambda) = \frac{2.303 \cdot A_{filter}(\lambda) \cdot S}{V \cdot \beta(\lambda)} \quad (2)$$

194 where $A_{filter}(\lambda)$ is the measured absorbance with QFT, S is the clearance area of the filter, V is the
 195 volume of filtered water, and $\beta(\lambda)$ is the amplification factor vector (Mitchell and Kiefer, 1988). The
 196 $\beta(\lambda)$ factor was calculated following Bricaud and Stramski (1990) with the equation:

197
$$\beta(\lambda) = 1.63 \cdot A_{filter}(\lambda)^{-0.22} \quad (3)$$

198 As standard procedure, a null point correction was set at 750 nm, where absorbance by
 199 particles is assumed to be negligible. Phytoplankton absorption coefficients [$a_{ph}(\lambda)$] were obtained
 200 by subtracting $a_{nap}(\lambda)$ from $a_p(\lambda)$. The chlorophyll-specific phytoplankton absorption coefficient [a^*_{ph}
 201 (λ)] was estimated as $a_{ph}(\lambda)/T-Chl\ a$, where *T-Chl a* concentration was determined by High
 202 Performance Liquid Chromatography (HPLC) and included the following pigments (*Chl a* + allomers
 203 and epimers + *DV-Chl a* + *chlorophyllide a*) (see section 2.4.1 for details).

204 The packaging effect was estimated by means of the dimensionless packaging index
 205 [$Q_a^*(440)$], calculated as the ratio of the phytoplankton absorption, $a_{ph}(\lambda)$, and the absorption of total
 206 pigments in solution (without packaging) [$a_{sol}(\lambda)$] (see equation 5).

$$207 \quad Q_a^*(440) = \frac{a_{ph}(440)}{a_{sol}(440)} \quad (4)$$

208 The $a_{sol}(\lambda)$ coefficient was reconstructed following the approach of Bricaud et al. (2004) (see
 209 equations 5 and 5') based on the weight-specific absorption coefficients of individual pigments
 210 proposed by Goericke and Repeta (1993) and Bidigare et al. (1990).

$$211 \quad a_{sol}(\lambda) = a_{pigm}(\lambda) + a_{miss}(\lambda) \quad (5)$$

212 with

$$213 \quad a_{pigm}(\lambda) = \sum C_i \cdot a^*_i(\lambda) \quad (5')$$

214 where $a_{pigm}(\lambda)$ is the sum of specific absorption coefficient of the *i*-th pigment [$a^*_i(\lambda)$, m² mg⁻¹]
 215 multiplied by their concentrations (C_i , mg m⁻³) in the medium; and $a_{miss}(\lambda)$, the so-called missing
 216 term, depends on *T-Chl a* concentration following $a_{miss}(440) = 0.0525 \cdot T-Chl\ a^{0.855}$; (Bricaud et
 217 al., 2004). The $Q_a^*(440)$ index can vary between 0 (maximum packaging effect) to 1 (no packaging
 218 effect). The chlorophyll-specific total pigment absorption coefficient [$a^*_{sol}(\lambda)$] is defined as *T-Chl a*
 219 normalized absorption coefficient of all the pigments in solution (a_{pigm}) plus the a_{miss} term.

220 We also made a distinction between the part of the chlorophyll-specific phytoplankton
 221 absorption coefficient associated with photosynthetically active pigments [$a^*_{ph-psp}(\lambda)$] and that
 222 associated with nonphotosynthetic (photoprotective) pigments [$a^*_{ph-ppc}(\lambda)$] following Babin et al.
 223 (1996) (see Supplementary Material for details).

224 The particulate beam attenuation coefficient at 660 nm [$c_p(660)$] was calculated from 1 m
 225 binned transmittance vertical profiles and determined subtracting the mean value of beam
 226 attenuation over the depth interval from 200-400 m (representing about 70% of the signal for the
 227 layer above it). These were considered representative of the background beam attenuation of
 228 phytoplankton-free water (Loisel et al., 2011; Loisel and Morel, 1998). Particle populations in the
 229 0.5–20 μ m size range make a dominant contribution to the $c_p(660)$ signal (Boss et al., 2001;
 230 Stramski and Kiefer, 1991). This size range encompasses phytoplankton, heterotrophs, as well as
 231 detrital and inorganic particles. Nevertheless, different field studies have demonstrated that in the
 232 open ocean, to first order, the $c_p(660)$ can successfully track phytoplankton biomass (e.g.,
 233 Behrenfeld and Boss, 2006; Dall'Olmo et al., 2009; Westberry et al., 2010). This was explained by
 234 either a rather constant contribution of phytoplankton to $c_p(660)$ or phytoplankton accounted for a

235 high proportion of c_p (660). Also, c_p (660) is less sensitive to physiological forcing (e.g., light and
236 nutrients) than pigments, thereby engendering the ratio of c_p (660) to $T-Chl a$ [c_p^* (660)] with
237 sensitivity to photoacclimation. Changes in c_p^* (660) with depth and seasons have indeed been
238 shown to follow a clear light-dependence, denoting changes in intracellular pigment concentration
239 due to phytoplankton photoacclimation (e.g., Fennel and Boss, 2003; Mignot et al., 2014; Mitchell
240 and Holm-Hansen, 1991). Following this, in the present study we evaluated the use of c_p (660) to
241 track the vertical and seasonal variation in phytoplankton biomass and c_p^* (660) as an optical index
242 of phytoplankton photophysiology.

243 **2.4. Phytoplankton pigment determinations, pigment-based phytoplankton size structure and** 244 **phytoplankton identification**

245 2.4.1. HPLC pigment determinations

246 Samples of 1.5 to 2 L from discrete depths were filtered through Whatman GF/F filters and
247 stored at -80 °C until return to the laboratory for pigment analysis. Concentrations of $T-Chl a$ and
248 accessory pigment (chlorophylls, carotenes and xanthophylls) were measured in using an HPLC
249 instrument (Spectra SYSTEM) following the method of Zapata et al. (2000). Calibration was made
250 using commercial pigment standards from the Institute for Water and Environment, Denmark.
251 Vertical fluorescence profiles were first corrected, when necessary, for the Non-Photochemical
252 Quenching effect following (Xing et al., 2012). Then, were converted into equivalent $T-Chl a$ profiles
253 (denoted $Fl-Chl a$) by using the $T-Chl a$ concentration of discrete samples obtained by HPLC
254 determinations following (Loisel et al., 2011).

255 In order to assess the effect of pigment composition on the variability of $a^*_{ph}(\lambda)$, we followed
256 the pigment classification of Bricaud et al. (2004): (a) photosynthetic carotenoids [PSC] [fucoxanthin
257 ($Fuco$), peridinin ($Peridi$), 19'-hexanoyloxyfucoxanthin (19'- HF), 19'- butanoyloxyfucoxanthin (19'-
258 BF)]; (b) photoprotective carotenoids [PPC] [$Zeaxanthin$ ($Zeax$), $Diadinoxanthin$ (Dd), $Alloxanthin$
259 ($Allo$), and β -carotene (β - $carot$)]; (c) total $Chlorophyll b$ ($T-Chl b$ as $Chl b + DV-Chl b$); and (d) total
260 $Chlorophyll c$ ($T-Chl c$). Following Babin et al. (1996), we also calculated the NPP index defined as
261 the ratio of the concentrations of photoprotective pigments to the concentrations of total pigments

262 2.4.2. Pigment-based estimation of phytoplankton size classes

263 Diagnostic pigment analysis (DPA) (Uitz et al., 2006; Vidussi et al., 2001) was used to
264 estimate the relative biomass proportions ($T-Chl a$ fractions) of pico- (<2 μm , F_{pico}), nano- (2–20
265 μm , F_{nano}) and microphytoplankton (>20 μm , F_{micro}). The biomass proportions were estimated from
266 the concentrations of the pigments that have a taxonomic significance and can be associated with
267 a certain size class, at least in oceanic case 1 waters. Seven pigments (i.e., $Fuco$, $Peridi$, 19'- HF ,
268 19'- BF , $Allo$, $T-Chl b$ and $Zeax$) were selected as diagnostic pigments of distinct phytoplankton
269 groups and used to calculate the $T-Chl a$ fractions belonging to the three size classes. The numerical
270 coefficients obtained by Uitz et al. (2006), were used to compute the biomass proportions associated
271 with each size class. It should be noted that there are some potential limitations in DPA, e.g., in
272 some cases, a given diagnostic pigment is shared by two size classes. Despite these drawbacks,
273 DPA is a useful approximation, and provides reasonable results at a global as well as at a regional
274 scale. (see Supplementary Material for details).

275 Then, the size index (*SI*) proposed by Bricaud et al. (2004) was used to assess the variations
276 of the dominant size class of the phytoplankton communities (see Supplementary Material for
277 details). As already acknowledged, the *SI* index is only an approximate indicator of the dominant
278 size of the phytoplankton assemblage (i.e., a unique central size is used to represent each size
279 class). Besides, this metric was originally derived for samples in the first optical depth. Therefore, in
280 this study, *SI* was only used to roughly synthesized, in a single continuous parameter, the size
281 structure of phytoplankton community throughout the three evaluated layers.

282 2.4.3. Phytoplankton identification

283 Water samples from a random subset of CTDs (8 for Sept-2011 and 4 for May-2012) were
284 used to describe the phytoplankton community composition. Samples of 250 mL of seawater were
285 placed in glass bottles, preserved with formaldehyde solution (4%) and stored in the dark until
286 analysis (within 4 months after the cruise). Micro- and nanohytoplankton were examined with an
287 inverted microscope following the method by Utermöhl (1958). Composite chambers, after 48 hours
288 of settling, were scanned at 125x to quantify the larger, less abundant organisms of the micro-
289 phytoplankton, and at least two transects were examined at 312x to enumerate the smaller and
290 more frequent nanophytoplankton forms. When possible, phytoplankton was identified to the
291 species level, but many organisms could not be adequately classified and were pooled in categories
292 like “Nanoflagellates 3-20 μm ”, “Small dinoflagellates (<20 μm)” or “small coccolithophores (<10
293 μm)”.

294 2.4.4. Cytometric determination of picoplankton community characteristics

295 Determination of picophytoplankton and bacterial abundance was performed using a Becton-
296 Dickinson FACScalibur flow cytometer (Gasol and Morán, 2015) equipped with a laser emitting at
297 488nm, using standard settings. Samples (1.2 mL) were fixed with a 1% paraformaldehyde and
298 0.05% glutaraldehyde solution and then deep-frozen in liquid N₂. Picophytoplankton samples were
299 analysed at high speed (ca. 100 $\mu\text{L min}^{-1}$) with thresholding in red fluorescence. Three main
300 populations (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) were discriminated according
301 to their scatter and fluorescence signals. Cell-specific pigment content was measured by the FL2
302 (*phycoerythrin* orange fluorescence) and FL3 (*chlorophyll a* red fluorescence) parameters
303 (Campbell and Vaultot, 1993; Lefort and Gasol, 2014). Bacterial abundances were stained with
304 SybrGreen I and enumerated at a low speed (ca. 15 $\mu\text{L min}^{-1}$) with thresholding in green
305 fluorescence. The cells were identified in plots of side scatter versus green fluorescence using
306 standard conditions (e.g., Gasol and Del Giorgio, 2000). Concentrations were obtained from weight
307 measurement of the volume analysed. Stock solutions of 1 μm yellow-green latex beads
308 (Polysciences) were added as an internal standard in both picophytoplankton and bacterial
309 measurements. FL2 and FL3 content in all picophytoplankton populations were standardized to the
310 FL2 and FL3 values of the beads to account for inter-sample machine variability.

311 2.5. Statistical analysis

312 Relationships between light exposure, pigment ratios, inherent optical properties, cell size
313 parameters and packaging index were analysed by Spearman rank correlation analysis. This non-
314 parametric method was chosen because environmental data often did not conform to the

315 requirements of parametric tests. Analyses of variance between studied environmental and bio-
 316 optical variables were performed using a one-way ANOVA test followed by the Holm–Sidak multiple
 317 pairwise comparison method to determine significance of differences between both cruises and
 318 layers. In the case of non-normal distributions, the non-parametric Kruskal-Wallis test was utilized.
 319 Graphs and statistics were performed with R software (R Development Core Team, 2004). Figure 1
 320 and 2 were plotted using Ocean Data View (ODV) software (version 5.0, Schlitzer, R., Ocean Data
 321 View, <https://odv.awi.de>, 2018).

322

323 3.0 RESULTS

324 3.1. Characteristics of the study area: continuous vertical profiles of temperature, 325 irradiance, *chlorophyll a*, and beam attenuation

326 Both cruises presented shallow SML with mean depths of 11.8 ± 4.0 m and 18.2 ± 1.5 m in
 327 Sept-2011 and May-2012, respectively. Sea surface temperature was on average 7 °C higher during
 328 Sept-2011. The thermally homogeneous layer was well above the limit of the euphotic depth
 329 (situated around 60 m), delimiting from the bottom of the SML up to $Z_{1\%}$ a sunlit, stratified layer (Fig.
 330 2a, c and Fig. 3a, b). In the SML, similar *T-Chl a* concentration was observed between cruises, with
 331 only slightly higher mean values in May-2012 (Table S1).

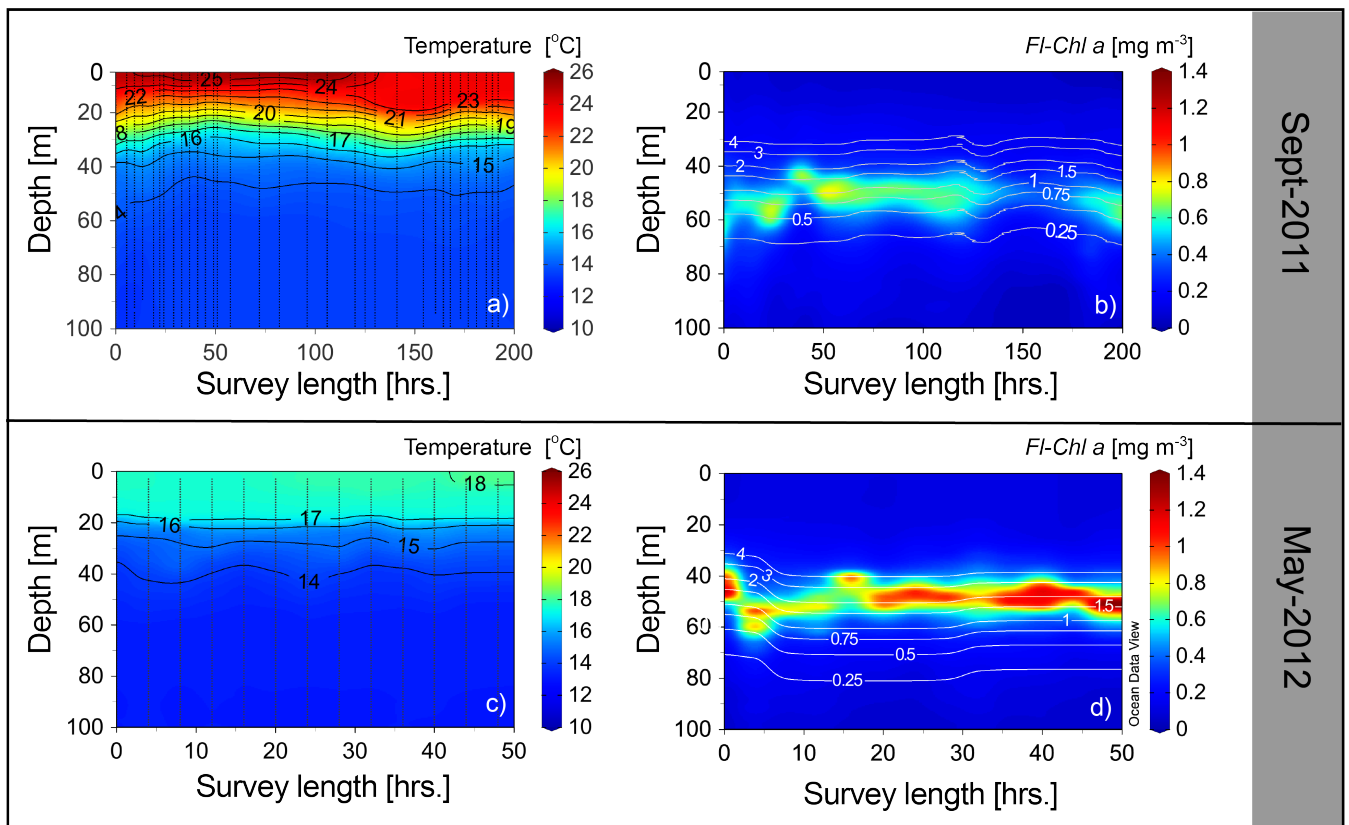


Fig. 2. Cross sections of temperature with isotherms every 1 °C (a, c) and HPLC converted *chlorophyll a* fluorescence (*FI-Chl a*) (b, d). In subplots a) and c) small black dots represent the 1m binned CTD data at each sampling station. In subplots b) and d) the white solid lines are the isolums (in mol quanta m⁻² d⁻¹) near the DCM level. Note the different interval length in x- axis scales between Sept-2011 (subplots a and b) and May-2012 cruises (subplots c and d).

332 The DCMs were well developed during both cruises, situated in the stratified part of the water
 333 column. Nevertheless, clear inter-cruise differences in either DCM magnitude or vertical distribution
 334 were observed. In Sept-2011, the DCM displayed on average 1.6-fold lower *T-Chl a* concentration
 335 (Table S1) and a significantly deeper vertical position ($Z_{DCM} = 54.1 \pm 4.5$ m in Sept-2011 and $47.5 \pm$
 336 2.2 m in May-2012, ANOVA, $p < 0.05$) (Fig. 2c, d and Fig. 3a, b).

337 Inter-cruise differences in incident solar radiation, explained by the solar seasonal cycle,
 338 resulted in dissimilar light exposure along the stratified water column. The Sept-2011 cruise
 339 presented a 22% lower mean values of incident daily PAR irradiance at the sea surface. Significant
 340 differences in \bar{E}_d (PAR) occurred between cruises at the Middle and DCM depths (ANOVA,
 341 $p \leq 0.003$). In Sept-2011 the DCM was placed at isolumes (the level where the daily integrated photon
 342 flux is constant) between 0.5 and 2 mol quanta $m^{-2} d^{-1}$ (mean value of 0.72 ± 0.28), while in May-
 343 2012 the DCM was placed at higher isolumes between 0.75 and 4 mol quanta $m^{-2} d^{-1}$ (mean value
 344 of 1.43 ± 0.6) (Fig. 2c, d; Fig. 3b; Table S1).

345 In addition to the observed vertical structure of *T-Chl a*, both cruises showed an uneven
 346 distribution of $c_p(660)$ with a pronounced deep maximum about 5 meters above the DCM (Fig. 3c).
 347 The average values of $c_p(660)$ increased from the SML to the DCM by a factor between 2.3 and 3
 348 in the two surveys. In contrast, *T-Chl a* depicted a much steeper increase, resulting in a significantly
 349 decrease with depth by a factor of ~ 4 of $c_p^*(660)$ (ANOVA, $p < 0.001$) (Table S1; Fig. 3d). The
 350 relationship between $c_p(660)$ and *T-Chl a* is further illustrated in Fig. S1. Superimposed to the regular
 351 vertical structure of either $c_p(660)$ or $c_p^*(660)$, clear differences between cruises were also found.
 352 The mean values of $c_p(660)$ and $c_p^*(660)$ were significantly higher in May-2012 in the three
 353 examined layers (ANOVA, $p \leq 0.005$) (Table S1; Fig. 3c, d).

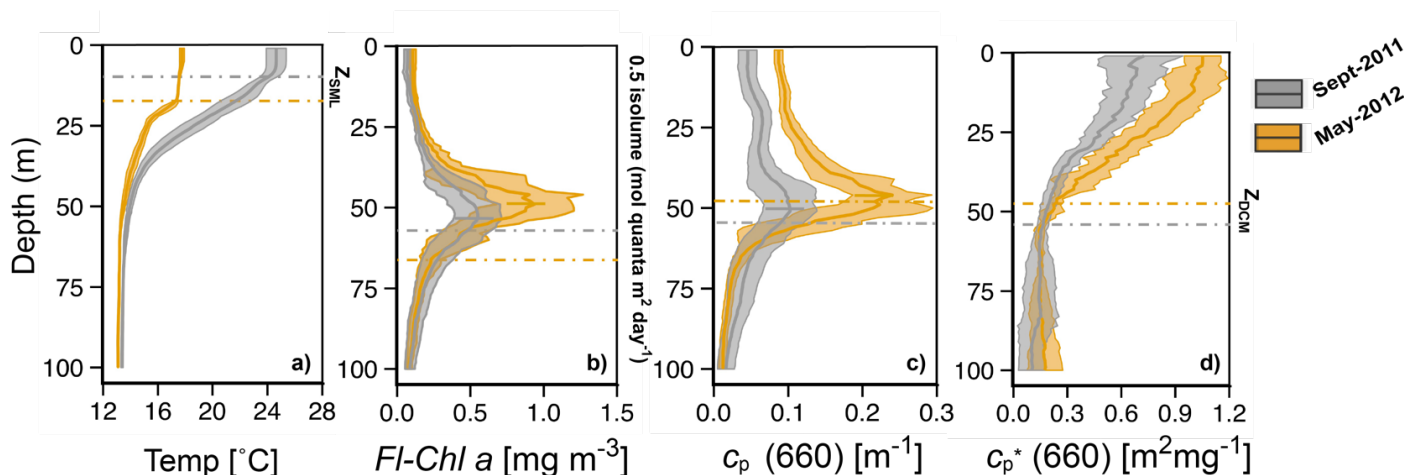


Fig. 3. The average depth distribution of (a) temperature, (b) corrected *chlorophyll a* fluorescence [*Fl-Chl a*], (c) beam attenuation by particles [$c_p(660)$] and (d) chlorophyll-specific beam attenuation [$c_p^*(660)$] for the Sept-2011 (22 stations) and May-2012 (6 stations) cruises. Shaded areas represent ± 1 SD. In subplot (a), the depth of SMLs is indicated with horizontal dashed lines. In subplot (b) the dashed lines indicate the 0.5 isolumes. In subplots (c and d) the dashed lines indicate the depth of DCMs.

354 3.2. Variation in the phytoplankton pigment composition and community structure

355 The contributions of the four groups of accessory pigments (i.e., *PSC*, *PPC*, *T-Chl b* and *T-*

356 *Chl c*), relative to *T-Chl a* concentration, were variable in the different layers and between cruises
357 (Fig. 4, see section 2.4. and Table 1 for symbols and definitions). The *PSC:T-Chl a* ratio showed an
358 even vertical distribution in Sept-2011, but a clear and significant increase (up to 17%) with depth in
359 May-2012 (ANOVA, $p = 0.008$) (Fig. 4a; Table S1). In Sept-2011, *PSC:T-Chl a* was mostly
360 determined by the *19'-HF:T-Chl a* ratio (Table S1). In May-2012, both the *19'-HF:T-Chl a* and *19'-*
361 *BF:T-Chl a* ratios increased with depth and mainly explained the *PSC:T-Chl a* vertical trend.

362 In contrast, the *PPC:T-Chl a* ratio showed a steeper and significant decrease with depth in
363 both cruises (ANOVA, $p \leq 0.005$), (Fig. 4b; Table S1). This decrease was mostly determined by the
364 *Zeax:T-Chl a* ratio in Sept-2011 and by the *Dd:T-Chl a* ratio in May-2012 (Table S1). Similarly, values
365 of NPP (the ratio of non-photosynthetic pigments to total pigment concentration) were significantly
366 higher than those at the DCM (ANOVA, $p \leq 0.005$) (Table S1). The *PPC:T-Chl a* ratio was significantly
367 higher in Sept-2011 than in May-2012 at the three evaluated layers (ANOVA, $p \leq 0.02$); despite higher
368 irradiances in the latter cruise. In contrast, the *PSC:T-Chl a* ratio was only significantly higher in
369 May-2012 at the DCM layer (ANOVA, $p = 0.0011$) (Table S1).

370 The accessory chlorophylls (*T-Chl b* and *T-Chl c*) to *T-Chl a* ratios increased significantly with
371 depth in both cruises (ANOVA, $p \leq 0.005$), (Fig. 4c, d; Table S1), and so did the ratios of the total
372 accessory photosynthetic pigments (i.e., *PSC + T-Chl b + T-Chl c*, herein after *T-APSP*) (ANOVA,
373 $p \leq 0.005$) (Table S1). Between cruises, *T-Chl b:T-Chl a* was higher in Sept-2011, and *T-Chl c:T-Chl*
374 *a* was higher in May-2012, for the three evaluated layers. No differences were found in the *T-*
375 *APSP:T-Chl a* ratio.

376 The diagnostic pigment data also revealed important vertical and inter-cruises differences in
377 the phytoplankton community structure. The *19'-HF* was the most abundant carotenoid in the entire
378 water column of both cruises, accounting on average for 30% and 38% of total carotenoids in Sept-
379 2011 and May-2012, respectively. This suggests that prymnesiophytes were the dominant group of
380 phytoplankton. On the other hand, *Fuco* was found in low amounts during both cruises (<10% of
381 total carotenoids). The surface waters of Sept-2011 also presented high concentrations of *Zeax*,
382 which accounted for 29% of the total carotenoids. This is indicative of a high abundance of
383 *Synechococcus spp.*, because diagnostic pigments of green algae (*Viola*, *Lut* and *Prasino*), that also
384 contain *Zeax*, were found in low abundance at the SML of this cruise. Besides, the contribution by
385 *Prochlorococcus spp.*, another *Zeax* producer, was deemed unimportant because *DV-Chl a* was
386 only 7% of *T-Chl a* in the SML. In contrast, the SML of May-2012 presented important concentrations
387 of *19'-BF* and *Dd*. The latter two pigments accounted for 35% of the total carotenoids, indicating
388 also the presence of chrysophytes/pelagophytes. *Zeax* was comparatively less abundant in this
389 cruise (<9% of total carotenoids), suggesting lower relative abundance of Cyanobacteria. Other

390 pigments like *DV-Chl a* and *Prasino* were undetected in the SML of May-2012.

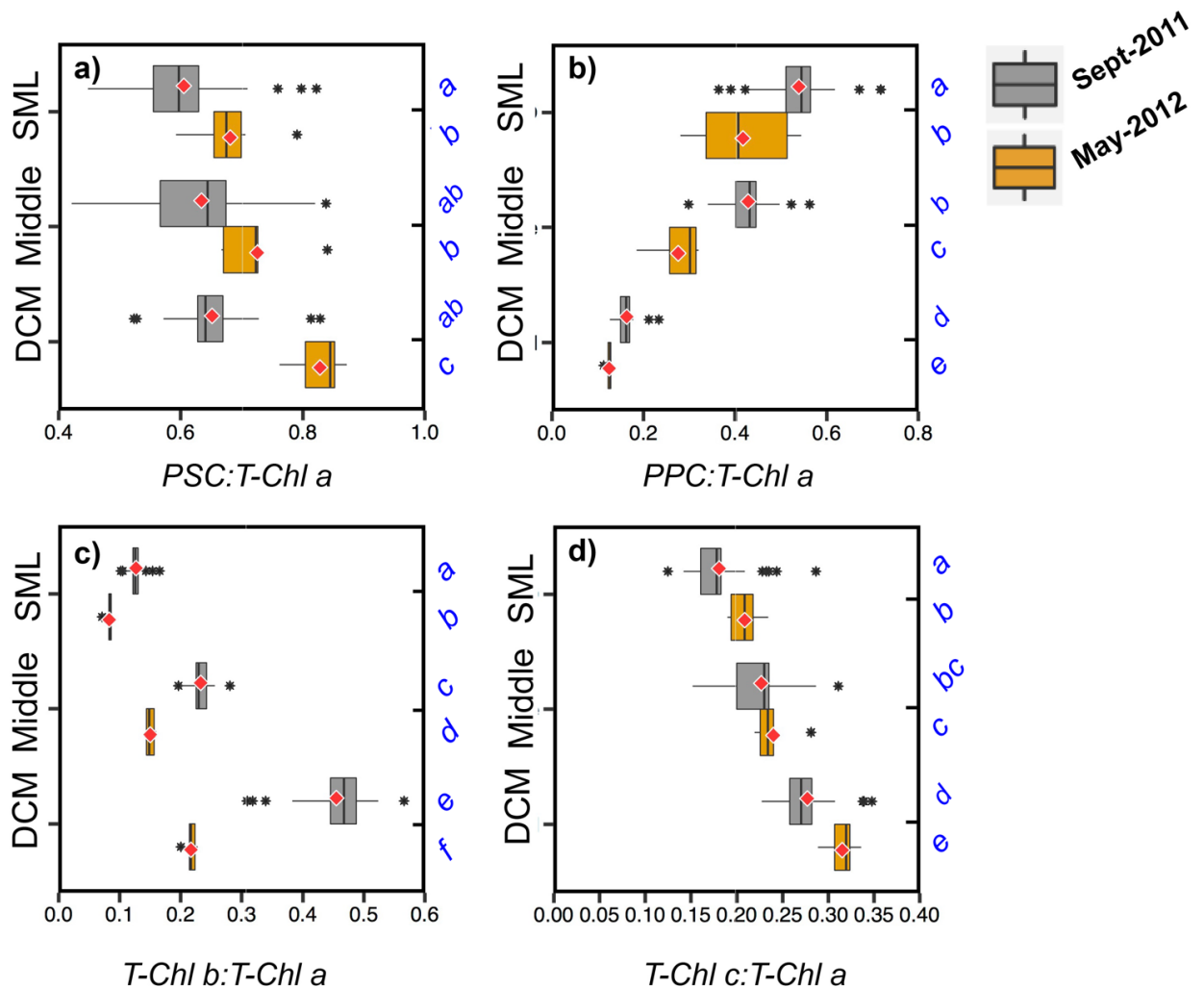


Fig. 4. Distribution of the ratios *PSC:T-Chl a* ratio (a); the *PPC:T-Chl a* ratio (b); the *T-Chl b:T-Chl a* ratio (c) and the *T-Chl c:T-Chl a* ratio for the three evaluated layers during the SUMMER cruises. The distributions are presented as boxplots where the central line is the median of the distribution of data and the red diamond is the mean; the edges of the boxes denote the 25th and 75th percentiles, while the whiskers denote the 10th and 90th percentiles; black asterisks represent outliers. Levels connected with the same blue letter were not significantly different at a $p > 0.05$ after ANOVA test.

391 In the vertical dimension, the Sept-2011 cruise was characterized by an increase of *DV-*
 392 *Chl a* at the Middle and DCM layers, accounting for up to 24% of *T-Chl a* at the DCM level and
 393 indicating high abundances of *Prochlorococcus spp.* *T-Chl b* showed an even steeper increase with
 394 depth (Table S1). The vertical trends of *DV-Chl a* and *T-Chl b* would indicate both a change in
 395 phytoplankton composition and the acclimation of *Prochlorococcus spp.* cells to low irradiance.
 396 DCMs of Sept-2011 also showed significant concentrations of *19'-BF* (accounting for 22% of total
 397 carotenoids), increasing their proportion relative to *19'-HF*. This is indicative of an increase in the
 398 relative importance of chrysophytes/pelagophytes vs. prymnesiophytes towards the base of the
 399 euphotic layer.

400 May-2012 showed less vertical variation in the proportion of taxonomic pigment

401 concentrations than Sept-2011. The DCM layer was characterized by high abundances of
 402 prymnesiophytes (*19'-HF* accounting for the 44% of total carotenoids) and high concentrations of
 403 *19'-BF* and *T-Chl c*. The carotenoid *19'-BF*, as in the Sept-2011, increased its proportion relative to
 404 *19'-HF* with depth. In addition, the observed increase in the *T-Chl b:T-Chl a* ratio at the DCM would
 405 suggest higher abundances of green algae (including prasinophytes), because *DV-Chl a*, a marker
 406 pigment of prochlorophytes, was only present at low concentrations (Table S1).

407 Light microscopy observations confirmed the scarcity of large diatoms (less than 2% of cell
 408 abundance) and the dominance of nanoflagellates (60-70%) in both cruises. Besides, naked small
 409 dinoflagellates (<20 μm) contributed on average to 35 and 28% of total cell counts in Sept-2011 and
 410 May-2012, respectively. Significant vertical and inter-cruise differences in the phytoplankton cell
 411 counts were observed (ANOVA, $p < 0.001$). SML presented around 3.5-fold lower mean cell
 412 abundance than the DCM in both cruises. Besides, May-2012 presented around 5-fold higher mean
 413 cell abundance ($9.2 \cdot 10^4 \text{ cell L}^{-1}$) than Sept-2011 ($1.9 \cdot 10^4 \text{ cell L}^{-1}$). In addition, enumerations by flow
 414 cytometry supported in general pigment analysis of picophytoplankton (Table 2). Photosynthetic
 415 prokaryotes (*Prochlorococcus* + *Synechococcus*) were more abundant at the three evaluated layers
 416 in Sept-2011. Besides, a clear increase of *Prochlorococcus* abundance was observed bellow SML
 417 in both cruises, with higher values in Sept-2011. The cytometry standardized fluorescence
 418 parameters: *phycoerythrin* orange fluorescence (FL2) and *chlorophyll a* red fluorescence (FL3),
 419 which indicate the cell-specific pigment content, increased towards the DCM (Table 2). In May-2012,
 420 *Synechococcus* (which constituted more than 80% of the picoplankton abundance in this cruise)
 421 showed lower relative fluorescence than in Sept-2011. Variation between cruises was less clear for
 422 *Prochlorococcus spp.* and picoeukaryotes, which only during May-2012 showed slightly lower FL3
 423 values at the DCM level (Table 2). Regarding heterotrophic bacteria, no significant differences in
 424 the bacteria:*T-Chl a* ratio were observed between cruises (ANOVA, $p < 0.005$).

Cruise	Sept-2011			May-2012		
	Average (\pm SD)			Average (\pm SD)		
Layers	SML	Middle	DCM	SML	Middle	DCM
<i>Synechococcus</i>						
Abundance ($10^4 \text{ cells ml}^{-1}$)	2.94 (\pm 0.59)	2.31 (\pm 0.26)	1.02 (\pm 0.86)	3.03 (\pm 3.53)	3.53 (\pm 4.85)	2.88 (\pm 1.41)
FL2 (rel. units)	0.07 (\pm 0.02)	0.19 (\pm 0.05)	2.58 (\pm 0.68)	0.06 (\pm 0.03)	0.05 (\pm 0.01)	1.57 (\pm 1.08)
FL3 (rel. units)	0.59 (\pm 0.13)	1.29 (\pm 0.31)	13.01 (\pm 3.42)	0.28 (\pm 0.04)	0.27 (\pm 0.02)	5.41 (\pm 2.18)
<i>Prochlorococcus</i>						
Abundance ($10^4 \text{ cells ml}^{-1}$)	1.80 (\pm 1.20)	9.45 (\pm 2.81)	5.98 (\pm 2.90)	0.23 (\pm 0.54)	0.22 (\pm 0.26)	0.98 (\pm 0.20)
FL3 (rel. units)	0.05 (\pm 0.01)	0.09 (\pm 0.02)	1.12 (\pm 0.36)	0.29 (\pm 0.10)	0.24 (\pm 0.07)	1.07 (\pm 0.47)
Picoeukariotes						
Abundance ($10^4 \text{ cells ml}^{-1}$)	0.12 (\pm 0.04)	0.11 (\pm 0.03)	0.15 (\pm 0.11)	0.16 (\pm 0.03)	0.17 (\pm 0.03)	0.22 (\pm 0.05)
FL3 (rel. units)	8.13 (\pm 1.15)	10.90 (\pm 2.20)	26.27 (\pm 5.79)	12.95 (\pm 3.06)	16.94 (\pm 5.01)	24.60 (\pm 7.97)
Bacteria Abundance ($10^5 \text{ cells ml}^{-1}$)	4.90 (\pm 1.70)	5.09 (\pm 1.58)	4.73 (\pm 1.91)	5.02 (\pm 1.63)	5.65 (\pm 2.06)	8.36 (\pm 2.73)

425 **Table 2.** Average values of different picoplankton community parameters and that of heterotrophic
 426 bacteria abundance. FL2 and FL3 stand for *phycoerythrin* orange fluorescence and *chlorophyll a*
 427 red fluorescence, respectively.

428

429

430 3.3. Variation of phytoplankton absorption coefficients

431 Over the entire water column, $a_{ph}(440)$ varied from 0.005 to 0.063 m^{-1} and from 0.008 to
 432 0.103 m^{-1} in Sept-2011 and May-2012, respectively. The values of $a_{ph}(675)$ ranged from 0.001 to
 433 0.023 m^{-1} in Sept-2011 and from 0.002 to 0.042 m^{-1} in May-2012. As reported in previous studies,
 434 $a_{ph}(440)$ and $a_{ph}(675)$ were positively and significantly ($p < 0.001$) related to $T-Chl a$ following a
 435 power-law functions. For $a_{ph}(440)$, the function was: $a_{ph}(440) = 0.062 T-Chl a^{0.785}$ ($R^2 = 0.95$,
 436 $p < 0.0001$, $n = 98$) (Fig. 5). This relationship is akin to the global average relationships reported by
 437 Bricaud et al. (1998, 1995), but shows a steeper slope. The slope of our relationship is closer to that
 438 obtained by Organelli et al. (2011) in the Mediterranean Sea over a similar $T-Chl a$ range, but our
 439 $a_{ph}(440)$ measurements are ~15% higher (Fig. 5).

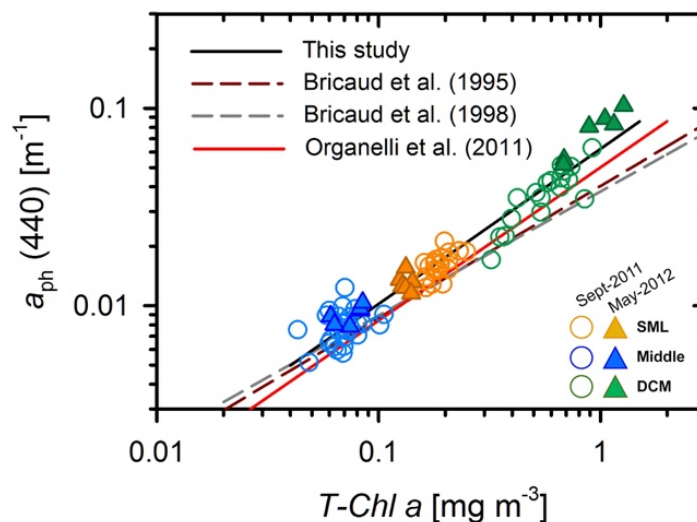


Fig. 5. The phytoplankton absorption coefficient at 440 nm as a function of $T-Chl a$ concentration. The black solid line represents the best fit regression (power law function) between $a_{ph}(440)$ and $T-Chl a$ (see text) considering both cruises and the three evaluated layers of this study. The relationships from Bricaud et al. (1995) (burgundy dashed line), Bricaud et al. (1998) (grey dashed line) and Organelli et al. (2011) (red solid line) are also displayed for comparison.

440

441 In addition, important vertical and inter-cruise variations in the chlorophyll-specific absorption
 442 coefficients were observed. Considering the entire data set, the ranges of $a^*_{ph}(440)$ and $a^*_{ph}(675)$
 443 were 0.041-0.174 $m^2 mg^{-1}$ and 0.014-0.051 $m^2 mg^{-1}$, respectively. Both cruises showed a clear
 444 vertical decrease of $a^*_{ph}(440)$ (Fig. 6c, d; Table S1), with average values significantly different
 445 between the SML and the DCM (ANOVA, $p \leq 0.005$) (Fig. 7a). In Sept-2011, the mean surface value
 446 of $a^*_{ph}(440)$ was ~1.7-fold higher than at the DCM, while in May-2012 this difference was about
 447 ~1.5-fold (Table S1). Vertical variation was less marked for $a^*_{ph}(675)$ (Table S1). In addition, values
 448 of $a^*_{ph}(440)$ and $a^*_{ph}(675)$ were always lower in Sept-2011 than in May-2012 (Table S1).

449 The vertical gradients of both $a_{ph}(\lambda)$ and $a^*_{ph}(\lambda)$ were accompanied by changes in the shape
 450 of the absorption spectra (Fig. 6). The $a_{ph}(\lambda)$ spectra, when normalized with respect to $a_{ph}(440)$,
 451 progressively increased around 470-480 nm and ~650 nm from the SML to the DCM (Fig. 6e, f).
 452 This can be attributed to the observed increase of accessory chlorophylls with depth. A strong
 453 correlation was found between $a_{ph}(475):a_{ph}(440)$ and $T-Chl b:T-Chl a$ in Sept-2011 ($R=0.81$,

454 $p < 0.001$, $n = 81$) and between $a_{ph}(475):a_{ph}(440)$ and $(T-Chl\ b + T-Chl\ c):T-Chl\ a$ in May-2012 ($R = 0.90$,
 455 $p < 0.001$, $n = 17$). In addition, an absorption increase between 515-533 nm was also evident towards
 456 deeper waters in May-2012 (Fig. 6f), likely related to the vertical trend of the $19'-BF:T-Chl\ a + 19'-$
 457 $HF:T-Chl\ a$ ratio ($R = 0.74$, $p < 0.001$, $n = 17$).

458

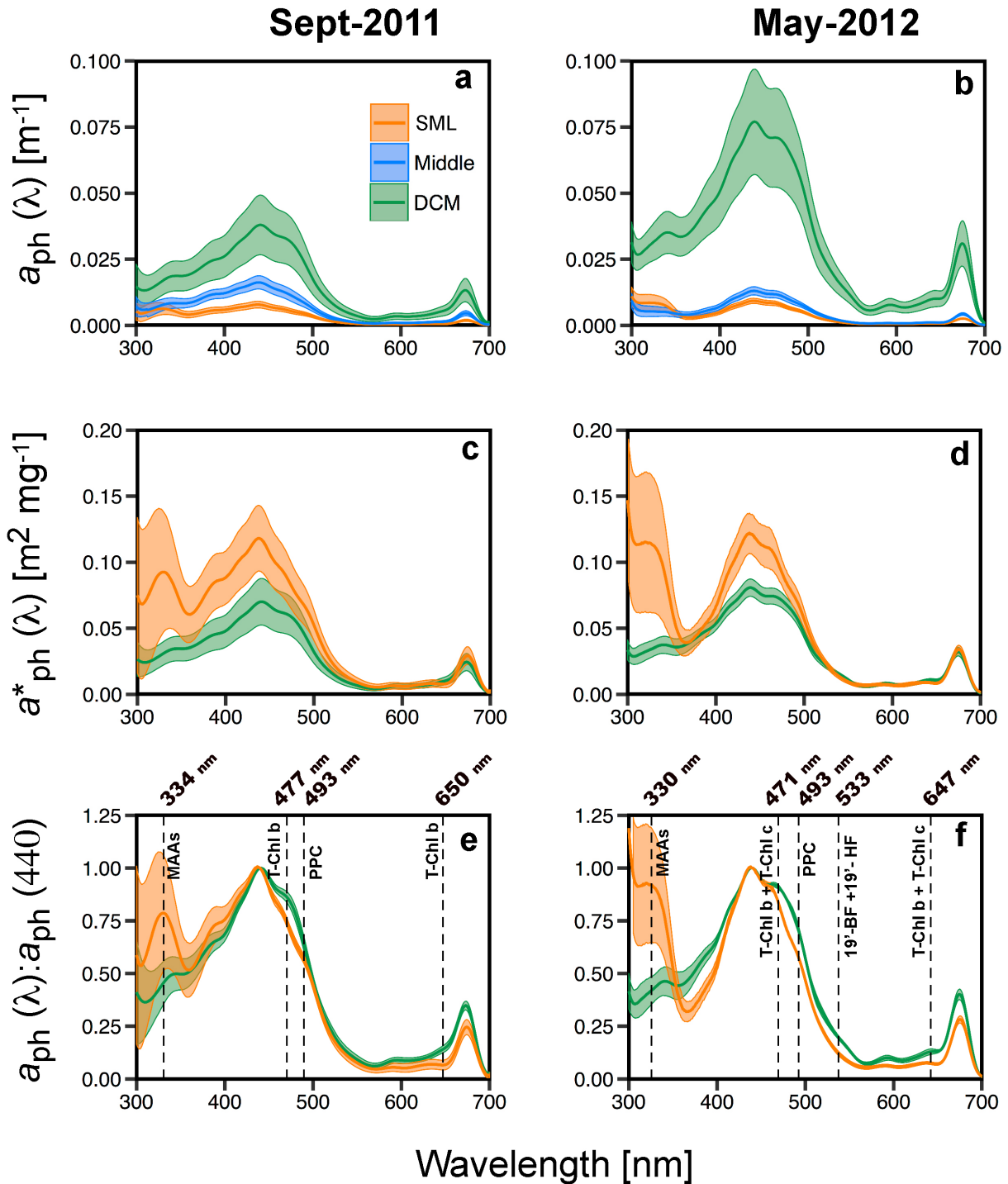


Fig. 6. Mean values of phytoplankton absorption spectra [$a_{ph}(\lambda)$] (a, b); chlorophyll-specific phytoplankton absorption [$a^*_{ph}(\lambda)$] (c, d) and phytoplankton absorption spectra normalized at 440 nm [$a_{ph}(\lambda):a_{ph}(440)$] (e, f), for the three evaluated layers in the Sept-2011 and May-2012 cruises (in c, d, e and f, the Middle layer was omitted for clarity). Shaded areas represent ± 1 standard deviation.

459 Although masked by the overlapping with chlorophyll absorption bands, an absorption
460 shoulder around 490 nm associated to PPC was identified in SML samples (Fig. 6e, f). This shoulder
461 progressively vanished with depth, reflecting the vertical decrease of *PPC:T-Chl a* in both cruises.
462 In Sept-2011 the $a_{ph}(493):a_{ph}(440)$ ratio was positively correlated with *Zeax:T-Chl a* ($R=0.63$,
463 $p<0.001$, $n=81$). Differences in $a_{ph}(\lambda)$ spectral shape were also evidenced by analysing the Blue:Red
464 ratio [i.e., $a_{ph}(440):a_{ph}(675)$, hereinafter B:R]. The B:R ratio was significantly higher at the surface
465 (~30%) than at the DCM in both cruises (ANOVA, $p<0.001$). These differences can be explained by
466 the varying proportions of *PPC*, being the B:R ratio positively and strongly related with the *NPP*
467 index ($R=0.79$, $p<0.0001$, $n=98$). Besides, special features were also detected in the UV spectral
468 region, indicating the presence of the UV sunscreens Mycosporine-like amino acids.

469 **3.4. Partitioning phytoplankton absorption: contribution of photoprotective and** 470 **photosynthetic fractions to $a^*_{ph}(440)$ variation**

471 The partitioning analysis, which takes into account the effects of pigment composition and
472 packaging, showed that the absorption associated to the photoprotective pigments, $a^*_{ph-ppc}(440)$,
473 represented about 30% of $a^*_{ph}(440)$ in the SML of both cruises, but was a minor component at the
474 DCM (~10%) (Table S1). Alike for $a^*_{ph}(440)$, the $a^*_{ph-ppc}(440)$ and $a^*_{ph-psp}(440)$ (the absorption by
475 photosynthetic fraction) also showed a significant decrease with depth in both cruises (ANOVA,
476 $p\leq 0.005$) (Fig. S2 and Fig. 7a, b and c). Surface $a^*_{ph-ppc}(440)$ was 3.8 and 4.7-fold higher than at
477 the DCMs in Sept-2011 and May-2012, respectively (Table S1). On the other hand, $a^*_{ph-psp}(440)$
478 presented a less pronounced vertical pattern, with mean values 1.3 and 1.2-fold higher at the surface
479 in Sept-2011 and May-2012, respectively (Table S1; Fig. 7b and c).

480 Therefore, around 60% of the observed $a^*_{ph}(440)$ decrease with increasing depth was
481 accounted for by changes in the photoprotective fraction of phytoplankton absorption. This was also
482 supported by the significant positive relationship between $a^*_{ph}(440)$ and the *PPC:T-Chl a* ratio ($R=$
483 0.72 , $p<0.0001$, $n=81$ and $R=0.83$, $p<0.0001$, $n=17$ in Sept-2011 and May-2012, respectively). In
484 contrast, the total accessory photosynthetic pigments to *T-Chl a* ratio, *T-APSP:T-Chl a*, increased
485 towards the DCM (Table S1). Therefore, the modest vertical decrease observed for the
486 photosynthetic fraction (Fig. 7c and Fig. S2c) indicates that the packaging effect is also an important
487 driver of $a^*_{ph}(440)$ vertical variation. This is further analysed in section 3.5.

488 Inter-cruise differences in $a^*_{ph}(440)$ were mainly attributed to the photosynthetic fraction of
489 phytoplankton absorption. While differences between cruises for the photoprotective absorption
490 fraction was minor (with slightly higher values during Sept-2011, Fig. 7b), we observed significantly
491 lower mean values of the photosynthetic fraction in Sept-2011 than in May-2012 at the three depths
492 (ANOVA, $p<0.008$) (Fig. 7c). The *T-APSP:T-Chl a* ratio, however, did not show significant
493 differences between surveys throughout the illuminated water column (Table S1), indicating that a
494 lower packaging effect in May-2012 (Fig. 7e) was responsible for the observed inter-cruise variation
495 of $a^*_{ph}(440)$.

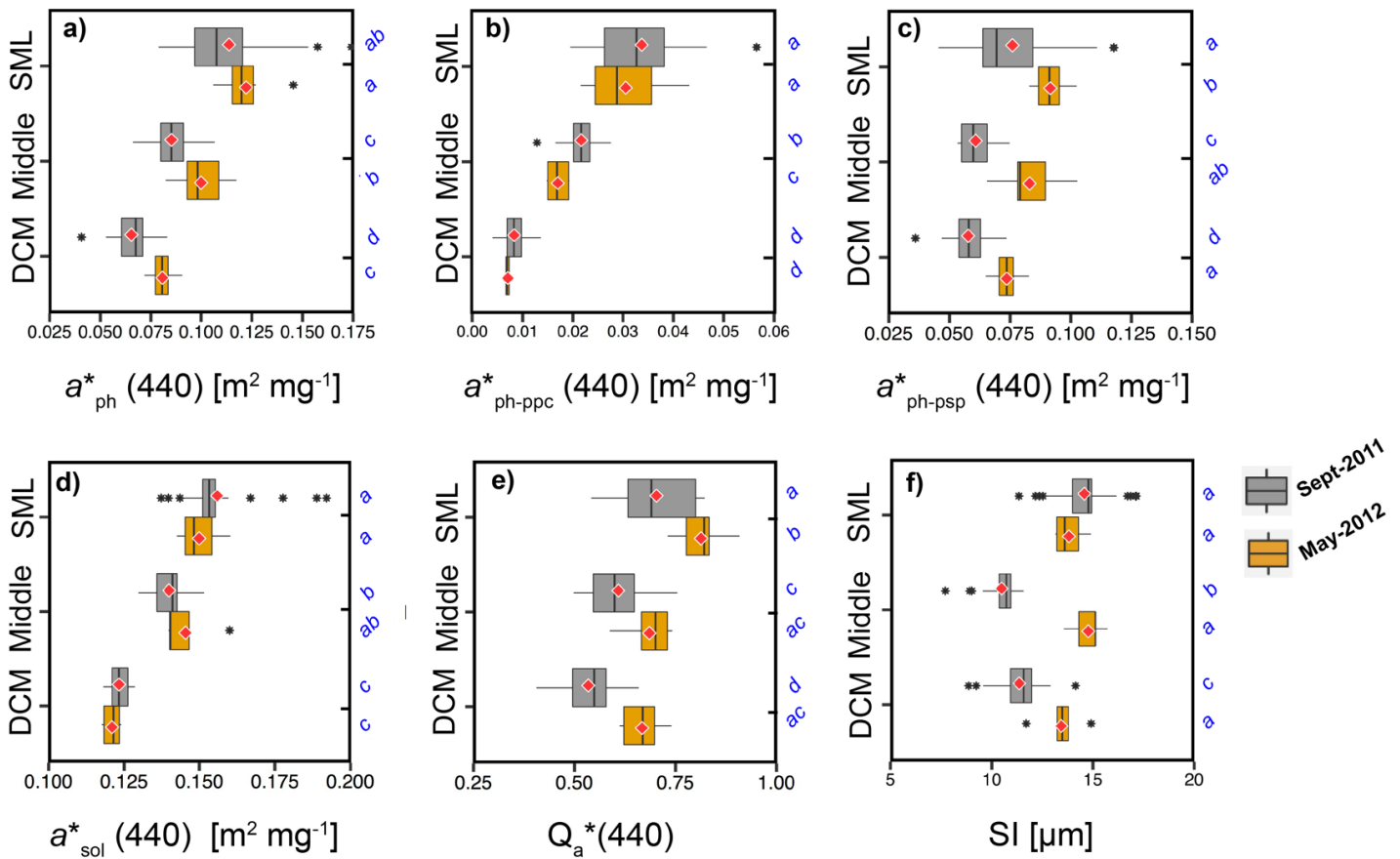


Fig. 7. Distribution of the phytoplankton chlorophyll-specific absorption coefficient, $a^*_{ph}(440)$ (a); the photoprotective absorption fraction, $a^*_{ph-ppc}(440)$ (b); the photosynthetic absorption fraction, $a^*_{ph-psp}(440)$ (c); the chlorophyll-specific absorption of total pigments in solution (without packaging), $a^*_{sol}(440)$ (d), the packaging index, $Q_a^*(440)$ (e) and the size index, SI (f) for the three evaluated layers in the SUMMER cruises. Description of the boxplots as in Figure 4

497 3.5. Effects of pigment composition and packaging on $a^*_{ph}(440)$ variability

498 Values of $a^*_{sol}(440)$, which indicate the unpackaged absorption of pigments, presented a
 499 moderate decrease with depth in both cruises (Fig. 7d; Table S1). This pattern resulted from the
 500 partial compensation between photosynthetic and photoprotective pigments that displayed opposite
 501 vertical patterns (see Fig. 4). The strong signature of photoprotective pigments through the
 502 illuminated water column remains evident, with mean surface values of $a^*_{sol}(440)$ significantly higher
 503 (~1.25-fold) than at the DCMs in both cruises (ANOVA, $p < 0.005$) (Fig. 7d; Table S1).

504 By comparing the vertical profiles of $a^*_{ph}(440)$, $a^*_{sol}(440)$ and packaging index [remember
 505 that $Q_a^*(440) = a_{ph}(440) / a_{sol}(440)$], we estimated that about half of the variation in the mean
 506 values of $a^*_{ph}(440)$ between the SML and the DCM were explained by differences in pigment
 507 composition. The depth decreases of $a^*_{sol}(440)$, ruled by photoprotective pigments, explained about
 508 46% and 52% of $a^*_{ph}(440)$ vertical variation in Sept-2011 and May-2012, respectively. The
 509 remaining variation was consequently attributed to the packaging effect. In both cruises, the Q_a^*
 510 (440) index showed a significant decrease with depth (ANOVA, $p \leq 0.008$), with surface values 1.3

511 and 1.2-fold higher (i.e., lower packaging) than at the DCMs of Sept-2011 and May-2012,
512 respectively (Table S1; Fig. 7e).

513 The minor inter-cruise differences in the average $a^*_{sol}(440)$ at the three depths (Fig. 7d; Table
514 S1) indicate that temporal differences in $a^*_{ph}(440)$ were caused by the different degrees of pigment
515 packaging. A similar conclusion can be deduced from the comparative analysis of $a^*_{ph-ppc}(440)$ and
516 $a^*_{ph-ppsp}(440)$ (section 3.4). Indeed, the mean values of $Q_a^*(440)$ were ~15% lower in Sept-2011 than
517 in May-2012 at both the SML and DCM (ANOVA, $p<0.05$) (Fig. 7e), indicating a stronger packaging
518 effect and explaining the lower values of $a^*_{ph}(440)$ in Sept-2011.

519

520 **3.6. Understanding the packaging effect: the effects of the size structure and intracellular** 521 **pigment concentration of the phytoplankton community**

522 The DPA used to derive the size structure of the phytoplankton community was in general in
523 good agreement with the light microscopy phytoplankton counts and cytometry determinations.
524 During Sept-2011 waters were co-dominated by picophytoplankton (42%) and nanophytoplankton
525 (38%), with a lower contribution of microphytoplankton (20%). In contrast, during May-2012 waters
526 were generally dominated by nanophytoplankton (57%), with lower contributions of pico and
527 microphytoplankton (~22% each) (Table S1). In both cruises, the fraction of picophytoplankton
528 biomass increased by around 20% from the SML to the DCM. Values of S/l index varied in a narrow
529 range with mean values between 10 and 15 μm . In Sept-2011, surface waters had a higher mean
530 S/l than the DCM (ANOVA, $p<0.001$), while no significant vertical differences occurred in May-2012.
531 Differences in S/l were also observed between cruises, with lower mean S/l values in Sept-2011 than
532 May-2012 at the Middle and DCM depths (ANOVA, $p<0.001$) (Fig. 7f; Table S1).

533 By comparing the figure 7e and the derived joint information of DPA, S/l index, light microscopy
534 observations and flow cytometry, we can see that neither the vertical patterns of $Q_a^*(440)$ nor its
535 differences between cruises can be explained by differences in the size structure of phytoplankton
536 community. Namely, during Sept-2011 the phytoplankton of the DCM, even though presenting
537 higher proportions of picophytoplankton and lower of microphytoplankton (i.e., small cells), had their
538 pigments more packaged [lower $Q_a^*(440)$] than phytoplankton at the SML (Table S1 and Fig 7e).
539 Likewise, the phytoplankton assemblages of Sept-2011 had more packaged pigments than in May-
540 2012, despite the community size structure in the latter cruise was composed by a lower proportion
541 of picophytoplankton (i.e., higher cells) (Table S1; Fig. 7e).

542 The other factor responsible for the packaging effect is the per cell concentration of pigments.
543 Using $c_p^*(660)$ as an optical index to track changes in phytoplankton photophysiology, we were able
544 to explain much of the variability of $Q_a^*(440)$ ($R=0.755$, $p<0.001$, $n=98$) (Fig. 8). Photoacclimation,
545 either to the decreasing light availability through the water column or to the inter-cruise seasonal
546 variation of solar irradiance, resulted in a higher intracellular *T-Chl a* concentration and stronger

547 pigment packaging [lower values of c_p^* (660) and Q_a^* (440)] with depth in both cruises, and during
 548 Sept-2011 relative to May-2012.

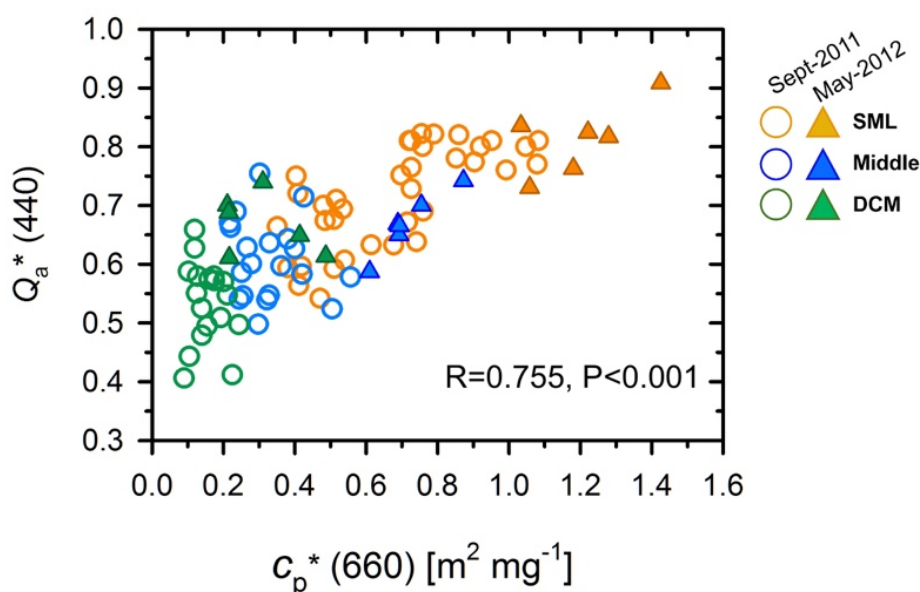


Fig 8. The relationship between the packaging effect index [Q_a^* (440)] and the chlorophyll-specific beam attenuation by particles [c_p^* (660)].

549

550 4.0. DISCUSSION

551 4.1 Vertical and inter-cruise differences in c_p (660), $T-Chl a$ and their relationship

552 The SUMMER cruises took place at the end (Sept-2011) and the beginning (May-2012) of
 553 the stratified season in the NWMS. Although both cruises had similarly low $T-Chl a$ concentration in
 554 the SML, the late-spring cruise (May-2012) presented on average 2-fold higher c_p (660) in the
 555 euphotic layer. This difference is likely indicative of higher phytoplankton biomass (in terms of
 556 carbon) in the latter cruise and could be explained by the hydrographic structure and the seasonality
 557 of nutrient concentrations. In the NWMS, the nitracline depth is maximal during July-September,
 558 when the water column is nitrate-depleted down to about 60 m. Conversely, shortly after the onset
 559 of stable stratification (March-May) the nitracline is shallower (<40 m) and turbulent mixing events
 560 can entrain nutrients into the upper layer (Pasqueron de Fommervault et al., 2015), enhancing
 561 phytoplankton biomass production.

562

563 In SUMMER cruises, the distinctive patterns of c_p (660) and $T-Chl a$ resulted in clear vertical
 564 and inter-cruise differences in c_p^* (660) (a proxy for Carbon: $Chl a$ ratio). Remarkably, these
 565 differences in c_p^* (660) were coincident with either the depth decreasing light availability or the lower
 566 mean daily PAR irradiance in the euphotic layer of Sept-2011, most likely indicating differences in
 567 phytoplankton photoacclimation. Related to the latter, a noteworthy feature of our observations was
 568 the position of DCM in terms of available light in absolute values. The benchmark study of Letelier
 569 et al. (2004) showed that, in some cases, the DCM seasonal dynamics were essentially irradiance
 570 driven. Our observations, conversely, indicate that DCM displacement also coincide with a change
 of isolume, indicating that other factors were also involved (see Cullen, 2015). These outcomes are

571 in agreement with contemporary works that observed seasonal differences in the photon flux at the
572 DCM (Barbieux et al., 2019; Lavigne et al., 2015; Mignot et al., 2014). Besides, the temporal
573 differences in the position and amplitude of the DCM described here are coincident with previous
574 observations in the NWMS (e.g., Estrada et al., 2014).

575 Even if different field studies have demonstrated that, to first order, the c_p (660) can track
576 phytoplankton biomass (see Dall'Olmo et al., 2009, and references therein), the drawback of using
577 c_p (660) is that it is not unique to phytoplankton. In the present study, however, we have evidence
578 supporting the idea of vertical and inter-cruise differences in phytoplankton biomass and
579 photoacclimation. First, we did not register important differences in the heterotrophic bacterial
580 abundance between cruises, with only higher values at the DCM of May-2012, but no significant
581 differences in bacteria: *T-Chl a* were observed. Second, light microscopy observations showed
582 important inter-cruise differences in the total phytoplanktonic cell counts with around 5-fold higher
583 mean cell abundance in May-2012 than in Sept-2011. Besides, these differences in cell counts were
584 higher than inter-cruise differences in *T-Chl a* concentration, indicating lower intracellular pigment
585 concentration during May-2012 than Sept-2011. Furthermore, in the vertical dimension and in both
586 cruises, a higher depth increases of *T-Chl a* concentration than that of the total cell abundance
587 explained the higher pigment concentration per cell towards the DCM. This vertical trend was also
588 confirmed by the cytometry standardized fluorescence for the picoplankton community. Third, in
589 both cruises, c_p^* (660) varied through the water column following expected changes in intracellular
590 pigmentation resulting from photoacclimation to the vertical light gradient, as previously observed in
591 several works (e.g., Fennel and Boss, 2003; Mignot et al., 2014; Mitchell and Kiefer, 1988). Overall,
592 these observations indicate that during the SUMMER cruises phytoplankton either dominate the c_p
593 (660) signal or contributes a rather constant fraction covarying with the remaining pool of particles.

594 **4.2. Phytoplankton pigments, community composition and size structure**

595 The structure of phytoplankton assemblage observed during SUMMER cruises could be
596 ascribed to the late-summer stratification (Sept-2011) and post-bloom (May-2012) periods of the
597 phytoplankton seasonal cycle (Estrada et al., 2014; Latasa et al., 2010). In the NWMS, except for
598 specific bloom events when the diatom-derived *Fuco* is dominant, the generally most abundant
599 carotenoid irrespective of depth and time is *19'-HF* (Latasa et al., 2010; Marty et al., 2002; Siokou-
600 Frangou et al., 2010), indicating that prymnesiophytes are the dominant group of phytoplankton.
601 This, in turn, is in consonance with Royer et al. (2016), who found extremely high ratio of DMSP_t
602 (the algal osmolyte dimethylsulphoniopropionate) per unit of *Chl a* in both SUMMER cruises (with
603 higher values in May-2012 than in Sept-2011), indicating the dominance of high DMSP producers,
604 such as prymnesiophytes.

605 In the Mediterranean Sea, prymnesiophytes frequently coexist with picoprokaryotes and
606 picoeukaryotes or alternate with dinoflagellates and other flagellates belonging to different algal
607 groups (Estrada et al., 2014; Latasa et al., 2010). As reported here, higher abundance of
608 *Synechococcus* and *Prochlorococcus* were observed in the Mediterranean Sea during the stably
609 stratified period, though the prokaryotic contribution was smaller during post-bloom conditions
610 (Latasa et al., 2010; Marty and Chiaverini, 2002; Vidussi et al., 2000).

611 The phytoplankton community size structure, estimated using the DPA approach, evidenced
612 waters mostly codominated by picophytoplankton and nanophytoplankton in Sept-2011 and by
613 nanophytoplankton in May-2012. Accordingly, the size index (*S_I*) showed lower values at the Middle
614 and DCM layers of Sept-2011 than May-2012, though both cruises presented a narrow range of
615 variation in this metric. Our observations of differences in the community size structure between
616 September and May were similar to that reported by Mayot et al. (2017) in the NWMS
617 (DYFAMED/BOUSSOLE site), using DPA derived biomass fractions. It must be noted that we made
618 strong assumptions when applying the DPA analysis (see Brewin et al., 2014, and references
619 therein). For example, a given diagnostic pigment could be shared by different size classes or some
620 taxonomic groups that harbour specific diagnostic pigments may vary in size. Besides and as
621 aforementioned, the *S_I* index was originally developed for the surface layer and can be used here
622 only as a rough indicator of the phytoplankton dominant size in the three evaluated layers. Even
623 though, it has been previously used in different studies either in the SML or below this layer
624 (Kheireddine et al., 2018; Organelli et al., 2011; Wang et al., 2014). Is encouraging, however, the
625 agreement between our observations (DPA, light microscopy counts and cytometry determinations),
626 as well as with the previously mentioned works and other studies conducted in the NWMS (see
627 Bricaud et al., 2004; Organelli et al., 2013). This suggests that DPA provides here a realistic
628 description of the “bulk size structure” of the phytoplankton assemblages.

629 **4.3. Comparing the impacts of photoacclimation and phytoplankton community size** 630 **structure on phytoplankton absorption**

631 The analysis of the chlorophyll-specific absorption coefficient, $a^*_{ph}(440)$, showed remarkable
632 vertical and inter-cruise differences. Several studies in the last three decades have tried to quantify
633 the intermingled effects of pigment packaging and pigment composition on $a^*_{ph}(440)$ (e.g., Bricaud
634 and Stramski, 1990; Ferreira et al., 2013; Lutz et al., 1996; Morel, 1978). In these studies, much of
635 the observed variability was explained by the interplay between short-term cellular acclimation to
636 the prevailing growth conditions and the species composition of phytoplankton community. The
637 comprehensive examination presented here indicates that around 50% of the vertical variability of
638 $a^*_{ph}(440)$ was explained by changes in the pigment composition with depth (ruled by the
639 photoprotective pigments) as a result of photoacclimation to the vertical light gradient. This
640 observation coincides with previous studies (Allali et al., 1997; Bouman et al., 2000; Letelier et al.,
641 2017), indicating that, if we aim to constrain estimates of pelagic primary production, we need to
642 improve our knowledge of how environmental factors affect the pigment composition of
643 phytoplankton assemblages and how much of the light absorbed by phytoplankton actually
644 translates into photosynthetic activity.

645 Changes in pigment composition, however, do not explain the observed vertical decrease in
646 the photosynthetic fraction of phytoplankton absorption nor the inter-cruise differences of $a^*_{ph}(440)$
647 in the SUMMER cruises. These were associated with the packaging effect, as observed for the
648 spatial and temporal variation in the packaging index [$Q_a^*(440)$], that accounted for a substantial
649 amount of the $a^*_{ph}(440)$ variability. The novelty of our analysis was the attempt to partition the
650 packaging effect to evaluate the interactive effects of the two intrinsic factors affecting them. Pigment

651 packaging is a positive function of both cell size and intracellular concentration of pigments and an
652 increase of either of these two terms will result in a corresponding decrease in $a^*_{ph}(\lambda)$ (Kirk, 1976;
653 Morel and Bricaud, 1981). In natural waters, these terms can vary differently with the environmental
654 factors (i.e., light history, nutrients, and temperature) and the phytoplankton community structure
655 (e.g., Bricaud and Stramski, 1990; Ciotti et al., 2002; Stæhr et al., 2002). The overall effect on the
656 packaging effect, and its consequences on the variability of $a^*_{ph}(440)$ (not explained by changes in
657 pigment composition), will depend on the relative contributions of cell size and intracellular pigment
658 concentration, and how they interact with each other.

659 In the SUMMER cruises, our main finding was to observe that differences in the $c_p^*(660)$
660 index, and therefore changes in photoacclimation, mainly explained the spatiotemporal variation in
661 the packaging effect. On the other hand, our observations are not fully consistent with the general
662 expectation that in natural waters changes in the packaging effect are mostly related to differences
663 in cell size (e.g., Bricaud et al., 2004; Ciotti et al., 2002; Ferreira et al., 2013). Several of these
664 studies, however, spanned a wide range of phytoplankton cell sizes and were carried out in surface
665 waters of different oceanic areas, with a constrained impact of photoacclimation. Even under these
666 conditions, a significant variation of the packing effect can be observed for a given value of SI (see
667 Bricaud et al., 2004, their figure 8). Authors attributed part of this variation to different
668 photoacclimation states. Therefore, is not surprising that at local scales photoacclimation can rule
669 the packaging effect, especially when analyzing the entire euphotic zone and there are no major
670 changes in the phytoplankton community structure.

671 Our observations are in line with different bio-optical studies that showed the significant
672 contribution of photoacclimation to phytoplankton absorption dynamics and how cell size and
673 intracellular pigment concentration can impact the packaging effect in different directions. For
674 instance, Organelli et al. (2011) found, in a study conducted in autumn 2006, 2007 and 2008 for the
675 upper and deeper euphotic layer, that the surface waters of the more oligotrophic areas of the
676 Mediterranean Sea were characterized by larger cells with lower pigment packaging than those
677 below the SML. Authors attributed this observation to vertical changes in cellular pigment
678 concentrations. Furthermore, in a study conducted in the SML of the Black Sea during different
679 seasons between 2011-2015, Churilova et al. (2017) showed a higher intracellular *Chl a*
680 concentration and lower values of $a^*_{ph}(\lambda)$ in winter, in response to the 10-fold lower daily irradiance
681 than that registered in summer, despite the dominance of large diatoms. Also in the Black Sea,
682 Churilova et al. (2019) reported in a study conducted during periods of seasonal stratification
683 between 1996-2016, a little variation of $a^*_{ph}(678)$ through the euphotic zone, due to the opposite
684 impact of the increase in the intracellular pigment concentration with depth and the decrease of cell
685 size. Moreover, during a cruise across the Atlantic Ocean between 21 October and 21 November
686 2014, Nunes et al. (2019) found that values of $a^*_{ph}(443)$ in the surface layer were uncorrelated with
687 indicators of the phytoplankton size structure and were mainly explained by differences in pigment
688 composition and the packaging effect due to changes in the intracellular pigment concentration.

689
690

691 **5.0 CONCLUSIONS**

692 In the presented analysis, we were able to use a suite of optical and biological parameters
693 to explicitly separate the contribution of changes in pigment composition and pigment packaging on
694 $a^*_{ph}(440)$ variability. Further, we partitioned the packaging effect into its two intrinsic terms (cell size
695 and intracellular pigment composition). Our observations indicate that the variability of $a^*_{ph}(440)$
696 was mainly determined by photoacclimation to the vertical light gradient and the seasonal changes
697 in light exposure.

698 Changes in the intracellular pigment concentration with depth increased the vertical effect
699 of the photoprotective carotenoids and explained the differences between cruises in the
700 phytoplankton chlorophyll-specific absorption. Our findings emphasize the need for increased efforts
701 to investigate the impact of photoacclimation on $a^*_{ph}(\lambda)$ variability with implications on the correct
702 evaluation of the packaging effect, thus constraining the natural variability of phytoplankton
703 absorption unexplained by changes in the community size structure. This requires bio-optical studies
704 that include a detailed analysis of the phytoplankton community size structure in the euphotic zone
705 using different approaches (e.g., DPA, size-fractionated filtration and cells counts) coupled with
706 robust indices of photoacclimation. This, in turn, should improve our comprehension of deviations of
707 bio-optical models that contemplate the variability of phytoplankton absorption.

708

709 **Acknowledgments**

710 The authors acknowledge the invaluable assistance and cooperation of the marine
711 technicians (UTM) and the crew aboard R/V Garcia del Cid. This research was funded by the
712 successive Spanish Ministries of Science through projects SUMMER “SURface Mixing Modulation
713 of the Exposure to solar Radiation” (CTM2008– 03309/MAR), BIOGAPS (CTM2016-81008-R) and
714 DOREMI (CTM2012– 34294), and through the CONICET and CONICET-CSIC scholarships to G.
715 L. Pérez. This is a contribution of the Research Group on Marine Biogeochemistry and Global
716 Change and that of Microbial Diversity and Function in Aquatic Environments, funded by the Catalan
717 Government.

718

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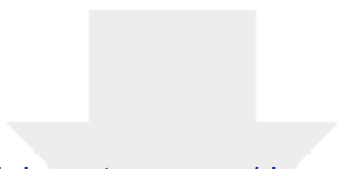
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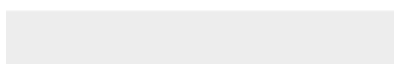
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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: