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11 Estimating phytoplankton size-fractionated primary production
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14 in the northwestern Iberian upwelling: is mixotrophy relevant in
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18 pigmented nanoplankton?
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ABSTRACT

Primary production of micro- (PP_{micro}), nano- (PP_{nano}) and picophytoplankton (PP_{pico}) in the NW Iberian margin were estimated by combining biomarker pigments to derive class-specific chlorophyll concentration and published class-specific photophysiological variables for large oceanic scales (Uitz et al., 2008). The accuracy of this approach was assessed comparing the predicted total primary production ($PP_p = PP_{\text{micro}} + PP_{\text{nano}} + PP_{\text{pico}}$) with the measured total primary production (PP_m). Despite the general agreement, PP_p overestimated PP_m when mixing in the water column was important. Therefore, the photophysiological variables originally derived from stratified and oligotrophic zones with strong influence of photoacclimation in the water column were re-evaluated to incorporate the particular conditions usually found in coastal upwelling systems, characterized by higher homogenization of the water column and lesser importance of photoacclimation. With this new fractionation we estimated the export capacity (f -ratio = new production) of the microbial plankton community, which can be assimilated to the fraction of primary production due to microphytoplankton. The NW Iberian margin showed f -ratios varying between the highest values recorded for coastal upwelling systems ($f > 0.75$) and the low values usually found in oligotrophic oceanic areas ($f < 0.1$). This size-fractionated primary production combined with phytoplankton size-fractionated biomass to obtain turnover rates allowed us to infer the existence of mixotrophy within nanophytoplankton. The occurrence of this type of nutrition was indirectly verified by comparing carbon fixation with estimates of gross primary production based on the metabolic theory of ecology. Realistic values of the photosynthetic quotient ($PQ = 1.78 \pm 0.17$; mol O_2 mol C^{-1}) were only obtained when heterotrophic nutrition of nanophytoplankton was considered.

1. Introduction

Matter and energy flows in oceanic pelagic food webs are strongly dependent on the phytoplankton size-classes prevailing at a given place and time. With dominance of picophytoplankton the carbon fixed by photosynthesis is mainly channeled through the microbial loop and so recycled within the microbial plankton realm (Pomeroy, 1974; Azam et al., 1982). The contrasting situation occurs under diatom dominance, when a significant fraction of the carbon fixed by phytoplankton is available to fuel higher trophic levels or be exported out of the pelagic environment (Goldman, 1988; Cushing, 1989).

Coastal upwelling systems have been traditionally viewed as zones where diatoms dominate in the phytoplankton community (Chavez et al., 1991; Tilstone et al., 2000). However, research conducted in the NW Iberian margin (Crespo et al., 2011; Espinoza-González et al., 2012) and in other coastal upwelling areas (Iriarte and González, 2004; Böttjer and Morales, 2007) clearly showed that small phytoplankton (< 20 µm, pico- and nanophytoplankton) is present in these regions as a permanent background where diatoms thrive in response to upwelling events. This condition has led to first hypothesize and later demonstrate that the microbial food web in coastal upwelling zones is basically multivorous (Legendre and Rassoulzadegan, 1995; Vargas et al., 2007; Teixeira et al., 2011). The microbial loop (based on pico- and nanoplankton) would occur as a permanent feature to which the diatom food web is added during upwelling episodes (Barber and Hiscock, 2006; Teixeira et al., 2011; Espinoza-González et al., 2012). Consequently, carbon fixation should take place in the three phytoplankton size-classes (pico-nano-and microphytoplankton), with picophytoplankton and nanophytoplankton fixing carbon continuously and microphytoplankton doing it sporadically in response to upwelling events.

1 Although phytoplankton photosynthesis constitutes the main supply of matter and
2 energy to the marine microbial food web, studies specifically planned to determine the
3 relative importance of primary production in each of the three phytoplankton size
4 fractions in coastal upwelling systems are scarce and limited to specific moments (Joint
5 et al., 2001; Teira et al., 2001; Iriarte and González, 2004). Studies covering a complete
6 seasonal cycle are still fewer (Bode et al., 1994; Wilkerson et al., 2000) and they do not
7 consider the three phytoplankton fractions. In this paper we attempt to fill this gap in
8 our knowledge by estimating the size-fractionated primary production at the continental
9 shelf of the NW Iberian margin throughout a seasonal cycle.

10 The approach used here to estimate size-fractionated primary production is based on
11 the photophysiological parameterization firstly proposed by Claustre et al. (2005) and
12 later improved by Uitz et al. (2008). This parameterization needs the chlorophyll
13 concentration in the three phytoplankton fractions, which were derived from the
14 phytoplankton pigment composition (Rodríguez et al. 2006) using seven accessory
15 pigments as taxonomic biomarkers to estimate the fraction of total chlorophyll
16 concentration ascribed to each phytoplankton size-class (Vidussi et al., 2001; Uitz et al.,
17 2006). This chlorophyll fractionation was later combined with the class-specific
18 photophysiological properties provided by Uitz et al. (2008) to estimate size-
19 fractionated and total primary production. The accuracy of this procedure was validated
20 by comparing the resulting total primary production to the total primary production
21 estimated from photophysiological variables actually measured in the region.

2. Materials and methods

2.1. Sampling

A station located on the NW Iberian shelf (150 m depth) in front of the Ría de Vigo (**42° 07.8'N, 9° 10.2'W**) was visited weekly from 15 May 2001 to 24 April 2002 on board R/V 'Mytilus' (Fig. 1). Sampling took place with a conductivity-temperature-depth (CTD) probe (SBE 9/11) fitted with a fluorometer and attached to a rosette equipped with 12 PVC Niskin bottles. Seawater samples to determine nitrate and chlorophyll *a* (chl *a*) concentrations were collected from the CTD upcasts at 7–8 depths in the water column from surface to bottom. Samples to characterize the size-structure and photophysiology of the phytoplankton community were also taken at several depths within the photic layer. These sampling depths were selected after inspecting the fluorescence profiles to ensure that the subsurface chlorophyll maximum was sampled when present. The spectral light field at sea surface and in the water column was also determined following the approach detailed by Lorenzo et al. (2004), which allows estimating the transmittance at the air-sea interface and the scalar spectral irradiance at each hour and depth in the water column.

2.2. Nitrate and chlorophyll

Nitrate concentrations ($\mu\text{mol kg}^{-1}$) were determined by segmented flow analysis according to Hansen and Grasshoff (1983). For chl *a*, seawater volumes of 100–250 mL were filtered through 25 mm Whatman GF/F filters using low vacuum. The filters were then frozen at $-20\text{ }^{\circ}\text{C}$ before pigments were extracted in 90% acetone over 24 h in the dark at $4\text{ }^{\circ}\text{C}$. Chl *a* concentration (mg m^{-3}) was determined by fluorometry in a Turner Designs fluorometer calibrated with pure chl *a* (Sigma Chemical).

2.3. Size-fractionated phytoplankton biomass

Samples to determine phytoplankton biomass and community size-structure were collected from 4 to 5 depths within the photic layer, which varied between 27 and 88 m (Fig 3D). Epifluorescence microscopy was used to identify pico-(< 2µm) and nanophytoplankton (2–20µm) in samples of 10 ml. These samples were fixed with buffered 0.2 µm filtered formaldehyde and then filtered through 0.2 µm black Millipore-Isopore filters placed on top of 0.45 µm Millipore backing filters. Excitation with blue light was used to enumerate autotrophic organisms that were identified by yellow (for the case of *Synechococcus*-type cyanobacteria) and red autofluorescence. This technique does not provide a correct identification of *Prochlorococcus*. However, *Prochlorococcus* is only present in the region at very low abundance during short time periods in autumn, when seasonal upwelling-downwelling transition occurs and oceanic waters are advected over the shelf (Rodríguez et al., 2006). This means that *Prochlorococcus* does not constitute an important part of the total phytoplankton biomass in this upwelling region. Dimensions were measured and cell volumes were calculated assuming a spherical shape or by approximation to the nearest geometrical shape (Hillebrand et al., 1999). Cell carbon was estimated according to Bratbak and Dundas (1984) for *Synechococcus*, Verity et al. (1992) for pico- and nanoflagellates and Strathmann (1967) for small (< 20µm) naked dinoflagellates.

Microphytoplankton (> 20 µm) was determined in samples of 100 ml preserved in **Lugol's iodine. The samples were sedimented in composite sedimentation chambers and** observed with an inverted microscope. The small species were enumerated from two transects scanned at 400x and 200x, while the whole slide was scanned at 100x to count the larger species. Phototrophic species of dinoflagellates, flagellates and ciliates were discriminated following Lessard and Swift (1986) and also using our historical records

of epifluorescence microscopy of fresh samples. All organisms with chloroplasts were assumed to be phototrophic. Biovolumes were estimated following Hillebrand et al. (1999) and cell carbon was calculated according to Strathmann (1967) for diatoms and dinoflagellates, Verity et al. (1992) for flagellates and Putt and Stoecker (1989) for aloricate ciliates. Chain-forming diatoms with cells <20 µm were assumed to be microphytoplankton, whereas single diatoms <20 µm and ciliates <20 µm were assigned to nanophytoplankton.

2.4. Phytoplankton absorption coefficients and photosynthetic parameters

Photophysiological properties of the phytoplankton community were determined at 3–4 depths within the photic layer; 3 depths during the IPC phase (see results and discussion) when water column was completely homogeneous, and 4 depths during all the other hydrographic phases.

Phytoplankton light absorption coefficients ($a_{ph}(\lambda)$, m^{-1}) were determined according to Arbones et al. (1996) after filtering 1–2 L of seawater through Whatman GF/F filters.

The photosynthetic parameters were determined by conducting photosynthesis–irradiance experiments (P–E curves) in linear incubators refrigerated with thermostatic baths (Arbones et al., 2000). The spectral quality of the incident light does not change significantly along these short incubators (Arbones et al., 2000). As photoinhibition was not observed, the data for each P–E curve were fitted to the following equation:

$$P_z^B = P_m^B [1 - \exp(-\alpha^B E_{PAR}/P_m^B)] \quad (1)$$

to estimate the light-saturate rate of photosynthesis P_m^B (mg C (mg chl a)⁻¹ h⁻¹) and the light-limited slope of the P-E curve α^B (mg C (mg chl a)⁻¹ h⁻¹ (μmol quanta m⁻² s⁻¹)⁻¹). P_z^B (mg C (mg chl a)⁻¹ h⁻¹) is the chlorophyll-specific rate of photosynthesis at each sampled depth and E_{PAR} (μmol quanta m⁻² s⁻¹) is the photosynthetic active radiation at each position in the incubator. The light saturation parameter for photosynthetic active radiation E_{kPAR} (μmol quanta m⁻² s⁻¹) was obtained from:

$$E_{kPAR} = P_m^B / \alpha^B \quad (2)$$

The photosynthetic parameters for light absorbed by phytoplankton were estimated as:

$$P_z^B = P_m^B [1 - \exp(-\alpha_{PUR}^B E_{PUR} / P_m^B)] \quad (3)$$

The light absorbed by phytoplankton (E_{PUR} , μmol quanta m⁻³ s⁻¹) was calculated from:

$$E_{PUR} = \int_{400}^{700} E(\lambda) a_{ph}(\lambda) d\lambda \quad (4)$$

where $E(\lambda)$ (μmol quanta m⁻² s⁻¹) is the spectral irradiance at each position in the incubator (Figueiras et al., 1999; Lorenzo et al., 2004). The light saturation parameter for light absorbed by phytoplankton E_{kPUR} (μmol quanta m⁻³ s⁻¹) is:

$$E_{kPUR} = P_m^B / \alpha_{PUR}^B \quad (5)$$

The maximum quantum yield for carbon fixation (ϕ_m , mol C fixed (mol quanta absorbed)⁻¹) was calculated as:

$$\phi_m = \alpha^B / 43.29 \bar{a}_{ph}^* \quad (6)$$

where 43.29 is a factor converting units and $\bar{a}_{ph}^*$ (m²(mg chl *a*)⁻¹) is the mean chlorophyll-specific absorption coefficient of phytoplankton weighted by the spectral distribution [$E(\lambda)$] of the light in the incubators used for P-E determinations:

$$\bar{a}_{ph}^* = \frac{\int_{400}^{700} a_{ph}^*(\lambda) E(\lambda) d\lambda}{\int_{400}^{700} E(\lambda) d\lambda} \quad (7)$$

The maximum quantum yield for carbon fixation can also be derived from the light-limited slope α_{PUR}^B , (mg C (mg chl *a*)⁻¹ h⁻¹ (μmol quanta m⁻³ s⁻¹)⁻¹) for the light absorbed by phytoplankton:

$$\phi_m = chl\ a\ \alpha_{PUR}^B / 43.29 \quad (8)$$

Then, combining equations 6 and 8 it can be deduced that both slopes (α_{PUR}^B and α^B) are related:

$$\alpha_{PUR}^B = \alpha^B / \bar{a}_{ph} \quad (9)$$

where $\bar{a}_{ph}$ (m^{-1}) is the mean absorption coefficient of phytoplankton weighted by the spectral distribution of the light in the incubators.

Vertical profiles of chl *a* concentration, hourly spectral light field, phytoplankton absorption coefficients and photosynthetic parameters for light absorbed by phytoplankton were combined in equation 3 to estimate integrated primary production (measured, determined) in the water column (PP_m , $g\ C\ m^{-2}\ d^{-1}$). Integration was done at hourly steps and 1 m intervals from sea surface down to 0.1% surface irradiance.

2.5. Estimation of size-fractionated primary production

Primary production of pico-, nano- and microphytoplankton was estimated following Uitz et al. (2008). This methodology is based on the depth-dependence of photophysiological variables and requires the chl *a* concentration of each size-fraction. The contribution of each phytoplankton size-class to total chl *a* concentration was estimated using the pigment composition determined by HPLC for the same sampling (Rodríguez et al. 2006) and following the biomarker pigment approach formerly proposed by Claustre (1994) to classify phytoplankton groups and later improved by Vidussi et al. (2001) and Uitz et al. (2006) to distinguish phytoplankton size-classes. The chl *a* concentration in each phytoplankton size-class was estimated according to Uitz et al. (2006).

As this fractionation approach relies on the existence of unambiguous relationships between biomarker pigments and taxonomic groups and on the precise ascription of taxonomic groups to size-classes, its accuracy was checked through comparing the chl *a* concentration estimated with this approach to the chl *a* concentration experimentally determined (Rodríguez et al., 2006) in two phytoplankton fractions (picophytoplankton and nano+microphytoplankton). The two comparisons showed highly significant linear

regressions with slopes close to 1 and intercepts close to 0 (Figs. 2A, B). The slight overestimation (7%) that the fractionation approach displayed for the picophytoplankton fraction could in fact be an underestimate of the measured chl *a* concentration, because some picophytoplankton cells could have been retained on the GF/D filter (2.7 μ m nominal pore size) used to sequentially separate the two phytoplankton fractions (Rodríguez et al., 2006). In addition, it could be expected that the low chl *a* values recorded in this fraction can increase the error in measurements and so rise the dispersion of values around the linear regression, lowering the determination coefficient and the slope accuracy. Therefore, we can agree with Vidussi et al. (2001) and Uitz et al (2006) who stated that the intrinsic uncertainty of this fractionation approach is of low importance when compared to natural variability in the ocean.

Once the chl *a* concentration of each phytoplankton fraction was known, the size-fractionated primary production was estimated following Uitz et al. (2008). This methodology provides the vertical distribution of photosynthetic parameters and chlorophyll-specific light absorption coefficients for each phytoplankton fraction as function of the relative available irradiance at each depth (*z*), which was estimated as z/Z_{eu} , where Z_{eu} is the depth of the photic layer. Taking into account that α^B and α_{PUR}^B are related through the equation 9, the depth integrated primary production for each phytoplankton fraction was estimated considering the light absorbed by phytoplankton using the equation (3). Integration was also done at hourly steps and 1 m intervals from sea surface down to 0.1% surface irradiance. Predicted total primary production (PP_p) was estimated as the sum of the primary production predicted for the three phytoplankton fractions: $PP_p = PP_{micro} + PP_{nano} + PP_{pico}$.

3. Results and discussion

3.1. Hydrography and phytoplankton

Detailed information on the hydrographic conditions found during this sampling have been provided by Crespo et al. (2007). In short, seven hydrographic phases were identified (Fig. 3). Sampling started during summer stratification (phase1), which remained up to the end of August (Fig. 3A, B). During this phase, nitrate levels were low ($< 1 \mu\text{mol kg}^{-1}$) in the surface layer (Fig. 3C) while a subsurface chl *a* maximum developed around the nitracline within the photic layer (Fig. 3D). After a short downwelling event at the end of this phase 1, upwelling alternated with downwelling during the following 3 phases, with upwelling occurring in phases 2 and 4 and downwelling (phase 3) taking place in between (Fig. 3A, B). The two upwelling events supplied nutrients to the surface layer (Fig. 3C) promoting the increase in chl *a* concentration (Fig. 3D), whereas the downwelling of phase 3 caused the advection of warm ($> 17 \text{ }^{\circ}\text{C}$; Fig. 3A) and nutrient poor ($< 1 \mu\text{mol kg}^{-1}$) oceanic water (Fig. 3C) with low chl *a* ($< 0.5 \text{ mg m}^{-3}$) concentration (Fig. 3D). The next phase 5 corresponded to the presence of the Iberian Poleward Current (IPC) in the region. This IPC is defined as a homogeneous water body of $14 \text{ }^{\circ}\text{C}$ (Fig. 3A) and salinity > 35.8 (Fig. 3B) with relatively low nitrate ($< 3 \mu\text{mol kg}^{-1}$; Fig. 3C) and chl *a* levels ($\sim 0.5 \text{ mg m}^{-3}$; Fig. 3D) that flows to the north during winter (Haynes and Barton, 1990). Water column mixing was still considerable during the following phase 6 (Fig. 3A, B, C) when chl *a* concentration increased showing a uniform vertical distribution exceeding the depth of the photic layer (Fig. 3D). The spring transition from winter to summer conditions occurred during the last phase 7. In this phase temperature stratification began (Fig. 3A), coinciding with the upwelling of nutrient rich water (Fig. 3C) and a noticeable chl *a* increase in the surface layer (Fig. 3D).

Phytoplankton community was clearly dominated by nanophytoplankton all year round (Fig. 4). This size-fraction, with an average $\pm$ SD integrated biomass of 2.5 ± 1.4 g C m⁻², accounted for $73 \pm 16\%$ of the total phytoplankton biomass. On the other hand, picophytoplankton (0.4 ± 0.3 g C m⁻²) only accounted for $12 \pm 8\%$ of the phytoplankton biomass and showed low temporal variability. Although the contribution of microphytoplankton ($14 \pm 19\%$ of phytoplankton biomass) was similar to the contribution of picophytoplankton, its variability was higher. Thus, microphytoplankton virtually disappeared from the phytoplankton community during downwelling (phase 3) and the IPC (phase 5) but remained during the rest of the hydrographic phases. Microphytoplankton attained higher importance during upwelling (phase 2), when diatoms dominated in this size fraction (Espinoza-González et al., 2012). Mean integrated biomass of microphytoplankton was 0.7 ± 1.2 g C m⁻² for the entire year.

3.2. Photophysiological variability

The photosynthetic parameters and the phytoplankton absorption coefficients measured during this sampling (Fig. 5) fell within the range of values previously reported for the NW Iberian margin (Tilstone et al., 2003; Lorenzo et al., 2005) and other coastal upwelling systems (e.g. Montecino et al., 2004). Average P_m^B tended to decrease with depth (Fig. 5A). However, significant differences between P_m^B values at the surface and at the bottom of the photic layer only occurred during summer stratification (phase 1) and the onset of spring (phase 7) (Table 1). In contrast, α^B displayed relatively homogeneous vertical profiles (Fig. 5B) and there were no significant differences between surface and deep values (Table 1). Homogenous vertical profiles were also observed for $\bar{a}_{ph}^*$ (Fig. 5d, Table 1) and ϕ_m (Table 1), since the two variables are related to α^B . Overall, E_{kPAR} (Fig. 5C) significantly decreased with depth

1 during all hydrographic phases except for the IPC (phase 5) (Table 1). This type of
2 profile with high P_m^B and E_{kPAR} values at surface and lower values at depth, has been
3 commonly interpreted as indication of phytoplankton photoacclimation to the vertical
4 distribution of irradiance in the water column (Falkowski, 1980). Nevertheless, the lack
5 of an opposite profile (low values at surface and higher values at depth) for α^B suggests
6 that either this type of photoacclimation commenced to develop or that it is never totally
7 reached in a highly dynamic environment as the water column in a coastal upwelling
8 system.

19 The seasonal variability in photosynthetic parameters showed high values of P_m^B
20 recorded in surface waters during summer stratification (Fig. 5A; phase 1). It is also
21 remarkable that there were high values of α^B measured during the strong water mixing
22 of IPC (Fig. 5B; phase 5). This and the absence of significant differences for E_{kPAR} in
23 the water column during the IPC (phase 5; Table 1) indicate that photoacclimation
24 and/or photoadaptation (through changes in species composition) also occurred
25 seasonally.

39 3.3. Primary production

41 Primary production (PP_m) estimated from the photophysiological variables
42 determined during this sampling was (Fig. 6) ranged between $0.07 \text{ g C m}^{-2} \text{ d}^{-1}$ recorded
43 at the end of the downwelling of phase 3 and $1.91 \text{ g C m}^{-2} \text{ d}^{-1}$ measured during summer
44 stratification (phase 1). These two values are lower than minimum and maximum values
45 reported for other coastal upwelling systems, such as Chile ($0.2\text{--}1.9 \text{ g C m}^{-2} \text{ d}^{-1}$;
46 Montecino et al., 2004), NW Africa ($0.3\text{--}2.3 \text{ g C m}^{-2} \text{ d}^{-1}$; Morel et al., 1996) and
47 California ($0.5\text{--}2.6 \text{ g C m}^{-2} \text{ d}^{-1}$; Pilskaln et al., 1996). The mean value of PP_m (1.1 ± 0.09
48 $\text{g C m}^{-2} \text{ d}^{-1}$) measured for the summer stratified period (phase 1) was the highest among
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all the mean values recorded for the seven hydrographic phases. However, PP_m values during summer stratification were not significantly different ($p \geq 0.48$, t-test for two samples) to values measured during summer upwelling (phase 2; $0.96 \pm 0.16 \text{ g C m}^{-2} \text{ d}^{-1}$) and during the onset of spring (phase 7; $0.99 \pm 0.11 \text{ g C m}^{-2} \text{ d}^{-1}$). The lowest values of PP_m ($0.27 \pm 0.07 \text{ g C m}^{-2} \text{ d}^{-1}$) occurred during the IPC (phase 5; Fig. 6) and they were similar to those recorded for other IPCs (Tilstone et al., 2003; Crespo et al., 2012).

The mean value of PP_m determined during this sampling ($0.77 \pm 0.44 \text{ g C m}^{-2} \text{ d}^{-1}$) was lower than mean annual values previously reported for the region (Joint et al., 2002; Arístegui et al., 2006), which could be attributed to the few and weak upwelling events that occurred during our seasonal sampling (Fig. 3). Primary production measured during stronger upwelling events in this region (Teira et al., 2001; Tilstone et al., 2003; Crespo et al., 2011) was appreciably higher ($\geq 3 \text{ g C m}^{-2} \text{ d}^{-1}$).

3.4. Measured primary production (PP_m) versus predicted primary production (PP_p)

Before analyzing the size-fractionated primary production estimated from Uitz et al. (2008) approach, it is logical to check whether the predicted total primary production $PP_p = PP_{\text{micro}} + PP_{\text{nano}} + PP_{\text{pico}}$ is able to reproduce the total primary production obtained from the photophysiological variables actually determined. This is an indirect way of determining the accuracy of the fractionation approach proposed by Uitz et al. (2008). From the comparison of both estimates (Fig. 7A) it can be seen that PP_p delivers a slight overestimation (6%) of PP_m . It is also apparent a certain degree of data dispersion around the regression slope, dispersion that results particularly evident for high PP_p values. The temporal evolution of the two estimates (Fig. 7B) shows that the overestimation of PP_m basically occurred for 4 samplings, 2 during summer upwelling (phase 2) and the other 2 during spring onset (phase 7). This suggests that the Uitz et al.

(2008) approach may not be accurate during hydrographic phases when the water column is well mixed, which may also suggest that this approach can only be accurately used for stratified and oligotrophic environments.

Considering that chl *a* fractionation does not produce unrealistic values (Fig. 2), the differences between PP_p and PP_m must be due to the input from photophysiological variables. This idea is founded on how the model by Uitz et al. (2008) was built. It was basically derived from photophysiological determinations obtained in the open ocean, mostly in stratified oligotrophic and mesotrophic regions of the Pacific, North Atlantic and Mediterranean, with very few determinations in coastal upwelling systems. Thus, the photosynthetic parameters provided by Uitz et al. (2008) for the three size classes show a clear variation with depth (photoacclimation) that is not so evident in the NW Iberian upwelling (Fig. 5). In this respect, the vertical homogeneity of α^B registered in our sampling (Fig. 5B) contrasts with the decrease (microphytoplankton) and the increase (pico- and nanophytoplankton) of this variable with depth as shown by Uitz et al. (2008). The vertical profiles in Fig. 8 clearly illustrate these differences for the whole phytoplankton community, with Uitz et al. (2008) providing α^B values increasing with depth and $\bar{\alpha}_{ph}^*$ values being considerably higher than those determined experimentally. Almost certainly, the phytoplankton composition during the summer upwelling (phase 2) and the spring onset (phase 7), with presence of diatoms (Fig. 4; see also Espinoza-González et al., 2012), contrasts with the typical phytoplankton composition commonly found in the open ocean where small phytoplankton dominate. Since the package effect is higher in large cells (diatoms) than in picophytoplankton, the light actually absorbed by phytoplankton during these two hydrographic phases should be lower than that predicted by Uitz et al. (2008), which could result in a PP_p higher than PP_m. Therefore, we can conclude that the model by Uitz et al. (2008) needs to be refined to incorporate

the characteristics of coastal upwelling systems where photoacclimation in the water column is not as important as in the stratified open ocean.

Keeping this in mind and following the approach developed by Uitz et al. (2008), we recalculate the new coefficients defining the depth variation (light dependence) of the photosynthetic parameters and light absorption coefficients for the 3 phytoplankton fractions during non-stratified conditions (Phase 2 to 7) in the NW Iberian upwelling (Table 2). The photophysiological variables measured during the 4 samplings with higher deviations between PP_m and PP_p (Fig. 7) were not used in this estimation. The new α^B and $\bar{\alpha}_{ph}^*$ estimated for the three size-classes do not show variations with depth (S slope not significant; Table 2) and they were more similar to values determined in the water column (Fig. 8).

With this new set of photophysiological variables (Table 2) we recalculate the size-fractionated primary production for the 4 samplings with higher differences between PP_p and PP_m . For all the other sampling days the size-fractionated primary production was estimated using the coefficients given by Uitz et al. (2008). The agreement between PP_m and this new PP_p was considerable improved (Fig. 9A) with a regression slope (Fig. 9B) not significantly different from 1 ($p = 0.995$, t-test for paired samples), suggesting that now size-fractionated primary production in the NW Iberian margin can be fairly estimated.

3.5. Size-fractionated primary production

After showing the agreement between PP_m and the new PP_p re-estimated with the new set of photophysiological variables, the size-fractionated primary production in the NW Iberian margin during this seasonal study can be analyzed.

1 Within the seasonal variability displayed by the three size fractions (Fig. 10A)
2 characterized by lowest primary production during the winter IPC (phase 5), the highest
3 variability was observed in the primary production due to microphytoplankton ($PP_{\text{micro}} =$
4 $0.35 \pm 0.36 \text{ g C m}^{-2} \text{ d}^{-1}$). $PP_{\text{micro}} > 1 \text{ g C m}^{-2} \text{ d}^{-1}$ were estimated at the end of the summer
5 stratification (phase 1) and during the following upwelling (phase 2). High PP_{micro}
6 values but $< 1 \text{ g C m}^{-2} \text{ d}^{-1}$ were also obtained for the winter upwelling (phase 4), winter
7 mixing (phase 6) and the spring onset (phase 7). In contrast, PP_{micro} was undetectable in
8 some samplings during the autumn downwelling (phase 3) and the winter IPC (Fig.
9 10A). PP_{nano} and PP_{pico} never dropped to undetectable values and both primary
10 productions showed lower short-term variability than PP_{micro} (Fig. 10A).

11 The contribution of each size fraction to total primary production over the entire
12 sampling period was similar (Table 3). Although the three size-fractions displayed high
13 variability within each hydrographic phase, the contribution of microphytoplankton
14 exhibited the highest seasonal variability or variability among hydrographic phases.
15 Microphytoplankton contribution to total primary production (PP_p) was particularly
16 important during summer upwelling (phase 2), showing similar levels to those reported
17 by Tilstone et al. (1999) and Arbones et al. (2008) for the nearby coastal environment of
18 Ría de Vigo. Microphytoplankton contributions $> 50\%$ of total PP_p were also estimated
19 for periods of high mixing with significant nutrient supply to the water column (winter
20 upwelling and winter mixing, phase 4 and phase 6, respectively). If the contribution of
21 microphytoplankton to total primary production is viewed as an estimate of the export
22 capacity (f -ratio) from the microbial plankton community (Uitz et al., 2010), the f -ratio
23 values > 0.5 obtained during the upwelling phases (phases 2 and 4) and during winter
24 mixing (phase 6) are within the range of values given for upwelling systems (Eppley
25 and Peterson, 1979; Kudela and Dugdale, 2000). In contrasts, the low f -ratio values

found during autumn downwelling (phase 3) and IPC (phase 5) coincide with the values reported for oligotrophic zones (Eppley and Peterson, 1979; Dugdale and Wilkerson, 1992). The mean f -ratio ($f = 0.36 \pm 0.28$) estimated for the whole sampling is similar to the value given by Joint et al. (2002) for this upwelling region.

3.6. Turnover rates and mixotrophy in nanophytoplankton

The allocation of primary production in these three fractions (Fig. 10A) contrasts with the distribution of autotrophic biomass in the same three fractions (Fig. 4). While PP_{micro} and PP_{pico} (biomass produced) frequently exceeded the corresponding standing stocks, which resulted in mean turnover rates of $1.3 \pm 1.3 \text{ d}^{-1}$ and $0.61 \pm 0.66 \text{ d}^{-1}$ respectively, the biomass produced by nanophytoplankton (PP_{nano}) was always lower than the standing stock, leading to extremely low turnover rates ($0.09 \pm 0.05 \text{ d}^{-1}$). High turnover rates ($>0.69 \text{ d}^{-1}$, > 1 doubling per day) indicate that the biomass produced does not accumulate in the system. Microphytoplankton is probably exported out of the microbial plankton community (fuelling benthos and/or pelagic consumers other than microbial), while picophytoplankton is possibly controlled inside the microbial plankton community by consumers belonging to the microbial loop. The low turnover rates showed by nanophytoplankton could be explained by the existence of mixotrophic nutrition in this group. Mixotrophy in nanophytoplankton is well documented (Unrein et al., 2007; Zubkov and Tarran, 2008; Hartmann et al., 2012) and its existence in the NW Iberian upwelling has been suggested by Crespo et al. (2011) who indicated that heterotrophy can supply 76% of carbon requirements of pigmented nanoflagellates.

The occurrence of mixotrophy in nanophytoplankton during this sampling can be indirectly demonstrated by comparing PP_p ($\text{mmol C m}^{-2} \text{ d}^{-1}$) with gross primary

production (GPP, mmol O₂ m⁻² d⁻¹) estimated according to the metabolic theory of ecology (López-Urrutia et al., 2006; Espinoza-González et al., 2012) (Fig. 10B):

$$GPP = \frac{1}{V} p_0 e^{-E_a/kT} \frac{PAR}{PAR + K_m} \sum_{i=1}^{n_a} M_i^{\alpha_a} \quad (10)$$

where p_0 is a normalization constant independent of body size, temperature and light, E_a is the activation energy, k is Boltzmann's constant (8.62×10^{-5} eVK⁻¹), T is the ambient absolute temperature, PAR is photosynthetic active radiation (mol photons m⁻² d⁻¹), K_m is the Michaelis-Menten half saturation constant, α_a is the allometric scaling exponent for body size and n_a is the number of autotrophic organisms in the volume V (m³) with individual carbon biomass M_i (pgC).

The relationship between PP_p and GPP (Fig. 10B) brings a photosynthetic quotient (PQ = 2.83 ± 0.27 ; the regression slope) which is excessively high and so unrealistic. However, these GPP estimates are based on cell size and assume that phytoplankton obtain energy exclusively from photosynthesis. When the regression (Fig. 10B) is re-calculated considering that only 24% of the GPP estimated for nanophytoplankton is due to photosynthetic carbon fixation (76% of carbon assimilated comes through heterotrophy; Crespo et al. 2011) the new PQ = 1.78 ± 0.17 obtained is not significantly different to the maximum theoretical value of 1.4 ($P = 0.76$, t-test for paired samples) given by Laws (1991) for predominance of protein synthesis. In addition, a PQ = 1.78 is similar to the PQ = 1.73 experimentally obtained by Arbones et al (2008) in the neighboring Ría de Vigo (Fig.1).

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References

- Arbones, B., Castro, C. G., Alonso-Pérez, F., Figueiras, F.G., 2008. Phytoplankton size structure and water column metabolic balance in a coastal upwelling system: the Ría de Vigo, NW Iberia. *Aquatic Microbial Ecology* 50 (2), 169–179.
- Arbones, B., Figueiras, F.G., Zapata, M., 1996. Determination of phytoplankton absorption coefficient in natural seawater samples: evidence of a unique equation to correct the pathlength amplification on glass-fiber filters. *Marine Ecology Progress Series* 137, 293–304.
- Arbones, B., Figueiras, F.G., Varela, R., 2000. Action spectrum and maximum quantum yield of carbon fixation in natural phytoplankton populations: implications for primary production estimates in the ocean. *Journal of Marine Systems* 26 (1), 97–114.
- Arístegui, J., Álvarez-Salgado, X.A., Barton, E.D., Figueiras, F.G., Hernández-León, S., Roy, C., Santos, A.M.P., 2006. Oceanography and Fisheries of the Canary Current/Iberian region of the Eastern North Atlantic. In: Robinson, A.R., Brink, K.H. (Eds.), *The Sea – The Global Coastal Ocean: Interdisciplinary Regional Studies and Synthesis*. Harvard University Press, Cambridge, UK, pp. 879–933.
- Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., 1982. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* 10, 257–263.
- Barber, R.T., Hiscock, M.R., 2006. A rising tide lifts all phytoplankton: Growth response of other phytoplankton taxa in diatom-dominated blooms. *Global Biogeochemical Cycles* 20, GB4S03, doi:10.1029/2006GB002726.

- 1 Bode, A., Casas, B., Varela, M., 1994. Size-fractionated primary productivity and
2 biomass in the Galician shelf (NW Spain): netplankton versus nanoplankton
3 dominance. *Scientia Marina* 58 (1-2), 131-141.
4
5
6
7 Böttjer, D., Morales, C.E., 2007. Nanoplanktonic assemblages in the upwelling area off
8 Concepción (~36°S), central Chile: Abundance, biomass, and grazing potential
9 during the annual cycle. *Progress in Oceanography* 75 (3), 415-434.
10
11
12
13
14 Bratbak, G., Dundas, I., 1984. Bacterial dry matter content and biomass estimation.
15
16
17 *Applied and Environmental Microbiology* 48 (4), 755-757.
18
19 Chavez, F.P., Barber, R.T., Kosro, P.M., Huyer, A., Ramp, S.R., Stanton, T.P.,
20
21 Mendiola, B.R., 1991. Horizontal transport and the distribution of nutrients in the
22 coastal transition zone off Northern California: Effects on the primary production,
23 phytoplankton biomass and species composition. *Journal of Geophysical Research*
24
25
26
27
28
29
30 96 (C8), 14833-14848.
31
32 Claustre, H., 1994. The trophic status of various oceanic provinces as revealed by
33 phytoplankton pigment signatures. *Limnology and Oceanography* 39 (5), 1206-1210.
34
35
36
37 Claustre, H., Babin, M., Merien, D., Ras, J., Prieur, L., Dallot, S., 2005. Toward a
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- Crespo, B.G., Figueiras, F.G., Groom, S., 2007. Role of across-shelf currents in the
dynamics of harmful dinoflagellates blooms in the northwestern Iberian upwelling.
Limnology and Oceanography 52 (6), 2668-2678.

- Crespo, B.G., Espinoza-González, O., Teixeira, I.G., Castro, C.G., Figueiras, F.G.,
2011. Possible mixotrophy of pigmented nanoflagellates: Microbial plankton
biomass, primary production and phytoplankton growth at the NW Iberian upwelling
in spring. *Estuarine, Coastal and Shelf Science* 94 (2), 172–181.
- Crespo, B.G., Espinoza-González, O., Teixeira, I.G., Castro, C.G., Figueiras, F.G.,
2012. Structure of the microbial plankton community in the NW Iberian margin at
the end of the upwelling season. *Journal of Marine Systems* 95, 50–60.
- Cushing, D.H., 1989. A difference in structure between ecosystems in strongly stratified
waters and in those that are only weakly stratified. *Journal of Plankton Research* 11
(1), 1–13.
- Dugdale, R.C., Wilkerson, F.P., 1992. Nutrient limitation of new production in the sea.
p. 107–122. In: Falkowski, P.G., Woodhead, A.D. (Eds.), *Primary productivity and
biogeochemical cycles in the sea*. Plenum Press, New York, pp. 107–122.
- Eppley, R.W., Peterson, B.J., 1979. Particulate organic matter flux and planktonic new
production in the deep ocean. *Nature* 282 (5740), 677–680.
- Espinoza-González, O., Figueiras, F.G., Crespo, B.G., Teixeira, I.G., Castro, C.G.,
2012. Autotrophic and heterotrophic microbial plankton biomass in the NW Iberian
upwelling: seasonal assessment of metabolic balance. *Aquatic Microbial Ecology* 67
(1), 77–89.
- Falkowski, P.G., 1980. Light-shade adaptation in marine phytoplankton. In: Falkowski,
P.G. (Ed.), *Primary productivity in the sea*. Plenum Press, New York, pp. 99–120.
- Figueiras, F.G., Arbones, B., Estrada, M., 1999. Implications of bio-optical modelling
of phytoplankton in Antarctic waters: further evidence of no light limitation in the
Bransfield Strait. *Limnology and Oceanography* 44 (7), 1599–1608.

- Goldman, J.C. 1988. Spatial and temporal discontinuities of biological processes in pelagic surface waters. In: Rothschild, B.J. (Ed.), Toward a theory on biological–physical interactions in the world ocean. Kluwer Academic Publisher, Dordrecht, pp. 273–296.
- Hansen, H.P., Grasshoff, K., 1983. Automated chemical analysis. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (Eds.), Methods of seawater analysis. Verlag Chemie, Weinheim, pp. 347–379.
- Haynes, R., Barton, E.D., 1990. A poleward flow along the Atlantic coast off the Iberian Peninsula. *Journal of Geophysical Research* 95 (C7), 11425–11442.
- Hartmann, M., Grob, C., Tarran, G.A., Martin, A.P., Burkill, P.H., Scanlan, D.J., Zubkov, V., 2012. Mixotrophic basis of Atlantic oligotrophic ecosystems. *Proceedings of the National Academy of Sciences USA* 109 (15), 5756–5760.
- Hillebrand, H., Dürselen, C., Kirschtel, D., Pollinger, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* 35 (2), 403–424.
- Iriarte, J.L., González, H.E., 2004. Phytoplankton size structure during and after the 1997/98 El Niño in a coastal upwelling area of the northern Humboldt Current System. *Marine Ecology Progress Series* 269, 83–90.
- Joint, I., Rees, A.P., Malcolm, E., Woodward, S., 2001. Primary production and nutrient assimilation in the Iberian upwelling in August 1998. *Progress in Oceanography* 51 (2–4), 303–320.
- Joint, I., Groom, S.B., Wollast, R., Chou, L., Tilstone, G.H., Figueiras, F.G., Loijens, M., Smyth, T.J., 2002. The response of phytoplankton production to periodic upwelling and relaxation events at the Iberian shelf break: estimates by ^{14}C method and satellite remote sensing. *Journal of Marine Systems* 32 (1–3), 219–238.

- 1 Kudela, R.M., Dugdale, R.C., 2000. Nutrient regulation of phytoplankton productivity
2 in Monterey Bay. California. Deep-Sea Research II 47 (5-6), 1023-1053.
3
4 Laws, E.A., 1991. Photosynthetic quotients, new production and net community
5 production in the open ocean. Deep-Sea Research 38 (1), 143-167.
6
7 Legendre, L., Rassoulzadegan, F., 1995. Plankton and nutrient dynamics in marine
8 waters. Ophelia 41 (1), 153-172.
9
10 Lessard, E.J., Swift, E., 1986. Dinoflagellates from the North Atlantic classified as
11 phototrophic or heterotrophic by epifluorescence microscopy. Journal of Plankton
12 Research 8 (6), 1209-1215.
13
14 López-Urrutia, A., San Martín, E., Harris, R.P., Irigoien, X., 2006. Scaling the
15 metabolic balance of the oceans. Proceedings of the National Academy of Sciences
16 USA 103 (23), 8739-8744.
17
18 Lorenzo, L.M., Arbones, B., Tilstone, G.H., Figueiras, F.G., 2005. Across-shelf
19 variability of phytoplankton composition, photosynthetic parameters and primary
20 production in the NW Iberian upwelling system. Journal of Marine Systems 54 (1-4),
21 157-173.
22
23 Lorenzo, L. M., Figueiras, F.G., Tilstone, G.H., Arbones, B., Mirón, I., 2004.
24 Photosynthesis and light regime in the Azores Front region during summer: are light-
25 saturated computations of primary production sufficient? Deep-Sea Research I 51
26 (9), 1229-1244.
27
28 Montecino, V., Astoreca, R., Alarcón, G., Retamal, L., Pizarro, G., 2004. Bio-optical
29 characteristics and primary productivity during upwelling and non-upwelling
30 conditions in a highly productive coastal ecosystem off central Chile (~36°S). Deep-
31 Sea Research II 51 (20-21), 2413-2426.
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- Morel, A., Antoine, D., Babin, M., Dandonneau, Y., 1996. Measured and modeled primary production in the northeast Atlantic (EUMELI JGOFS program): The impact of natural variations in photosynthetic parameters on model predictive skill. Deep-Sea Research I 43 (8), 1273–1304.
- Pilskaln, C.H., Paduan, J.B., Chavez, F.P., Anderson, R.Y., Berelson, W.M., 1996. Carbon export and regeneration in the coastal upwelling system of Monterey Bay, central California. Journal of Marine Research 54 (6), 1149–1178.
- Pomeroy, L.R., 1974. The ocean's food web, a changing paradigm. BioScience 24 (9), 499–504.
- Putt, M., Stoecker, D.K., 1989. An experimentally determined carbon: volume ratio for **marine “oligotrichous” ciliates from estuarine and coastal waters**. Limnology and Oceanography 34 (6), 1097–1103.
- Rodríguez, F., Garrido, J.L., Crespo, B.G., Arbones, B., Figueiras, F.G., 2006. Size-fractionated phytoplankton pigment groups in the NW Iberian upwelling system: impact of the Iberian Poleward Current. Marine Ecology Progress Series 323, 59–73.
- Strathmann, R., 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnology and Oceanography 12 (3), 411–418.
- Teira, E., Serret, P., Fernández, E., 2001. Phytoplankton size-structure, particulate and dissolved organic carbon production and oxygen fluxes through microbial communities in the NW Iberian coastal transition zone. Marine Ecology Progress Series 219, 65–83.
- Teixeira, I.G., Figueiras, F.G., Crespo, B.G., Piedracoba, S., 2011. Microzooplankton feeding impact in a coastal upwelling system on the NW Iberian margin: The Ría de Vigo. Estuarine, Coastal and Shelf Science 91 (1), 110–120.

- 1
2
3
4
5
6
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47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- Tilstone, G.H., Míguez, B.M., Figueiras, F.G., Fermín, E.G., 2000. Diatom dynamics in a coastal upwelling ecosystem affected by upwelling: coupling between species succession, circulation and biogeochemical processes. *Marine Ecology Progress Series* 205, 23–41.
- Tilstone, G.H., Figueiras, F.G., Fermín, E.G., Arbones, B., 1999. Significance of nanophytoplankton photosynthesis and primary production in a coastal upwelling system (Ría de Vigo, NW Spain). *Marine Ecology Progress Series* 183, 13–27.
- Tilstone, G.H., Figueiras, F.G., Lorenzo, L.M., Arbones, B., 2003. Phytoplankton composition, photosynthesis and primary production during different hydrographic conditions at the Northwest Iberian upwelling system. *Marine Ecology Progress Series* 252, 89–104.
- Uitz, J., Claustre, H., Morel, A., Hooker, S.B., 2006. Vertical distribution of phytoplankton communities in the open ocean: An assessment based on surface chlorophyll. *Journal of Geophysical Research* 111, C08005, doi:10.1029/2005JC003207.
- Uitz, J., Claustre, H., Gentili, B., Stramski, D., 2010. Phytoplankton class-specific **primary production in the world's oceans: Seasonal and interannual variability from satellite observations.** *Global Biogeochemical Cycles* 24, GB3016, doi:10.1029/2009GB003680.
- Uitz, J., Huot, Y., Bruyant, F., Babin, M., Claustre, H., 2008. Relating phytoplankton photophysiological properties to community structure on large scales. *Limnology and Oceanography* 53 (2), 614–630.
- Unrein, F., Massana, R., Alonso-Sáez, L., Gasol, J.M., 2007. Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. *Limnology and Oceanography* 52 (1), 456–469.

- Vargas, C.A., Martínez, R.A., Cuevas, L.A., Pavez, M.A., Cartes, C., González, H.E.,
Escribano, R., Daneri, G., 2007. The relative importance of microbial and classical
food webs in a highly productive coastal upwelling area. *Limnology and
Oceanography* 52 (4), 1495–1510.
- Verity, P.G., Robertson, C.Y., Tronzo, C.R., Andrews, M.G., Nelson, J.R., Sieracki,
M.E., 1992. Relationships between cell volume and the carbon and nitrogen content
of marine photosynthetic nanoplankton. *Limnology and Oceanography* 37 (7), 357–
366.
- Vidussi, F., Claustre, H., Manca, B.B., Luchetta, A., Marty, J.C., 2001. Phytoplankton
pigment distribution in relation to upper thermocline circulation in the eastern
Mediterranean Sea during winter. *Journal of Geophysical Research* 106 (C9),
19,939–19,956.
- Wilkerson, F.P., Dugdale, R.C., Kudela, R.M., Chavez, F.P., 2000. Biomass and
productivity in Monterey Bay, California: contribution of the large phytoplankton.
Deep-Sea Research II 47 (5–6), 1003–1022.
- Zubkov, M.V., Tarran, G.A., 2008. High bacterivory by the smallest phytoplankton in
the North Atlantic Ocean. *Nature* 455 (7210), 224–226.

Table 1. Average ($\pm$ SD) values of the photophysiological variables (P_m^B , α^B , E_{kPAR} , ϕ_m and $\bar{a}_{ph}^*$) determined at the surface (SS, surface samples) and at the bottom of the photic layer (DS, deep samples) during the seven hydrographic phases identified. The statistical significance (t-test for two samples) of the differences between SS and DS is shown: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant.

Hydrographic phase		P_m^B	α^B	E_{kPAR}	$\bar{a}_{ph}^*$	ϕ_m
Phase 1 Summer stratification	SS	4.16 $\pm$ 0.76	0.024 $\pm$ 0.008	194 $\pm$ 88	0.012 $\pm$ 0.003	0.050 $\pm$ 0.020
	DS	1.42 $\pm$ 0.54 ***	0.026 $\pm$ 0.015 ns	66 $\pm$ 41 ***	0.009 $\pm$ 0.003 *	0.063 $\pm$ 0.028 ns
Phase 2 Summer upwelling	SS	2.89 $\pm$ 0.83	0.017 $\pm$ 0.006	177 $\pm$ 69	0.008 $\pm$ 0.001	0.050 $\pm$ 0.014
	DS	1.48 $\pm$ 0.96 ns	0.025 $\pm$ 0.013 ns	70 $\pm$ 38 *	0.008 $\pm$ 0.002 ns	0.072 $\pm$ 0.031 ns
Phase 3 Autumn downwelling	SS	3.13 $\pm$ 0.65	0.024 $\pm$ 0.004	136 $\pm$ 48	0.010 $\pm$ 0.002	0.056 $\pm$ 0.012
	DS	2.08 $\pm$ 0.95 ns	0.031 $\pm$ 0.005 ns	66 $\pm$ 22 *	0.013 $\pm$ 0.005 ns	0.061 $\pm$ 0.024 ns
Phase 4 Winter upwelling	SS	2.02 $\pm$ 0.70	0.016 $\pm$ 0.007	128 $\pm$ 17	0.008 $\pm$ 0.003	0.050 $\pm$ 0.030
	DS	1.43 $\pm$ 0.85 ns	0.021 $\pm$ 0.006 ns	64 $\pm$ 24 **	0.007 $\pm$ 0.002 ns	0.072 $\pm$ 0.031 ns
Phase 5 IPC	SS	3.16 $\pm$ 1.00	0.034 $\pm$ 0.018	102 $\pm$ 36	0.011 $\pm$ 0.001	0.073 $\pm$ 0.033
	DS	3.12 $\pm$ 0.65 ns	0.044 $\pm$ 0.019 ns	75 $\pm$ 16 ns	0.011 $\pm$ 0.001 ns	0.093 $\pm$ 0.037 ns
Phase 6 Winter mixing	SS	3.23 $\pm$ 1.67	0.022 $\pm$ 0.010	150 $\pm$ 50	0.007 $\pm$ 0.002	0.077 $\pm$ 0.027
	DS	2.12 $\pm$ 0.30 ns	0.026 $\pm$ 0.004 ns	82 $\pm$ 12 *	0.007 $\pm$ 0.002 ns	0.092 $\pm$ 0.038 ns
Phase 7 Spring onset	SS	3.32 $\pm$ 1.11	0.021 $\pm$ 0.004	157 $\pm$ 29	0.009 $\pm$ 0.003	0.057 $\pm$ 0.017
	DS	1.72 $\pm$ 0.30 *	0.037 $\pm$ 0.022 ns	57 $\pm$ 21 ***	0.009 $\pm$ 0.003 ns	0.093 $\pm$ 0.032 ns

Table 2. Coefficients ($\pm$ SE) of the equations describing the variation with depth ($X^* = X^*(0)e^{-S z/Z_{eu}}$) of P_m^B [(mg C mg chl a)⁻¹ h⁻¹], α^B [mg C (mg chl a)⁻¹ h⁻¹ (μ mol quanta m⁻² s⁻¹)⁻¹] and $\bar{a}_{ph}^*$ [m² (mg chl a)⁻¹] for the three phytoplankton size-classes (micro-, nano- and picophytoplankton) during not stratified conditions (phases 2 to 7) in the NW Iberian upwelling. The experimental data were fitted to the equation (13) in Uitz et al. (2008) that assumes that the photophysiological properties of the phytoplankton population result from the addition of the photophysiological properties of the three phytoplankton size classes. $X_{micro}^*(0)$, $X_{nano}^*(0)$ and $X_{pico}^*(0)$ are the values of P_m^B , α^B or $\bar{a}_{ph}^*$ at the surface ($z/Z_{eu} = 0$), and S_{micro} , S_{nano} and S_{pico} are the slopes describing the depth dependence. The level of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant) and the correlation coefficient (r) are given.

Non-stratified regime (n = 97)	P_m^B		α^B		$\bar{a}_{ph}^*$	
$X_{micro}^*(0)$	3.58 $\pm$ 0.47	***	0.015 $\pm$ 0.004	***	0.006 $\pm$ 0.001	***
S_{micro}	0.69 $\pm$ 0.31	*	-0.340 $\pm$ 0.380	ns	0.036 $\pm$ 0.339	ns
$X_{nano}^*(0)$	1.55 $\pm$ 0.86	*	0.021 $\pm$ 0.008	*	0.011 $\pm$ 0.002	***
S_{nano}	-0.17 $\pm$ 0.91	ns	-0.280 $\pm$ 0.740	ns	0.281 $\pm$ 0.337	ns
$X_{pico}^*(0)$	4.25 $\pm$ 0.87	***	0.036 $\pm$ 0.008	***	0.009 $\pm$ 0.002	***
S_{pico}	0.70 $\pm$ 0.43	*	0.037 $\pm$ 0.397	ns	-0.41 $\pm$ 0.202	ns
r	0.48		0.50		0.72	

Table 3. Average ($\pm$ SD) values of the contributions (%) of microphytoplankton (PP_{micro}), nanophytoplankton (PP_{nano}) and picophytoplankton (PP_{pico}) to total primary production predicted (PP_p) during each hydrographic phase and for the whole sampling.

Hydrographic phase	PP_{micro}/PP_p (%)	PP_{nano}/PP_p (%)	PP_{pico}/PP_p (%)
Phase 1 (n = 16) Summer stratification	36 ± 22	34 ± 14	29 ± 12
Phase 2 (n = 4) Summer upwelling	75 ± 3	10 ± 4	15 ± 5
Phase 3 (n = 4) Autumn downwelling	17 ± 17	51 ± 18	31 ± 5
Phase 4 (n = 4) Winter upwelling	52 ± 32	32 ± 25	16 ± 7
Phase 5 (n = 8) IPC	7 ± 6	46 ± 7	47 ± 9
Phase 6 (n = 5) Winter mixing	53 ± 37	30 ± 25	17 ± 13
Phase 7 (n = 5) Spring onset	37 ± 23	34 ± 16	30 ± 12
Whole sampling (n = 46)	36 ± 28	35 ± 18	29 ± 14

Figure captions

Fig. 1. The NW Iberian margin showing the position of the sampled station. The location of the 4 Rías Baixas (Vigo, Pontevedra, Arousa and Muros) is also shown.

Fig. 2. Relationships between chlorophyll *a* concentration (chl *a*) measured and predicted for (A) picophytoplankton fraction (< 3 µm) and (B) nano + microphytoplankton fraction at the sampled station on the NW Iberian shelf. Chl *a* predicted was deduced from biomarker pigments following the approach by Uitz et al. (2006). See Rodríguez et al. (2006) for further details on analytical procedure.

Fig. 3. (A) Temperature (°C), (B) salinity, (C) nitrate concentration (µmol kg⁻¹), and (D) chlorophyll *a* concentration (mg m⁻³) at the sampled station during the study year. The dashed line in (D) shows the depth of the photic layer (0.1% of sea surface irradiance). The seven identified hydrographic phases (1 to 7) are given on the top of panel (A).

Fig. 4. Integrated biomass over the photic layer of picophytoplankton (Pico), naophytoplankton (Nano) and microphytoplankton (Micro). The seven hydrographic phases (1 to 7) are shown on the top.

Fig. 5. Mean vertical profiles for (A) maximum photosynthetic rate P_m^B , (B) broadband light limited slope α^B , (C) light saturation parameter for PAR irradiance E_{kPAR} and (D) mean chlorophyll-specific light absorption coefficient $\bar{a}_{ph}^*$ for the seven hydrographic phases identified and the whole sampling period.

Fig. 6. Primary production measured and integrated over the photic layer. Vertical arrow shows the sampling at the end of the downwelling of phase 3 when the minimum value of primary production ($0.07 \text{ g C m}^{-2} \text{ d}^{-1}$) was recorded. Horizontal dashed line denotes the hydrographic phase 5 (IPC) with lowest values of primary production. The seven hydrographic phases (1 to 7) are shown on the top.

Fig. 7. (A) Relationship between measured total primary production (PP_m) and total primary production predicted (PP_p) according to Uitz et al. (2008) and (B) time evolution of PP_m and PP_p . Points inside circles in (A) correspond to samplings with the highest difference between PP_m and PP_p that are denoted with arrows in (B). The seven hydrographic phases (1 to 7) are shown on the top of panel (B).

Fig. 8. Vertical profiles for (A) maximum photosynthetic rate P_m^B , (B) broadband light limited slope α^B and (C) mean chlorophyll-specific light absorption coefficient $\bar{a}_{ph}^*$ empirically determined, estimated according to Uitz et al. (2008) and newly estimated in this study (see text for more details) for one of the four sampling days (24th April) when differences between PP_m and PP_p where the highest (see Fig. 7).

Fig. 9. (A) Measured total primary production (PP_m) and primary production newly predicted (PP_p) after estimating the new photophysiological variables for the four days with deviations between both PP_m and PP_p (see Table 2 and text for details) and (B) relationship between PP_m and PP_p newly estimated. The seven hydrographic phases (1 to 7) are shown on the top.

Fig. 10. (A) Primary production predicted for picophytoplankton (PP_{pico}), nanophytoplankton (PP_{nano}) and microphytoplankton (PP_{micro}) and (B) relationship between total primary production predicted (PP_p) and gross primary production predicted according to the metabolic theory of ecology (GPP). Open circles and dashed line show the relationship without correction for mixotrophy in nanophytoplankton $Y = (17.6 \pm 22.6) + (2.83 \pm 0.27) X$; $r^2 = 0.71$; $n = 46$; $P < 0.001$. Filled circles and continuous line show the relationship when GPP was estimated assuming 76% of heterotrophy in nanophytoplankton $Y = (-20.0 \pm 14.1) + (1.78 \pm 0.17) X$; $r^2 = 0.71$; $n = 46$; $P < 0.001$. The seven hydrographic phases (1 to 7) are shown on the top of panel (A).

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.

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Figueiras et al.