Possible mixotrophy of pigmented nanoflagellates: microbial plankton biomass, primary production and phytoplankton growth in the NW Iberian upwelling in spring

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ABSTRACT

Microbial plankton biomass, primary production (PP) and phytoplankton growth rates (μ) were estimated along the NW Iberian margin during an upwelling relaxation event.

Although the interaction between wind forcing and coastline singularities caused high spatial variability in PP ($0.4\text{--}8.4 \text{ g C m}^{-2} \text{ d}^{-1}$), two domains (coastal and oceanic) could be distinguished regarding microbial plankton biomass and μ . At the coastal domain, with higher influence of upwelling, diatoms showed an important contribution ($27 \pm 17\%$) to total autotrophic biomass (AB). Nonetheless, AB was dominated by autotrophic nanoflagellates (ANF) at both realms, accounting for $62 \pm 16\%$ and $89 \pm 6\%$ of the integrated AB at the coastal and oceanic domain respectively. AB and

heterotrophic biomass (HB) were significantly higher at the oceanic than at the coastal domain, with both biomasses covarying according to $HB:AB = 0.33$. Whereas the low phytoplankton carbon to chlorophyll *a* ratio ($C_{ph}:chl\ a = 38 \pm 3$) and the high $\mu = 0.54 \pm 0.09\ d^{-1}$ registered at the coastal stations suggest that phytoplankton was not nutrient limited at this domain, the values ($C_{ph}:chl\ a = 157 \pm 8$; $\mu = 0.17 \pm 0.02\ d^{-1}$) recorded at the oceanic domain point to severe nutrient limitation. However, the high Fv/Fm fluorescence ratios (0.56 ± 0.09) measured at the sea surface in the oceanic domain suggest that nutrient limitation did not occur. To reconcile these two apparently opposite views, it is suggested the occurrence of mixotrophic nutrition of ANF, with heterotrophic nutrition supplying about 75% of carbon requirements.

Keywords: Microbial plankton biomass, autotrophic nanoflagellates, mixotrophy, coastal upwelling, NW Iberia

1. Introduction

The biogeochemistry of the ocean is largely governed by the structure and composition of planktonic communities, with all possible scenarios lying between the two extremes defined by oligotrophy and eutrophy (Legendre and Rassoulzadegan, 1995). Small phytoplankton prevails in oligotrophic areas of the ocean (Ryther, 1969) where heterotrophic biomass often exceeds autotrophic biomass (Gasol et al., 1997) and recycling is favoured. In contrast, export can be considered as one of the main features of eutrophic areas (Eppley and Peterson, 1979), in which large phytoplankton acquires higher importance (Duarte et al., 2000) and autotrophic biomass surpasses heterotrophic biomass (Gasol et al., 1997; Duarte et al. 2000).

Coastal upwelling zones have been traditionally viewed as eutrophic systems, because high nutrient concentrations injected into the surface layer by upwelling induce

1 the dominance of large diatoms (Chavez et al., 1991) and stimulate primary production,
2 which is frequently higher than planktonic respiration (Daneri et al., 2000; Teira et al.,
3 2003; Cermeño et al., 2006; Arbones et al., 2008). Nevertheless, there is emerging
4 evidence of the importance of small phytoplankton in these regions (Iriarte et al., 2000;
5 Tilstone et al., 2003; Iriarte and González, 2004; Lorenzo et al. 2005; Rodríguez et al.,
6 2006; Böttjer and Morales, 2007), with pico- and nanophytoplankton making significant
7 contributions to both autotrophic biomass and primary production during some periods.
8 As the presence of several phytoplankton size-fractions determines the existence of a
9 multivorous food web (Legendre and Rassoulzadegan, 1995) which in turn means
10 several energy flow pathways, it is important to know the oceanographic processes that
11 lead to the dominance of each phytoplankton fraction in coastal upwelling systems. This
12 knowledge will improve our understanding of the role of these relatively small but
13 highly productive zones in biogeochemical cycles, and would also help to anticipate
14 future scenarios resulting from climate change.

15 The NW Iberia (Fig.1a), located on the northern boundary of the Canary Current
16 upwelling ecosystem, experiences seasonal upwelling. Northerly winds, favouring
17 upwelling, blow in the region from April to September, whereas downwelling favouring
18 southerly winds predominate between October and March (Figueiras et al., 2002).
19 Despite this seasonality, short-term variability in the wind regime is recurrent and
20 significant, with wind relaxations and even opposing events occurring within each
21 season (Figueiras et al., 2002). Coastline singularities are also prominent in the region,
22 the two more conspicuous being the change in coastline orientation that takes place
23 around Cape Finisterre and the presence of four large bays, the Rías Baixas, on the west
24 coast (Fig. 1a). The change in coastline orientation determines that northerly winds only
25 cause upwelling at the west coast, whereas upwelling at the north coast is induced by

1 easterly winds. On the other hand, the Rías Baixas, owing to their orientation and
2 bathymetry, shallower in their inner parts, act intensifying the across-shelf transport
3 imposed by upwelling and downwelling. Thus, the Rías export material to the shelf
4 during upwelling, whereas they receive material from the shelf during downwelling
5 (Tilstone et al., 2003; Lorenzo et al., 2005).

6 Previous research in the NW Iberia margin showed that small plankton can be
7 numerically dominant in the microbial plankton community, especially during
8 relaxation and downwelling periods (e.g. Castro et al. 1997; Crespo and Figueiras,
9 2007). However, few studies considered the size structure (Varela et al., 1991; Tilstone
10 et al., 2003; Lorenzo et al., 2005; Rodríguez et al., 2006) and even fewer analysed the
11 biomass distribution in the microbial community (Bode et al., 2005; Figueiras et al.,
12 2006). In this paper we revisited a data set collected almost 20 years ago (May 1991) to
13 analyse the size structure and biomass distribution in the microbial plankton community
14 in relation to the hydrographic conditions prevailing during a spring upwelling event.
15 The spatial distributions of primary production and phytoplankton growth rates, taking
16 into account the microbial community structure, were also examined.

17 **2. Materials and methods**

18 *2.1. Sampling*

19 The northwestern Iberian margin was almost synoptically sampled in spring 1991,
20 when 33 stations located along 8 transects perpendicular to the coastline (Fig. 1a) were
21 visited during 5 days (May 10 to 14) on board the R/V *Investigador*. Sampling, which
22 began at the northernmost transect (Stns 11 to 14), was conducted with a conductivity-
23 temperature-depth (CTD) probe (SBE 9/11) equipped with a fluorometer and a rosette
24 with 12 PVC Niskin bottles. After inspecting the fluorescence profiles, seawater
25

1 samples were collected from the CTD upcasts at 7-8 depths, from sea surface to bottom
2 at the stations on the shelf and from sea surface to ~100 m at the other stations.
3 Nutrients, chlorophyll *a* (chl *a*) and *in vivo* fluorescence were determined at all stations,
4 whereas biomass and structure of the microbial plankton community were analysed at
5 21 stations located along 5 of the 8 transects (black symbols in Fig. 1a). Photosynthesis-
6 irradiance relationships and light penetration in the water column were also determined
7 at 9 stations unevenly distributed on the sampled area (triangles in Fig. 1a). For
8 photosynthesis-irradiance experiments water was collected from 3-4 depths within the
9 photic layer, which varied between 22 and 50 m.

11 2.2. Ekman transport, nutrients and chl *a*

12 Geostrophic winds at 43° N, 11° W (150 km off Cape Finisterre), deduced from surface
13 pressure charts provided at 6 h intervals by the Spanish Institute of Meteorology, were
14 used to estimate the cross-shelf Ekman transport according to Bakun (1973). Nutrients
15 ($\mu\text{mol kg}^{-1}$) were determined on board by segmented flow analysis (Technicon AAI
16 system); nitrate according to Hansen and Grasshoff (1983) and ammonium following
17 Grasshoff and Johansen (1972). Chl *a* concentration (mg m^{-3}) was determined by
18 fluorometry using a Turner Designs fluorometer calibrated with pure chl *a* (Sigma).
19 Sub-samples of 100 ml were filtered under low-vacuum pressure through 25 mm
20 Whatman GF/F filters, which were immediately frozen at -20°C for at least 24 h. The
21 pigments were then extracted in 90% acetone for 24 h in the dark at 4°C.

23 2.3. In vivo Fluorescence

24 Measurements of *in vivo* fluorescence were made with the Turner Designs
25 fluorometer. Before readings, samples were dark-adapted at ambient temperature for 30

1 min (Falkowski and Kiefer, 1985). Two measurements were performed: *in vivo*
2 fluorescence (F_o) and maximum *in vivo* fluorescence (F_m) after adding dichlorophenyl
3 dimethylurea (DCMU) dissolved in ethanol to a final concentration of 10 µmol l⁻¹.
4 Although errors in measuring *in vivo* fluorescence with conventional fluorometers are
5 large, these readings are approximately correlated with those obtained using other more
6 sophisticated fluorometers based on saturating flashes (Geider et al., 1993). Therefore,
7 the readings derived from conventional fluorometers can be used to qualitatively assess
8 the photosynthetic capacity and efficiency of phytoplankton. Thus, variable
9 fluorescence (F_v), defined as F_m-F_o, can be viewed as an estimate of the photosynthetic
10 capacity of the sample, and the ratio F_v/F_m has been considered an estimate of the
11 relative phytoplankton growth rate (Kolber et al., 1990; Falkowski et al., 1991).

12 Although the F_v/F_m ratio may be affected by several intrinsic (Geider et al., 1993;
13 Suggett et al., 2009) and extrinsic (Juneau et al., 2002) factors to phytoplankton
14 community, this ratio has also been used to assess nutrient limitation of phytoplankton
15 photosynthesis in the sea (Kolber et al., 1990; Falkowski et al., 1991; Geider et al.,
16 1993). Commonly, F_v/F_m approaches 0.6 in nutrient replete conditions and declines to
17 around 0.3 under nutrient limitation.

18 19 2.4. Microbial plankton biomass

20 Autotrophic and heterotrophic pico- (<2 µm) and nanoplankton (2-20 µm) were
21 determined by epifluorescence microscopy (Porter and Feig, 1980) in subsamples of 10
22 ml (see details in Figueiras et al., 2006). We realize that *Prochlorococcus* is not
23 correctly identified and counted with this technique, but their presence in the NW Iberia
24 is restricted to late summer-beginning of autumn (Rodriguez et al., 2006). Bacterial
25 biomass was estimated according to Lee and Furhmann (1987). For the other groups

dimensions were taken and their cell volumes were calculated assuming a spherical shape. Biovolumes were converted to cell carbon following Verity et al. (1992) for pico- and nanoflagellates and Bratbak and Dundas (1984) for *Synechococcus*-type cyanobacteria (*Syn*).

Microplankton (20-200 µm) was determined in subsamples of 100 ml preserved in Lugol's iodine solution. Samples were allowed to settle in composite sedimentation chambers and diatoms, dinoflagellates and ciliates were identified and counted to the species level, when possible, using an inverted microscope (Crespo and Figueiras, 2007). Phototrophic and heterotrophic species were differentiated following Lessard and Swift (1986) and also using epifluorescence microscopy. Dimensions were taken to determine cell volumes and plasma volumes by approximation to the nearest geometrical shape (Edler, 1979). The plasma volume of diatoms and the biovolume of dinoflagellates and ciliates were converted to cell carbon following Strathman (1967) for diatoms and dinoflagellates and Putt and Stoecker (1989) for ciliates.

2.5. Light in the water column and at sea surface

The penetration of photosynthetic active radiation (PAR, $\lambda = 400-700$ nm, $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) in the water column was determined at 2 m intervals with a Li-Cor spherical quantum sensor LI-193SA that provided scalar measurements. Incident PAR_{0+} at sea surface was also determined simultaneously using a Li-Cor cosine-sensor (LI-190SA). PAR just below the sea surface (PAR_{0-}) was estimated by adjusting the PAR profile in the water column to the equation describing the light attenuation with depth (z):

$$\text{PAR}_z = \text{PAR}_{0-} e^{-kz} \quad (1)$$

where k (m^{-1}) is the light attenuation coefficient.

The ratio PAR_{0-}/PAR_{0+} gives the light transmittance at the air-sea interface. The daily solar PAR radiation was measured at 1 min intervals using a LI-190SA sensor placed on the deck of the research vessel. Readings were integrated at hourly intervals to provide the time distribution of incident irradiance at sea surface.

2.6. Photosynthesis-irradiance experiments, primary production and phytoplankton growth rates

The photosynthesis-irradiance determinations were carried out in linear incubators (Arbones et al., 2000) illuminated on the front side with tungsten-halogen lamps (50W 12 V) and refrigerated by circulating surface seawater. Each incubator houses 14 subsamples collected in 75 ml tissue culture Corning flasks that were inoculated with 1.85×10^5 Bq (5 μ Ci) of 14 C-labeled bicarbonate. The PAR at the position of each bottle was measured with a LI-190SA sensor, but the flask at the end of the incubator was wrapped in aluminium foil to check dark carbon fixation. After 2 h of incubation, samples were filtered through 25 mm Whatman GF/F filters at low-vacuum pressure. The filters were then exposed to HCl fumes for 12 h to eliminate unincorporated 14 C. The incorporated activity was determined with a liquid scintillation counter using the external standard and the channel ratio methods to correct for quenching.

As photoinhibition was not observed, the data were fitted to the model of Webb et al. (1974) to obtain chlorophyll-specific light-saturated rates of photosynthesis [P_m^B , mg C (mg chl *a*)⁻¹ h⁻¹] and light-limited slopes [α^B , mg C (mg chl *a*)⁻¹ h⁻¹ (μ mol quanta m⁻² s⁻¹)⁻¹]:

$$P_z^B = P_m^B [1 - \exp(-\alpha^B PAR / P_m^B)] \quad (2)$$

where P_z^B is the chlorophyll-specific rate of photosynthesis [mg C (mg chl *a*)⁻¹ h⁻¹] at each sampled depth.

The daily integrated primary production (PP, g C m⁻² d⁻¹) was estimated by combining chl *a* concentration, light field and the photosynthetic variables P_m^B and α^B . Integration was done at hourly steps and 1 m intervals from the sea surface down to 1% surface irradiance.

Mean gross growth rates of phytoplankton ($\bar{\mu}$, d⁻¹) in the photic layer ($z_{1\%}$) were calculated as:

$$\bar{\mu} = \frac{1}{z_{1\%}} \int_{z_{1\%}} \ln \left(1 + \frac{dC/dt}{C_{ph}} \right) \quad (3)$$

where dC/dt is carbon fixation (mg C m⁻³ d⁻¹) and C_{ph} (mg C m⁻³) phytoplankton carbon.

3. Results

3.1. Hydrography and nutrients

Although meteorological and hydrographic conditions during this cruise have already been described (Castro et al., 1994; Figueiras et al., 2006), the main features must be mentioned here to help to understand the oceanographic framework of the area. The cruise took place when strong northerly winds favourable to upwelling on the western shelf prevailed (Fig. 1b). However, the wind relaxation that occurred before the beginning of the cruise caused a shift towards the west coast of relatively warm (> 13 °C) oceanic surface water (Fig. 2a). This advection induced downwelling and confined upwelled waters to a narrow band near the coast (Figs. 4b, c, e, f), which partially masked the typical upwelling signals that could be expected to find in surface waters. Thus, upwelled cold (<12.8 °C) and nitrate replete waters (>1 µmol kg⁻¹) only outcropped around Cape Finisterre and in front of the northernmost Rías Baixas (Figs. 2a, b). In contrast, the water column at the northern coast appeared relatively well

stratified (Figs. 4a, d), as northerly winds did not provoke upwelling in this zone. Although nitrate concentrations in surface waters were relatively low ($<1 \mu\text{mol kg}^{-1}$) outside the upwelling area (Fig. 2b), ammonium levels were remarkably high ($>0.6 \mu\text{mol kg}^{-1}$, Fig. 2c), particularly at both sides (north and south) of Cape Finisterre upwelling centre.

3.2. *Chl a and in vivo fluorescence*

The chl *a* distribution at sea surface (Fig. 3a) largely reflected the hydrographic situation. Highest concentrations ($>8 \text{ mg m}^{-3}$) were located on the western shelf, where the outflow from the Rías Baixas was confined on the inner shelf by the advected oceanic water characterised by lower chl *a* values. However, in the upwelling area around Cape Finisterre, chl *a* concentration was higher at the oceanic stations than on the shelf (Fig. 3a), where upwelled waters containing high nutrients (Fig. 4e) and low chl *a* values (Fig. 4h) reached the sea surface. Whereas high chl *a* values were found in surface waters around the Cape Finisterre upwelling centre and on the western shelf (Figs. 4h, i), at the northernmost transect, without upwelling influence (Figs. 4a, d), a subsurface chl *a* maximum associated with the nitracline was evident (Fig. 4g).

The sea surface Fv/Fm ratio was >0.6 in the upwelling area of Cape Finisterre and in front of the Rías Baixas (Fig. 3b) but was <0.4 at the two southernmost oceanic stations (Fig. 3b). In spite of that, Fv/Fm values approached 0.6 in the upper layer of the water column (Figs. 4j, k, l). The Fv/Fm ratio was not significantly correlated either with nitrate ($r = 0.026$; $P > 0.05$) or total inorganic nitrogen concentration ($r = 0.032$; $P > 0.05$) for the upper 50 m (approximately the average depth of the nitracline) of the water column. The average value of Fv/Fm at sea surface (0.56 ± 0.09) was not significantly

different ($P = 0.89$, t-test for two samples) from the mean value for the upper 50 m (0.56 ± 0.12).

3.3. Microbial plankton biomass

According to the distribution of autotrophic plankton biomass (Fig. 5), two domains (coastal and oceanic) could be distinguished (Table 1). At the coastal domain, formed by the most inshore stations (2 or 3 stations) at each transect (Table 1), diatoms made up a significant contribution ($27 \pm 17\%$) to total autotrophic biomass (AB). In contrast, diatoms were of minor importance ($1 \pm 2\%$) at the oceanic domain (Table 1). AB was significantly higher ($P < 0.001$, t-test for two samples) in the ocean ($24.5 \pm 9.6 \text{ g C m}^{-2}$) than in coastal waters ($8.7 \pm 4.1 \text{ g C m}^{-2}$) and this pattern was observed for other autotrophic plankton groups, except for diatoms and autotrophic ciliates (AC). The biomass of diatoms was significantly higher at the coastal domain ($P < 0.001$), whereas that of AC did not show differences between domains. Autotrophic nanoflagellates (ANF) dominated in both realms, representing $62 \pm 16\%$ and $89 \pm 6\%$ of the integrated AB at the coastal and oceanic domain, respectively. Although the contribution of autotrophic dinoflagellates (AD) to AB was relatively important at the two domains (7-8%), the contributions of the other groups were nearly irrelevant (Table 1). Differences also occurred within the coastal domain, where AB was significantly higher ($P < 0.001$) at the stations south of Cape Finisterre ($12.4 \pm 2.1 \text{ g C m}^{-2}$) than at the northern stations ($5.6 \pm 2.2 \text{ g C m}^{-2}$). Higher biomass in front of the Rías Baixas, though observed in practically all groups (AC was the exception), was especially important for diatoms (Table 1). Integrated chl *a* concentration was also significantly higher ($P < 0.001$) in front of the Rías than at the northern coastal stations (Table 1).

Differences within the coastal domain were not recorded for the phytoplankton carbon: chl *a* ratio ($C_{ph}:chl\ a$), which however showed contrasting values at the coastal and oceanic domains (Fig. 6). Thus, this ratio was appreciably lower in coastal (38 ± 3) than in oceanic samples (157 ± 8).

Heterotrophic biomass (HB), dominated by heterotrophic nanoflagellates (HNF) and bacteria ($68 \pm 6\%$ and $22 \pm 5\%$ of the total integrated HB, respectively), did not show such evident differences between domains (Fig. 7, Table 2). Nevertheless, HB was significantly higher ($P < 0.01$) in the ocean ($15.7 \pm 5.2\ g\ C\ m^{-2}$) than on the shelf ($9.5 \pm 3.6\ g\ C\ m^{-2}$), owing to higher biomass of HNF and bacteria (Table 2). In contrast to that with AB, there were no differences between northern and southern stations within the coastal domain for HB and any of the heterotrophic plankton groups.

With regard to the integrated values of AB and HB (Fig. 8), the oceanic domain could be considered autotrophic, where only 2 stations (Stns. 64 and 14) showed HB higher than AB. However, HB ($9.5 \pm 3.6\ g\ C\ m^{-2}$) and AB ($8.7 \pm 4.1\ g\ C\ m^{-2}$) were not significantly different ($P = 0.51$, t-test for paired samples) in the coastal domain. The equation describing AB vs HB relationship shows that both biomasses covaried according to a HB:AB ratio of 0.33. This relationship also indicates that the balance between HB and AB ($HB:AB = 1$) occurred at a value ($\sim 10\ g\ C\ m^{-2}$) close to the mean AB at the coastal domain.

3.4. Photophysiological variables, primary production and phytoplankton growth rates

The light-limited slopes (α^B) of the photosynthesis-irradiance relationships were significantly higher ($P < 0.01$) at the oceanic than at the coastal domain (Table 3). This difference between domains was due to the lower values recorded at the stations north of Cape Finisterre within the coastal domain, because the values at the coastal stations

1 south of Cape Finisterre were not different from those obtained at the oceanic stations.

2 The maximum photosynthetic rates (P_m^B), though lower at the northern coastal stations
3 (Table 3), did not show significant differences between domains or coastal subdomains.

4 Photophysiological variables not normalised to chl *a*, P_m (mg C m⁻³ h⁻¹) and α [mg
5 C m⁻³ h⁻¹ (μmol quanta m⁻² s⁻¹)⁻¹], were strongly correlated ($r = 0.91$, $P < 0.001$, $n = 30$),
6 and both showed significant relationships with variable fluorescence ($F_v = F_m - F_o$):

$$P_m = (828.85 \pm 56.38) F_v; r^2 = 0.77; P < 0.001 \quad (4)$$

$$\alpha = (5.47 \pm 0.33) F_v; r^2 = 0.72; P < 0.001 \quad (5)$$

9 Using these relationships, the chlorophyll-specific rates (P_m^B and α^B) and PP (g C
10 m⁻² d⁻¹) were estimated at the 9 stations where the photosynthesis-irradiance
11 experiments were conducted. High agreement was achieved between the PP estimated
12 values and the PP values obtained (PP measured) using the photophysiological variables
13 actually determined:

$$PP_{\text{estimated}} = (0.99 \pm 0.05) PP_{\text{measured}}; r^2 = 0.91 \quad (6)$$

15 Therefore, equations 4 and 5 allowed mapping PP at the sampled area (Fig. 9a), after
16 knowing that the empirical relationship describing the dependency of the light
17 attenuation coefficient (k , m⁻¹) on chl *a* concentration (mg chl *a* m⁻³) was:

$$k = (0.056 \pm 0.008) + (0.026 \pm 0.003) \text{ chl } a; r^2 = 0.76; P < 0.001 \quad (7)$$

19 where the slope is the chlorophyll-specific light attenuation coefficient [m² (mg chl *a*)⁻¹]
20 and the intercept the attenuation coefficient due to seawater and other suspended or
21 dissolved material.

22 Primary production in NW Iberia during May 1991 (3.29 ± 1.56 g C m⁻² d⁻¹) was
23 highly variable (Fig. 9a). The highest values (5.04 ± 1.88 g C m⁻²) were found at the
24 coastal stations in front of the Rías Baixas, whereas the lowest (< 2 g C m⁻²) occurred at
25 the northernmost transect and in the advected oceanic water at the southwest corner.

1 The oceanic stations around the main upwelling area of Cape Finisterre, where chl *a*
2 concentration was relatively high (Fig. 3a), showed PP values (3-4 g C m⁻²) in between.

3 As phytoplankton growth rates estimated (equation 4) with the C_{ph} derived from the
4 C_{ph}: chl *a* ratios obtained for the two domains (Fig. 6) were not significantly different (P
5 = 0.67; t-test for paired samples) from the rates estimated using the phytoplankton
6 carbon actually determined, mean gross growth rates in the photic layer were also
7 mapped for all sampled area (Fig. 9b).

8 Contrasting with PP (Fig. 9a) and autotrophic biomass (Fig.5, Table 1), which
9 showed alongshore and across-shelf variability, across-shelf variability predominated in
10 phytoplankton growth rates, with phytoplankton growing faster near the coast ($0.54 \pm$
11 0.09 d^{-1}) than at the ocean ($0.17 \pm 0.02 \text{ d}^{-1}$).

13 **4. Discussion**

14 *4.1. Spatial variability in phytoplankton biomass and primary production under an* 15 *upwelling scenario*

16 As with other coastal upwelling systems (Shannon et al., 1985; Largier et al., 1993),
17 at the NW Iberia the interaction between coastline features and wind forcing also caused
18 high spatial variability. This is the only cruise were a quasi-synoptic spatial variability
19 of primary production and biomass under an upwelling scenario along the entire
20 Galician coast has been established.

21 Northerly winds had a lesser effect at the northern coast, where the water column
22 remained relatively well stratified allowing the development of a subsurface chl *a*
23 maximum at the nitracline (Fig. 4a, d, g). This clearly contrasted with the situation
24 observed at the other zones of the sampling area, where northerly winds induced
25 stronger upwelling near the coast (Figs. 2, 4) promoting phytoplankton growth (Fig.

9b). However, the most remarkable feature was the accumulation of phytoplankton biomass at the western coast in front of the Rías Baixas (Fig. 3a; Table 1), a recurrent feature for this region during upwelling relaxation and downwelling (Castro et al., 1997). Under upwelling conditions, a positive estuarine circulation is established in the Rías Baixas becoming an extension of the continental shelf. In this scenario, nutrient rich subsurface water flows into the Rías along their bottom regions, while there is an outflow of surface waters exporting fixed organic matter towards the ocean (Figueiras et al. 2002). This offshore transport of material can be hindered due to the onshore shift of salty oceanic waters as it occurred in May 1991, provoking an accumulation of the exported organic matter on the shelf.

The high contribution of ANF to total AB recorded during our cruise in May 1991 should not be considered exceptional; instead, it must be now viewed as a general feature in coastal upwelling systems (Varela et al., 1991; Iriarte et al., 2000; Rodríguez et al., 2006; Böttjer and Morales, 2007), even during upwelling conditions. Diatoms, which are expected to be dominant in these regions, however restricted their presence to the nearshore band directly affected by upwelling (Fig. 5). This distribution has also been described for other coastal upwelling zones (Iriarte et al., 2000) and previously for the NW Iberian shelf during summer upwelling (Tisltone et al. 2003). Nevertheless, coastal upwelling systems are essentially dynamic, with wind forcing varying within relatively short-time periods (Fig. 1b). Thus, the presence of diatoms in oceanic waters could occur during moments of strong upwelling, when offshore transport is enhanced. For the case reported here, the export of diatoms towards the ocean was probably limited by the wind relaxation occurring before the cruise. The higher contribution of diatoms to total AB observed in front of the Rías Baixas (Table 1) is due to the intensification of upwelling inside the Rías, which simultaneously stimulates diatom

growth and their transport to the adjacent shelf (Tilstone et al., 2000; Lorenzo et al., 2005).

The spatial variability forced by the interaction between wind and shoreline singularities also affected PP (Fig. 9a). The range of PP values ($0.4\text{--}8.4\text{ g C m}^{-2}\text{ d}^{-1}$) was comparable to those reported for other coastal upwelling systems (e.g. Brown and Field, 1986; Daneri et al., 2000; Iriarte and González. 2004). The high values measured in front of the Rías ($5\text{--}8\text{ g C m}^{-2}\text{ d}^{-1}$; Fig. 9a) lied within the upper range of values recorded for the Rías interior during upwelling (Teira et al., 2003; Cermeño et al., 2006) and were also similar to maximum values ($5 \pm 1\text{ g C m}^{-2}\text{ d}^{-1}$) registered at the shelf during strong upwelling events (Teira et al., 2001). The average PP ($3.3 \pm 1.6\text{ g C m}^{-2}\text{ d}^{-1}$) during this spring cruise was slightly higher than the mean value ($2.5 \pm 2.8\text{ g C m}^{-2}\text{ d}^{-1}$) for the upwelling season in the Rías (Álvarez-Salgado et al., 2010), and it was significantly higher than the mean value ($1.6 \pm 0.8\text{ g C m}^{-2}\text{ d}^{-1}$) reported for the coastal transition zone (Joint et al., 2002; Tilstone et al., 2003). According to these comparisons, it can be concluded that this spring upwelling event was sufficiently strong to cause a significant increase in PP at the NW Iberian margin, well above the mean value.

4.2. Phytoplankton growth: is mixotrophy significant for autotrophic nanoflagellates?

Although the low growth rates obtained at the most oceanic stations ($0.17 \pm 0.02\text{ d}^{-1}$; Fig. 9b) were similar to those reported for oligotrophic regions in the Atlantic Ocean (Marañón, 2005) and hence suggest nutrient limitation of the growth of ANF (Marañón, 2005), contrasting conclusions can be extracted looking at other variables related to phytoplankton growth. Thus, the high Fv/Fm values recorded at sea surface (0.56 ± 0.09) indicates that phytoplankton growth was not nutrient limited in the photic layer

(Kolber et al., 1990; Falkowski et al., 1991; Suggett et al., 21009), whereas the high $C_{ph}:chl\ a$ ratio (157 ± 8) points to strong nutrient limitation (Marañón, 2005). A way of reconciling these two opposite views is to appeal to the mixotrophic nutritional mode of ANF (Unrein et al., 2007).

Mixotrophy, regarded here as the combination of phototrophic and phagotrophic nutrition, is widespread within the microbial community. It extends from large dinoflagellates (Stoecker, 1999) to the smallest phytoplankton (Zubkov and Tarran, 2008). Mixotrophs always show preference for heterotrophic nutrition under not limiting conditions for both heterotrophic and autotrophic growth, with food supplying ~ 90% of their carbon requirements (Sanders et al. 1990; Skovgaard et al., 2000). Pigmented nanoflagellates can account for 60-80% of total bacterivory in the sea (Unrein et al., 2007; Zubkov and Tarran, 2008), where they exert a tight control on bacterial abundance.

Knowing this mixotrophic nutrition of ANF and also that $C_{ph}:chl\ a$ ratios vary from ~40 for unfed cells to ~150 for fed cells (Skovgaard et al., 2000), we can estimate that the fraction of carbon obtained by ANF at the oceanic domain through heterotrophic nutrition was 76% [$(157-38)/157 = 0.76$]; $70 \pm 17\%$ considering individual samples. For this estimate it was assumed that at the coastal domain, where bacterial biomass was lower (Table 2) and $C_{ph}:chl\ a$ was 38 (Fig. 6), the nutrition of ANF was entirely phototrophic. Certainly, this is an approximate estimate that, however, produces a carbon fraction obtained by heterotrophic nutrition that is very close to the fraction derived from laboratory experiments (Sanders et al. 1990; Skovgaard et al., 2000). It also provides indirect evidence for the possible mixotrophic mode of nutrition of ANF in coastal upwelling systems.

1 Photosynthesis at the oceanic domain was still taking place, as suggested by the high
2 values of Fv/Fm and PP. High Fv/Fm ratios in surface waters (Figs. 3b; 4j, k, l) are near
3 the maximum value (0.6) usually attained under nutrient replete conditions (Kolber et
4 al., 1990; Falkowski et al., 1991). This can be in part due to ammonium concentrations
5 (Fig. 2c), probably resulting from nutrient recycling in the euphotic zone favoured by
6 the possible heterotrophic nutrition of ANF.

7 The low HB:AB ratios recorded at the oceanic domain (Fig. 8) contrast with the
8 expected situation derived from comparative (Gasol et al., 1997) and experimental
9 studies (Duarte et al., 2000) that predict high HB:AB ratios under low nutrient
10 conditions. The relative high AB at this domain undoubtedly resulted from considering
11 pigmented nanoflagellates as autotrophs. But, when it is assumed that ~76% of the
12 biomass of ANF was obtained through heterotrophy and this carbon fraction is
13 reassigned to heterotrophs, the resulting HB ($32 \pm 10 \text{ g C m}^{-2}$) and AB ($8 \pm 3 \text{ g C m}^{-2}$)
14 produce a HB to AB ratio > 1 .

15 Whether mixotrophy of ANF is common or not in coastal upwelling zones requires
16 further assessment especially as it is essential to understand the role that these abundant
17 and ubiquitous organisms play in biogeochemical cycles. It is also needed for the
18 accurate inclusion of ANF in plankton functional types (PFT) models (Le Quéré et al.,
19 2005).

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Figure legends

Fig. 1. (a) Sampled region in the NW Iberian margin showing the four Rías Baixas, Cape Finisterre and the transects selected to represent vertical representations in Figs. 4, 5 and 7. Black symbols denote the stations where microbial plankton biomass was determined. The nine stations where photophysiological variables and light penetration in the water column were determined are marked with triangles (black and open). (b) Cross-shore Ekman transport component ($-Q_x$) from May 1 to 20, 1991, estimated at 43° N, 11° W. Positive values of $-Q_x$ correspond to offshore transport (upwelling) of surface waters.

Fig. 2. Sea surface distributions of (a) temperature, (b) nitrate concentration and (b) ammonium concentration.

Fig. 3. Sea surface distributions of (a) chl *a* concentration and (b) variable fluorescence to maximum *in vivo* fluorescence ratio (F_v/F_m , dimensionless).

Fig. 4. Vertical distribution at 3 selected transects (see Fig. 1a) of (a to c) temperature, (d to f) nitrate concentration, (g to i) chl *a* concentration and (j to l) variable fluorescence to maximum *in vivo* fluorescence ratio (F_v/F_m , dimensionless).

Fig. 5. Vertical distributions at 3 selected transects (see Fig. 1a) of (a to c) total autotrophic microbial biomass (AB), (d to f) biomass of autotrophic nanoflagellates (ANF) and (g to i) biomass of diatoms.

Fig. 6. Phytoplankton carbon (C_{ph}) to chl *a* relationship at the coastal (black circles) and oceanic (open circles) domains (see text for details). The equations defining these relationships are: for the coastal domain $C_{ph} = (18 \pm 10) + (38 \pm 3) \text{ chl } a$, $r^2 = 0.74$; for the oceanic domain $C_{ph} = (8 \pm 18) + (157 \pm 8) \text{ chl } a$, $r^2 = 0.83$. Intercepts are not significant ($P > 0.05$) for the two equations.

Fig. 7. Vertical distributions at 3 selected transects (see Fig. 1a) of (a to c) total heterotrophic microbial biomass (HB), (d to f) biomass of heterotrophic nanoflagellates (HNF) and (g to i) bacterial biomass.

Fig. 8. Integrated total autotrophic biomass (AB) *versus* integrated total heterotrophic biomass (HB). Solid line, $HB = (7.04 \pm 1.64) + (0.33 \pm 0.08)AB$, $r^2 = 0.45$, defines the relationship considering all points, which is not markedly different from that excluding Stns. 64 and 63 (dashed line), $HB = (6.13 \pm 1.10) + (0.33 \pm 0.06)AB$, $r^2 = 0.67$. The intersection of both lines with the 1:1 relationship (dotted line) corresponds to the mean AB value ($9\text{--}11 \text{ g C m}^{-2}$) from which the system shifts from heterotrophy to autotrophy.

Fig. 9. Horizontal distributions of (a) integrated primary production (PP) and (b) mean gross growth rate of phytoplankton (μ) in the photic layer.

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Figure 1

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Table 1. Integrated (surface-bottom on the shelf and 0-100 m at the ocean) values of chl *a* concentration (mg m⁻²) and autotrophic plankton biomass (g C m⁻²) at each station and domain. The coastal domain is divided in subdomains North and South of the Cape Finisterre upwelling centre. AB: total autotrophic biomass; *Syn*: *Synechococcus*; APF: autotrophic picoflagellates; ANF: autotrophic nanoflagellates; Diat: diatoms; AD: autotrophic dinoflagellates; AC: autotrophic ciliates.

Domain	Stn	chl <i>a</i>	AB	<i>Syn</i>	APF	ANF	Diat	AD	AC
Coastal (North)	11	66	4.62	0.008	0.07	3.07	0.77	0.57	0.13
	12	122	6.23	0.001	0.09	4.08	1.37	0.54	0.15
	31	151	9.65	0.003	0.13	7.37	1.69	0.34	0.11
	32	115	4.76	0.005	0.13	3.39	0.38	0.62	0.24
	41	117	3.10	0.002	0.07	1.63	1.23	0.17	0.00
	42	71	5.27	0.006	0.13	4.63	0.27	0.20	0.03
Coastal (South)	61	333	14.85	0.003	0.13	4.29	9.20	1.23	0.01
	62	221	9.82	0.004	0.17	4.66	4.10	0.87	0.01
	63	271	13.58	0.015	0.26	7.63	4.71	0.81	0.15
	71	438	13.20	0.003	0.23	8.69	2.94	1.34	0.00
	72	228	10.69	0.003	0.25	6.49	2.65	1.28	0.02
Average North (SD)		107 (33)	5.6 (2.2)	0.004 (0.003)	0.10 (0.03)	4.03 (1.93)	0.95 (0.57)	0.41 (0.20)	0.11 (0.09)
Average South (SD)		298 (90)	12.4 (2.1)	0.006 (0.005)	0.21 (0.06)	6.35 (1.89)	4.72 (2.64)	1.11 (0.25)	0.04 (0.07)
Average domain (SD)		194 (117)	8.71 (4.12)	0.005 (0.004)	0.15 (0.07)	5.08 (2.18)	2.67 (2.61)	0.73 (0.42)	0.08 (0.08)
Oceanic	13	117	17.15	0.036	0.43	16.25	0.06	0.33	0.04
	14	76	9.28	0.008	0.25	8.77	0.01	0.23	0.00
	33	143	24.06	0.039	0.30	21.73	0.11	1.73	0.14
	34	208	36.85	0.107	1.17	30.05	0.01	5.52	0.00
	46	162	24.42	0.070	0.61	21.91	0.21	1.57	0.06
	44	164	29.85	0.078	0.63	28.07	0.02	1.04	0.01
	45	140	17.06	0.046	0.51	13.51	0.03	2.92	0.05
	64	156	18.33	0.032	0.40	15.32	1.17	1.39	0.02
	73	225	40.71	0.175	1.44	36.80	0.13	2.03	0.14
	74	136	27.64	0.133	0.60	24.39	0.30	2.07	0.15
Average domain (SD)		153 (42)	24.54 (9.62)	0.07 (0.05)	0.64 (0.38)	21.68 (8.50)	0.21 (0.35)	1.88 (1.51)	0.06 (0.06)

Table 2. Integrated (surface-bottom on the shelf and 0-100 m at the ocean) values of microbial heterotrophic plankton biomass (g C m⁻²) at each station and domain. HB: total heterotrophic biomass; HPF: heterotrophic picoflagellates; HNF: heterotrophic nanoflagellates; HD: heterotrophic dinoflagellates; HC: heterotrophic ciliates.

Domain	Stn	HB	Bacteria	HPF	HNF	HD	HC
Coastal	11	8.88	1.36	0.37	6.18	0.67	0.30
	12	12.17	2.19	0.74	8.83	0.28	0.12
	31	7.44	1.72	0.29	5.23	0.19	0.01
	32	4.88	1.23	0.28	2.61	0.34	0.43
	41	5.59	1.75	0.29	3.47	0.08	0.00
	42	8.18	1.83	0.35	5.77	0.22	0.02
	61	8.31	1.73	0.40	5.80	0.21	0.16
	62	11.30	2.13	0.47	7.90	0.78	0.02
	63	18.16	3.75	0.75	12.79	0.39	0.48
	71	9.95	1.98	0.35	7.29	0.32	0.01
	72	9.56	2.48	0.47	6.15	0.45	0.01
	Average (SD)	9.49 (3.60)	2.01 (0.68)	0.43 (0.17)	6.55 (2.73)	0.36 (0.21)	0.14 (0.18)
Oceanic	13	7.72	1.67	0.56	4.84	0.49	0.15
	14	12.37	1.76	0.54	9.97	0.09	0.01
	33	12.18	2.93	0.57	8.36	0.21	0.11
	34	13.74	3.81	0.78	8.81	0.26	0.08
	46	13.83	3.93	0.52	8.36	0.40	0.61
	44	17.69	4.04	0.75	11.21	1.12	0.56
	45	13.83	3.09	0.60	8.76	0.40	0.99
	64	24.74	3.51	0.79	19.73	0.57	0.13
	73	22.67	3.94	0.54	15.99	1.69	0.51
	74	18.57	4.55	0.70	12.24	0.85	0.23
	Average (SD)	15.73 (5.17)	3.32 (0.97)	0.64 (0.11)	10.83 (4.28)	0.61 (0.49)	0.34 (0.32)

Table 3. Average and standard deviations of P_m^B (mg C (mg chl a)⁻¹ h⁻¹) and α^B (mg C (mg chl a)⁻¹ h⁻¹ (μmol quanta m⁻² s⁻¹)⁻¹) at the coastal (n = 14) and oceanic (n = 16) domains and at the two coastal subdomains, North (n = 8) and South (n = 6) of Cape Finisterre.

Domain	Subdomain	P_m^B	α^B
Coastal	Coastal (North)	3.71 ± 2.04	0.024 ± 0.011
	Coastal (South)	4.66 ± 1.54	0.037 ± 0.006
		4.12 ± 1.84	0.030 ± 0.011
Oceanic		4.08 ± 1.49	0.049 ± 0.018