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**Outside the paradigm of upwelling rias in NW Iberian Peninsula:
Biogeochemical and phytoplankton patterns of a non-upwelling ria**

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ABSTRACT

The Atlantic Galician rias show the effect of natural eutrophication during ENACW upwelling episodes when large amounts of nutrients are injected. However, the Cantabrian Galician rias do not appear to be disturbed by upwelling events and it can be hypothesized that eutrophy is not inherent to the Galician rias. This question was tackled regarding the biogeochemical and phytoplankton characteristic in the Ria of Barqueiro, located on the western boundary of the Cantabrian Sea, from January 2008 to January 2009. Thermohaline, PAR and fluorescence profiles, dissolved oxygen, nutrient salts, DOC, DON and chlorophyll concentrations, phytoplankton abundances and primary production were monthly determined in a monitoring station (43° 45.509° N-07° 39.493° W); moreover, the daily upwelling index information and fluvial contributions from Sor River were considered. Throughout the year only four annual oceanographic periods were observed: spring, summer stratification, autumn and winter, unlike the Atlantic rias, where a long upwelling period occurs with usually outstanding upwelling processes. Nutrient concentrations (a maximum of 6.2 mM of nitrate, 0.5 mM of nitrite, 2.8 mM of ammonium, 0.4 mM of

phosphate and 5.7 mM of silicate), organic matter (98 mM of DOC, 14 mM of DON, 16.4 mM of POC and 2.0 mM of PON), Chl-a (1.1 mg L^{-1}), primary production ($280 \text{ gC m}^{-2} \text{ yr}^{-1}$) and phytoplankton abundance were clearly lower (about half) when compared to those reported for the other rias during upwelling. Diatoms dominated the phytoplankton assemblages especially during spring and autumn blooms. In summer, although the wind conditions were favorable, upwelling was not observed in the Ria and a mixing of diatoms and dinoflagellates co-dominated the phytoplankton community with a lower contribution of diatoms in comparison to the Atlantic rias. The paradigm of natural eutrophication reported for the Galician rias was not observed in the Ria of Barqueiro, which may be classified as an oligo-mesotrophic ria-type, according to its environmental patterns.

Keywords

oceanography, thermohaline, nutrients, phytoplankton, primary production, pristine, Spain.

1. Introduction

Along the coastal regions there is a great variety of estuarine systems, which are one of the most common geographical features in the world coasts. The variety of river-sea boundaries is so large that several ways have been used to define and classify them (Perillo, 1995; Dyer, 1997). Among them, a ria is a submersed incised valley where its inner part can be only considered as an estuary from both the hydrographic and the resulting sedimentological considerations (Evans and Prego, 2003). Eighteen rias which are located at the northwest corner of the Iberian Peninsula form the Galician Ria System. Research on hydrographical conditions, nutrient inputs, primary production and phytoplankton biomass has been mainly carried out in the system of rias located at the western (Margalef et al., 1955; Figueiras and Pazos, 1991; Cermeño et al., 2006; Varela et al., 2008) and north-western (Bode and Varela, 1998; Varela et al., 2001, 2005; Bode et al., 2005a) coast of Galicia. These studies recognized the existence of seasonal changes of environmental and biological conditions. Accordingly, several similar oceanographic periods found across the Galician rias open to the Atlantic Ocean (north-western and western aforementioned rias) have been described: mixing in winter, stratification in summer and phytoplankton blooms in the transition periods, i.e. mixing-stratification (spring bloom) and stratification-mixing (autumn bloom) related to the seasonal variability of this type of ecosystem (Margalef et al., 1955). However, this cycle is altered, usually during late spring and summer, by

the pulses of Eastern North Atlantic Central Water (ENACW), which supplies nutrients (Fraga, 1981) and causes phytoplankton blooms of similar magnitude to those occurring in spring and autumn (Varela et al., 2005, 2008). Therefore, the Atlantic Galician rias may be considered as upwelling rias where diatoms are dominant throughout the year, even in summer, due to the continuous upwelling pulses. It is only during the winter, mainly due to the decrease in solar radiation, when their abundance and biomass exhibit very low values (Varela et al., 2008). However, during late winter, pre-bloom may occur under favorable upwelling conditions. In the Sound of Corcubión, near Cape Finisterre, the observed phytoplankton proliferation in February 2005 was more the result of increased environmental irradiance associated to upwelling favorable winds, i.e. persistent clear skies, than a consequence of nutrient upwelling supply as these exhibit high concentrations due to winter mixing (Varela et al., 2010).

The Galician upwelling is part of the north boundary of the Eastern North Atlantic Upwelling System (Wooster et al., 1976; Barton, 1998). Ospina-Alvarez et al. (2010) pointed out that this upwelling influence might not extend beyond the Atlantic rias. Hence, one of the questions arising from these studies has to do with determining the typical oceanographic patterns in the Cantabrian Galician rias located on the north coast of Galicia. Unlike the Galician Atlantic, the Galician Cantabrian rias have not been widely investigated. Current oceanographic knowledge of this region is based on remote sensing (Anderson et al., 1990) and hydrographical information (Alvarez et al., 2009; Ospina-Alvarez et al., 2010; Alvarez et al., 2010a,b) showing the shelf influence of upwelling occurring only offshore the rias, as has already been observed by Prego et al. (1999).

The Atlantic Galician rias are under the effect of natural eutrophication during upwelling episodes when large amounts of nutrients are injected through the upwelled seawaters (Alvarez-Salgado et al., 1996; Prego, 2002). Moreover, the Galician Cantabrian rias do not seem to be affected by significant human activities (Bode et al., 2011). As these rias are not disturbed by natural (upwelling) and anthropogenic eutrophication processes, it can be hypothesized that eutrophy is not inherent to all Galician rias. Therefore, it is of value to define the biogeochemical and phytoplankton patterns of a non-upwelling ria-type such as the Ria of Barqueiro located in the northern Galician coast. In this respect the following objectives are searched: 1) determining the nutrient availability and phytoplankton assemblages during the own oceanographic periods of the Ria of Barqueiro, and 2) reconsidering the paradigm of natural eutrophication of Galician rias, which is based on the well-known (Atlantic) upwelling rias, with the Cantabrian ria results.

2. Materials and methods

2.1. Study area

The Ria of Barqueiro and the neighboring Rias of Ortigueira, Viveiro, Foz and Ribadeo are the Northern Galician rias (Torre-Enciso, 1958), also known as the Galician Cantabrian rias, since they are located on the Cantabrian coast. This differentiates them from those located west of Cape Ortegal (Fig. 1), i.e. on the Atlantic coast, so that they will be called as the Atlantic Galician rias.

The Ria of Barqueiro, open to the north with a SWeNE orientation, is located on the western boundary of the Cantabrian coast next to Cape Estaca-de-Bares, the northernmost point on the Iberian Peninsula (Fig. 1). The Ria has an area of 10 km² and a mouth-depth of 27 m. Tides are semidiurnal with a range of 2-4 m. Marine processes control the dynamics in the middle-outer zone of the Ria, but in the inner or estuarine zone the fluvial processes have a greater influence. The most important freshwater source is the Sor River, which had an average flow of 19.3 m³ s⁻¹ in 2008. According to the Köppen classification system (Peel et al., 2007) the climate is wet temperate oceanic (Cfb type). The wind regime is characterized by westerly winds in autumn/winter, while in spring and summer easterly winds prevail. From the meteorological data of the station in Estaca-de-Bares during the sampling year (2008) the annual average air temperature was 10.4°C with a minimum and maximum of -0.3°C and 23.2°C, respectively; rainfall was 1084 mm with approximately 168 days of rain and a mean relative humidity of 80%, and 36% of the prevailing winds during 2008 blew from the east (upwelling favorable winds).

2.2. Water column sampling and analysis

Monthly cruises were carried out on board the R/V Mytilus and R/V Lura from January 2008 to January 2009 at a fixed station located in the central part of the Ria at 43° 45.509'N and 07° 39.493'W (18 m depth in equinoctial tides). Temperature and salinity profiles were obtained with a General Oceanic Rosette, including a Sea-Bird 25 CTD with PAR (Li-COR) and Fluorescence (SCUFA) sensors. The water column was sampled using General Oceanic (5 or 12 L) bottles. Aliquots were collected at standard depths (0, 5, 10 and 20 m) from the surface to near bottom.

The water was sampled to determine:

- (1) Dissolved oxygen concentration and saturation: samples were fixed in situ and analyzed within 24 h using an automatic titrator (702-SM Titrino, Metrohm) according to the Winkler method (Aminot, 1983). Error percentage in dissolved oxygen analysis was 0.16%, calculated from ten replicate analyses

of equal concentration.

- (2) Particulate organic carbon (POC) and nitrogen (PON) contents: after aliquot filtration through Whatman GF/F filters both were analyzed using a FlashEA 11-12 Termoquesth CNH analyzer. Considering the low contribution of carbonate to total carbon (Fernández et al., 1995), carbonate was not removed from the filters of POC. Filter blanks showed contents of POC and PON lower than 0.01%. The molar POC:PON ratio was calculated.
- (3) Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations: analyses were carried out on a Shimadzu TOC-VCSH analyzer with a TNM-1 unit by high temperature catalytic oxidation (HTCO) method and Non-dispersive infrared (NDIR) and quimiluminiscence detection, respectively, following the procedure described by Alvarez-Salgado and Miller (1998). The precision was $\pm 0.8 \text{ mmolC L}^{-1}$ and $\pm 0.3 \text{ mmolN L}^{-1}$.
- (4) Nutrient salt (nitrate, nitrite, ammonium phosphate, silicate) concentrations: samples were frozen during sampling and then analyzed in an Integral Futura (Alliance Instruments) autoanalyzer system with separate lines according to the standard colorimetric methods of Grasshoff et al. (1999). The precision were: $\pm 0.01 \text{ }\mu\text{M}$ for nitrite and phosphate, and $\pm 0.02 \text{ }\mu\text{M}$ for nitrate, ammonium and dissolved silicate.
- (5) Chlorophyll-*a* concentration. Aliquots were filtered through Whatman GF/F filters, and then chlorophyll was extracted with 90% acetone following the JGOFS protocols (UNESCO, 1994) and measured by spectrofluorimetry with a Perkin Elmer Luminescence Spectrometer LS50B, according to Neveux and Panouse (1987). Error in analyses was lower than 1%.
- (6) Phytoplankton abundances. Samples were preserved with Lugol's solution and the technique described by Utermöhl (1958) was used. Between 500 and 1000 cells were counted per sample and the nomenclature for species was based on Tomas (1997). Taxa were identified at species level when possible. Small phytoplankters less than 5-10 μm were usually grouped, at Genus level or included into a more general category as Cryptophyceae, smaller (<30 μm) and large (>30 μm), Dinoflagellates and large and small Diatoms (both Centric and Pennates). The diatom Genus *Pseudo-nitzschia* is not possible to be identified unless observed under electron microscope (Lundholm et al., 2012). Species identification is based on density of striae and fibula and presence of central node which are not revealed by light microscope. Therefore it has named *Pseudo-nitzschia* observed at micro-scope, as *Pseudo-nitzschia* group "pungens" or *Pseudo-nitzschia* group "delicatissima" when larger or smaller than 3 μm . On the other hand, Cryptophyceae include several species belonging to Genera *Cryptomonas* (mainly brackish species), *Chroomonas* or *Rhodomonas*. Phytoplankton carbon was calculated following Strathmann

(1967). As empty diatoms valves were frequently observed, an estimation of their abundances was carried out.

- (7) Primary production: this was measured after 2 h incubation on board simulating in situ conditions, as described in Bode et al. (1994a) and references therein. Light levels were simulated using neutral density screens. Samples were filtered through GF/F filters, and primary production rates for each depth (light intensity level) within the photic zone, estimated by the ^{14}C method using a Liquid Scintillation Analyzer Tri-Carb 2800 TR (PerkinElmer). Production in $\text{mgC m}^{-2} \text{h}^{-1}$ for total water column was calculated through integration of individual values for each depth. Daily primary production rates were calculated by multiplying hourly rates by the total number of hours during the day, excluding the first 2 h after sunrise and the last 2 h before sunset (Varela et al., 2005).

2.3. Meteorological and hydrological data

Surface wind fields were obtained from the QuikSCAT satellite at the positions marked in Fig. 1. Wind data, available from July 1999, were retrieved from the Jet Propulsion Laboratory web site (http://podaac.jpl.nasa.gov/quikscat/qscat_data.html). The data set consists of global grid values of meridional and zonal components of wind measured twice daily on an approximately $0.25^\circ \times 0.25^\circ$ grid with global coverage. QuikSCAT data are given in an ascending and descending pass. Data corresponding to one pass present numerous shadow areas, therefore, an average between both passes was considered to increase the coverage. Wind speed measurements range from 3 to 20 ms^{-1} , with an accuracy of 2 m s^{-1} and 20° in direction.

Ekman transport components can be defined in terms of wind speed, W , the sea water density, $\rho_w = 1025 \text{ kg} \cdot \text{m}^{-3}$, a dimensionless drag coefficient, $C_D = 1.4 \times 10^{-3}$, and the air density $\rho_a = 1.22 \text{ kg} \cdot \text{m}^{-3}$, following:

$$Q_x = \frac{\rho_a C_D}{\rho_w f} (W_x^2 + W_y^2)^{1/2} W_y \text{ and } Q_y = -\frac{\rho_a C_D}{\rho_w f} (W_x^2 + W_y^2)^{1/2} W_x$$

where f is the Coriolis parameter defined as twice the vertical component of the Earth's angular velocity, Ω , about the local vertical given by $f = 2 \Omega \sin(\theta)$ at latitude θ . The subscripts x and y corresponds to the zonal and meridional component respectively.

Due to the northern orientation of the Cantabrian Galician coast and the fact that UI is defined as the component of Ekman transport in a perpendicular direction to the shoreline (Nykjaer and Van Camp, 1994), the averaged values of the UI for

the Cantabrian Galician rias (UI_C) corresponds to the Q_y component, which is used in this study. Positive values of UI_C ($\text{m}^3 \text{s}^{-1} \text{km}^{-1}$) indicate favorable upwelling conditions. Note that the UI_A component of upwelling favorable conditions considered in the Atlantic Galician rias is -Q_x due to its western coastal orientation.

In addition to the measured variables, daily data from the Sor River flow during 2008/09 supplied by “Augas de Galicia” (hydrological station number 440) were also studied. Flows were corrected considering the total basin area for the river (202 km^2).

2.4. Data analysis

According to the thermohaline properties (water column gradients), nutrient concentrations and phytoplankton biomass, samples were grouped into four categories, related to oceanographic periods previously defined for the Galician rias: Winter, Spring, Summer and Autumn (see, Casas et al., 1997; Varela and Prego, 2003; Varela et al., 2001, 2005, 2008). A non-upwelling period was considered in this ria due to the fact that it is not affected by upwelling events, even though favorable upwelling conditions were detected in the area (see Ospina-Alvarez et al., 2010). In order to verify the aforementioned classification into four periods, a one-way ANOVA followed by a post-hoc Tukey (HDS) test was performed (Zar, 1984). Data normality was tested with a KolmogoroveSmirnov test and then transformed using $\ln(x + a)$. All statistical analyses were conducted using SPSS 16.0 for Mac OS-X (StatSoft, 2008). Datasets of date, depth, and measured value (X, Y, Z respectively) were gridded using the Kriging method with linear semi-variogram model and anisotropy ratio equal to 1. Filled contour plots were generated without smoothing option with Surfer 9.0 (Golden Software, 2009). The values presented as results in text and tables are expressed as the mean \pm standard deviation.

To test whether or not this assumption of phytoplankton periods was correct, all phytoplankton samples for each sampling date and depth, were a priori classified into the above defined periods and a Discriminant Analysis (DIS) was performed on the transformed ($\log x + 1$) data of those taxa with a presence equal or higher than 20% (e.g. Estrada, 1984; Fernández and Bode, 1994). Statistical software JMP from SAS was employed for these analyses.

3. Results

3.1. Upwelling index, hydrography and river flow

Persistent conditions favorable to upwelling events were observed during April (9 days with UI_N of $1030 \pm 370 \text{ m}^3 \text{s}^{-1} \text{km}^{-1}$), July (9 days with UI_C of $1100 \pm 550 \text{ m}^3$

$\text{s}^{-1} \text{ km}^{-1}$), and mainly in September (19 days with UIC of $860 \pm 490 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$). Conditions favorable to downwelling were also detected during the period of study, mainly in winter, although sporadic positive indices were seen (Fig. 2).

Water temperature showed seasonal differences (ANOVA, $F = 35.75$, $p < 0.001$; Table 1) in the water column of the sampling station. There, the minimum value of temperature during the monitoring was observed in December ($11.6 \pm 0.3^\circ\text{C}$) and the maximum in August ($18.9 \pm 0.1^\circ\text{C}$; Fig. 2). Temperature was characterized by a temporal stratification from May to October, most evident in the first 10 m of depth (Fig. 2). Salinity in 2008 varied seasonally between 34.1 and 35.8, indicating an oceanic predominance during the whole year. The mean monthly freshwater discharge was high during November ($42.1 \text{ m}^3 \text{ s}^{-1}$) and December ($38.9 \text{ m}^3 \text{ s}^{-1}$) and low during August ($6.5 \text{ m}^3 \text{ s}^{-1}$).

3.2. Nutrient salt concentrations

The temporal variability of nitrate was evident (ANOVA, $F = 26.31$, $p < 0.001$; Table 1), exhibiting a decreasing trend until the summer, followed by a gradual increase toward winter. The lowest concentrations of nitrate were observed in August ($0.19 \pm 0.15 \text{ mM}$) with the entire water column being almost nitrate depleted (Fig. 3), and the highest concentration in December ($5.82 \pm 0.23 \text{ mM}$) associated with the peaks of the river inputs and the light limitation during winter for photosynthetic activity (Fig. 3). Nitrite also showed seasonal differences (ANOVA, $F = 5.20$, $p < 0.01$; Table 1) with low concentrations throughout the year, reaching the lowest values in November ($0.09 \pm 0.03 \text{ mM}$) and the highest in March ($0.38 \pm 0.06 \text{ mM}$; Fig. 3). Ammonium also exhibited seasonal differences (ANOVA, $F = 46.22$, $p < 0.001$; Table 1) increasing during the month of June ($2.6 \pm 0.2 \text{ mM}$) mainly near the bottom.

Silicate (contour plot not showed) was seasonably variable (ANOVA, $F = 4.39$, $p < 0.01$; Table 1) but remained low through the year (generally less than 3 mM). Particular exception was observed in November 2008 - January 2009 when the concentrations increased in the surface layer (0-10 m depth) up to 5.7 mM in parallel with the highest fluvial contributions and lowest salinities (34e35 in the surface layer). Phosphate (contour plot not showed) also differed during the year (ANOVA, $F = 11.71$, $p < 0.001$; Table 1), with the lowest values in May ($0.04 \pm 0.03 \text{ mM}$) and the highest in November 2008 e January 2009 ($0.40 \pm 0.01 \text{ mM}$). Nitrate (Fig.3), phosphate and silicate patterns, according their respective contour plots, were similar.

3.3. Oxygen, organic matter and C:N ratio distributions

The water column was well-oxygenated throughout the year with >90% (Table 1, Fig. 4). The highest values were observed in the upper layers with a maximum

of over-saturation in April ($110 \pm 1\%$) associated with high levels of chlorophyll and primary production. The concentration of DOC (contour plot not showed) was seasonably variable (ANOVA, $F = 9.92$, $p < 0.001$; Table 1); DOC increased in summer up to 95 mM in June (entire water column) and September (near bottom: 10e20 m depth) while decreases about 20% during autumn/winter. A similar pattern showed DON (contour plot not shown) with concentration higher than 8 mM during summer and a seasonal variability (ANOVA, $F = 12.15$, $p < 0.001$; Table 1). Temporal patterns in vertical profiles of PON (Fig. 3) and POC (Fig. 4) were similar. The highest values of PON and POC were observed in the bottom waters in April (1.5 ± 0.5 and 13.7 ± 3.4 mM, respectively). PON also showed high values in June and October in deep waters. POC displayed high values in summer, and in early autumn and winter. The lowest values were measured in late winter and mid summer. The particulate C:N molar ratio ranged from 5 to 12 (Fig. 4), with subsurface maximum values in winter and during the spring bloom (>8).

3.4. Chlorophyll-a and primary production

Chlorophyll-a (Chl-a) values showed a temporal variability (ANOVA, $F = 4.58$, $p < 0.01$; Table 1) in the water column between January (0.39 ± 0.05 mg L⁻¹) and July (0.34 ± 0.08 mg L⁻¹) as the months with the lowest values, and April (1.47 ± 0.57 mg L⁻¹) and June (1.30 ± 0.74 mg L⁻¹) with maximum concentrations. Small increases in the Chl-a concentration were also detected in September (0.86 ± 0.10 mg L⁻¹; Fig. 4). The lowest Chl-a values were measured in early winter and mid summer. A late spring Bloom occurred in April, showing the highest Chl-a concentration for the 2008 period (Fig. 4). Primary production differed significantly between seasons in 2008 (ANOVA, $F = 3.42$, $p < 0.05$; Table 1), with three important peaks greater than 12 mgC m⁻² h⁻¹ during the spring and late summer season (Fig. 4). The seasonal pattern was similar to that of Chlorophyll-a, with lower values during winter and mid summer (<5 mg C m² h⁻¹). Maxima were found in April (12 ± 4 mg C m² h⁻¹) and later in September (13 ± 5 mg C m² h⁻¹) during stratification. The main difference between blooms is the sub-superficial location in spring months, while during the stratification period the maximum is at the surface level. In autumn, values decreased to almost winter levels (Table 1).

3.5. Phytoplankton assemblages

A total of 161 species or taxa were identified during the study, of which 69 corresponded to dinoflagellates and 68 to diatoms. Other species counted were chrysophytes, prymnesophytes, and cryptophytes in addition to small flagellates that

were not identified at the species level. Benthic diatom empty valves of brackish, marine and freshwater were not included in the tables and figures. However, they were usually found in some particular periods and were taken into account since they were indicative of strong river flow (continental runoff) or resuspension. Small flagellates (<10 mm) were very abundant in early spring and summer (Fig. 5). Dinoflagellates followed a similar pattern to that of flagellates, with sub-surface maxima in late winter-early spring and surface maxima in early and late summer (Fig. 5). Most species were unidentifiable (Table 2), but clearly belonged to the Genus *Gymnodinium* or *Gyrodinium*. Among the specimens recognized, *Heterocapsa niei* showed a constant presence throughout the year, *Lingulodinium polyedrum* during stratification, *Scrippsiella trochoidea* in winter-spring and *Prorocentrum minimum* in winter and during the early summer stratification.

Diatoms had maxima late in June and later in autumn (Fig. 5) and the maxima were always subsuperficial with abundances of over 300 cells mL⁻¹. Chaetoceros species were present during all seasons, but the highest abundances corresponded to spring and autumn. *Lauderia annulata*, *Leptocylindrus danicus* and *Pseudo-nitzschia* group *delicatissima* were the most abundant taxa and dominant during spring. *Thalassionema nitzschioides* was especially predominant in winter, and some benthic species from the Genus *Rhabdonema* were common during the summer months. Chrysophyceans showed a reduced maximum in late winter and almost disappeared until November when they reached their highest abundance levels (Fig. 5). The most representative species were *Distephanus speculum*, in winter, and *Rhizomonas setigera* in autumn. Cryptophyceans showed a pattern opposite to that of diatoms and almost identical to that of flagellates and dinoflagellates with clear maxima in late winter-early spring and later at the end of the summer.

Fig. 6 shows the distribution of cases (samples) in the discriminant space defined by canonical (discriminant) functions 1 and 2. These first two functions accounted for 90% of the variance; ellipses represent a 95% confidence region to contain the true mean of the group. According to the analysis, more than 90% of the samples were correctly categorized, confirming the existence of four oceanographic periods (Tables 1 and 2 and Fig. 6). More than 230 species or taxonomic entities were identified during the study of the annual cycle. Diatoms accounted for 60%, Dinoflagellates for 36%, and Chrysophyceans for 4% of the total taxa respectively. Most of the species observed were present all year round. Approximately 30 species (20% of presence) were used for DIS analysis. Fig. 6 shows the distribution of cases (samples, circles) in the discriminant space defined by canonical (discriminant) functions 1 and 2, which accounted for 90% of the variance. More than 90% of the a priori samples were correctly categorized by DIS confirming the existence of the four oceanographic periods initially predicted (Tables 1 and 2 and Fig. 6). The characteristic species for each period are also shown in Fig. 6. *Pseudo-nitzschia* group *delicatissima* and *Leptocylindrus danicus* were found to be related to the spring period upwelling favorable winds during February (UI in Fig. 2)

in the Ria of Barqueiro is the increase in solar radiation due to the persistent clear skies because at that month nutrient supply (Fig. 3) is not critical due to water column mixing (Fig. 2).

4. Discussion

4.1. Oceanographic seasonality in the Ria of Barqueiro

In 2008 in the Ria of Barqueiro, prior to the spring bloom dominated by diatoms, another bloom of flagellates occurred. These flagellates were composed of nanoflagellates (<20 μm), small dinoflagellates, *Cryptophyceae* and *Chrysophyceae* (Fig. 5). These types of blooms have been described for other European coastal areas (Heiskanen, 1993; Spilling and Tamelander, 2006; Olli and Trunova, 2010) but in Galicia very few studies reported early spring blooms occurring during winter (Varela et al., 2008, 2010). Now, as in Corcubión Sound (Varela et al., 2010), the main effect of upwelling favorable winds during February (UI in Fig. 2) in the Ria of Barqueiro is the increase in solar radiation due to the persistent clear skies because at that month nutrient supply (Fig. 3) is not critical due to water column mixing (Fig. 2).

The typical spring bloom, dominated by diatoms occurred late in the Ria of Barqueiro, starting to develop in April and extending until June (Fig. 5). Such a persistent bloom suggests the existence of favorable conditions for these events alternating with periods of nutrient depletion (Fig. 3) and decreasing phytoplankton biomass (Fig. 4). This assumption is supported by the taxonomic composition of phytoplankton (Table 2). At the start of this period the dominant diatoms were *Lauderia annulata* and *Chaetoceros socialis* which are typical of late winter and early spring in the Atlantic Galician rias (Varela et al., 2001, 2008). On the contrary, in May and June, dominant species, such as *Asterionellopsis glacialis*, *Guinardia delicatula*, *Leptocylindrus danicus*, and *Pseudo-nitzschia* group *delicatissima* (Table 2 and Fig. 6), are in general, indicative of more advanced stages of phytoplankton succession in spring (Varela et al., 2001, 2005; Varela and Prego, 2003). In parallel with the depletion of nitrate, ammonium increased during the month of June (Fig. 3), which was most likely due to the remineralization processes occurring immediately after the spring bloom (see oxygen saturation percentage in Fig. 4).

Upwelling events are a typical feature of the Galician coast mainly during the late spring and summer (Fraga, 1981), as a consequence of prevailing northerly winds in this area of the NW Iberian Peninsula. On the northern Galician coast there were remarkably favorable conditions for upwelling in July and August 2008, although the upwelled seawater did not penetrate into the Ria of Barqueiro (Fig. 2). In this area, the upwelling remained outside the rias (Ospina-Alvarez et al., 2010), affecting only the neighboring shelf as forecasted by Prego and Bao (1997). Therefore, the summer stratification period presented a depletion of nitrate

and ammonium concentrations (Table 1 and Fig. 3). The absence of summer upwelling within the Ria explains the low phytoplankton biomass and abundances (Table 2 and Figs. 5 and 7). Several species of *Chaetoceros*, *Leptocylindrus*, *Pseudonitzschia*, *Thalassiosira* and *Rhizosolenia* were characteristic of this summer period (Fig. 6). The contribution of diatoms to total phytoplankton biomass was about 30%, similar to that of flagellates, and lower than that of dinoflagellates, which represented almost 50% of the biomass.

The most striking characteristic of the autumn maximum is the lack of a sign in the chlorophyll-a (Fig. 4) in spite of increased abundances (Fig. 5 and Table 2), which is exactly the opposite when compared with the spring bloom. When observed under a microscope, the autumn phytoplankton exhibited a poor physiological condition. Cells were smaller than those usually observed in other studies in Galicia (Varela et al., 2008, 2010). On the other hand, the diatom frustules appeared to be deformed, weakly silicified and with a reduced Chlorophyll-a content as well. All of the above indicates that growth is limited by some nutrients (Fig. 3). This is supported by the stoichiometric nutrient limitation criterion established by Justic et al. (1995). Nutrient ratios show nitrogen as being the limiting factor for phytoplankton growth in October (Figs. 3-5), which would explain the poor condition of the cells. In fact, nitrogen limitation existed from late spring to autumn, even though it was less-evident under the microscope as in October. Despite this, most diatom species from spring to autumn were small in size and had a degraded appearance. This supports the idea of nutrient limitation for diatoms, compared to other smaller-sized phytoplankters which are more efficient in nutrient uptake, and the persistent presence of dinoflagellates (Fig. 5 and Table 2), with most species having the ability to carry out mixotrophic nutrition (Sanders and Porter, 1988; Moorthi et al., 2009). In early November the water mixing had been completed (Fig. 2).

Therefore, the annual cycle in the Ria of Barqueiro (Fig. 6) only consisted of a spring bloom and an autumn bloom, during the transition periods of mixing-stratification (spring) and stratification-mixing (autumn). This cycle resembles that of the cycle of temperate areas (Margalef, 1978; Smayda, 1980; Harris, 1986). Chlorophyll-a concentrations barely exceeded 2 mg L^{-1} , only in the deep water column in spring, and did not reach 1 mg L^{-1} in autumn (Fig. 4).

4.2. Comparison of the Barqueiro Ria with the upwelling rias

The Ria of Barqueiro was characterized by a low concentration of nutrients (Table 1) with values in the range of those reported for unpolluted coastal areas (Ketchum, 1969). In general, chlorophyll and primary production were linked to

the mixing-stratification cycle of the water column, mainly governed by temperature. The role of the river is of limited relevance, as indicated by the salinity profiles and nutrient concentrations at the station studied (Figs. 2 and 3). However, some influence must exist since the nutrients were never completely depleted even in the shallow, stratified surface layer. The possibility of a nutrient supply from low turbulence caused by wind, together with small fluvial contributions during summer cannot be completely discounted. This supply would allow for diatom growth, even with levels that were sufficiently low to cause diatom stress by nutrient limitation. Nevertheless, this nutrient limitation favors a relatively high contribution of dinoflagellates and flagellates in the phytoplankton community (Table 2). Nogueira et al. (2000) described a similar community as being responsible for non-upwelling late-summer and autumn blooms in the Ria of Vigo.

During the most productive periods - spring and autumn - phytoplankton in the Barqueiro Ria was typically constituted by chain-forming, but made up of small individual cell-size diatom species, such as *Chaetoceros socialis*), *Pseudo-nitzschia* group *delicatissima* and *Leptocylindrus minimus*. However, most diatom species, such as *Leptocylindrus danicus*, *Lauderia annulata*, *Detonula pumila*, *Guinardia delicatula* or *Asterionellopsis glacialis*, and the dinoflagellates *Heterocapsa niei* and *Scrippsiella trochoidea*, also reached high abundances in spring (Tables 2 and 3). These species may be considered ruderal or disturbance-tolerant and are typical of situations where disturbances were moderate and allowed for phytoplankton growth (Nogueira et al., 2000). Their persistence in the community for most of the annual cycle (Table 2) indicates that disturbances are frequent throughout the year. In the Atlantic Galician rias (Bode et al., 2005a,b), the ruderal phytoplankton species would benefit from the influence of upwelling directly or through the shelf water interchange to cause summer upwelling blooms. In contrast, in the Barqueiro Ria, despite the presence of upwelling events in the neighboring shelf during summer (Ospina- Alvarez et al., 2010), the upwelling did not supply nutrients into the Ria (Fig. 3). Nutrient concentrations in Barqueiro seawaters (up to 6.2 mM of nitrate, 0.4 mM of phosphate and 5.7 mM of silicate), were much lower than in Atlantic rias (up to 12 mM of nitrate, 1.0 mM of phosphate and 9.0 mM of silicate; Prego et al., 1995, 1999; Varela et al., 2008; Doval et al., 2013). So, ruderal species did not bloom in the Ria of Barqueiro but DOC and DON concentrations (with maximum of 98 and 14 mM, respectively) were in the order of the upwelling rias during summer (140 mM of DOC and 17 mM of DON; Prego, 1993, 2002; Doval et al., 1997, 2013). By contrast, particule organic matter increased considerably, from a maximum of 16 mM of POC and 2mM of PON in the Barqueiro Ria to 60 and 9 mM of POC and PON, respectively, in the Atlantic rias (Doval et al., 1997).

Studies carried out on the Galician coast (Casas et al., 1999; Varela et al.,

2001; Varela and Prego, 2003; Varela et al., 2005, 2008) reported a high abundance of small naked flagellates and their presence throughout the year. This is also one of the most notable aspects of the phytoplankton of the Ria of Barqueiro found in 2008. The average values of flagellates were approximately $1100 \text{ cells mL}^{-1}$, accounting for 85% of the total phytoplankton abundance in the Ria while diatoms and Dinoflagellates, represented only 12 and 3% respectively (Table 4). In oligotrophic ecosystems, small phytoplankton has increased in importance (Malone, 1980; Kosaki et al., 1985; Bode et al., 1994a,b). The abundance of flagellates seems to highlight the oligo-mesotrophic character of the Ria of Barqueiro as the contribution of flagellates is greater e 85% e than in the more productive upwelling (Atlantic) rias, where this small component accounts for approx. 65% of phytoplankton abundance (Table 4).

The larger specimens of phytoplankton provide more information since microscope observation makes it possible to recognize different indicator groups and species. When comparing the Ria of Barqueiro (Cantabrian) with the upwelling rias (Atlantic), the average number of taxa in the Galician rias was about 150 per ria, less than 40% of which corresponded to dinoflagellates and over 50% to diatoms (Varela et al., 2001, 2005, 2008; Bode et al., 2005a). The Ria of Barqueiro showed a similar number of taxa, although the contribution of dinoflagellates to total phytoplankton taxa was higher than in the Atlantic rias, and equal to that of diatoms (nearly 50% for both groups). This would suggest a relatively higher contribution of the former, and would therefore indicate a less productive ecosystem. Nevertheless, the oligo-mesotrophic character of the Ria of Barqueiro is more consistently supported by the absolute and relative abundances and biomasses of both groups which, as in the case of flagellates, confirm the low productivity of the Ria of Barqueiro in comparison to the upwelling rias (Table 3). Densities of dinoflagellates are lower in the Ria of Barqueiro, but the relative contribution accounts for 3% versus only 1% in the upwelling rias (Table 4), while the contribution of this group to the total phytoplankton carbon in the Ria of Barqueiro is about 30% compared to 15% of other rias. Given that dinoflagellates as a group are considered to be indicators of low productivity, the higher contribution of this group to the abundance and biomass of phytoplankton indicate the low productivity of the Ria of Barqueiro.

Diatoms are the group with the most notable differences when comparing both types of rias. Mean annual abundances in the Ria of Barqueiro are about $100 \text{ cells mL}^{-1}$, while in the Atlantic these values reach almost $3000 \text{ cells mL}^{-1}$ (Table 3). However, these values do not give a clear idea of the real differences in abundances between both groups of rias. In the Ria of Barqueiro during the most productive period, spring, the higher punctual densities were displayed by diatoms *Pseudo-nitzschia* group *delicatissima* with $400 \text{ cells mL}^{-1}$ followed by *Leptocylindrus*

danicus, *Chaetoceros socialis* and *Lauderia annulata* with 350, 170 and 130 cells mL⁻¹, respectively (Table 2). These densities may not be considered blooms; rather they are a moderately high concentration of phytoplankton, at least in the context of what is known on phytoplankton abundances in Galician rias (Varela et al., 2001, 2005). Densities over 10,000 cells mL⁻¹ of *C. socialis* and *L. danicus* have been reported for the Atlantic rias of Ferrol and Coruña (Varela et al., 2001; Varela and Prego, 2003; Bode et al., 2005b) respectively. More than 8000 cells mL⁻¹ of *Skeletonema costatum* were also observed in the Atlantic Rias of Laxe and Pontevedra (Varela et al., 2005, 2008). Between 1000 and 3000 cells mL⁻¹, were also reported for the same species in the Rias of Ferrol and Coruña. The diatom *S. costatum*, a frequent blooming species in Galician rias, showed negligible abundances in the Ria of Barqueiro. This species is usually linked to low salinity during late winter and early spring (Varela and Prego, 2003). The scarce continental supply could explain this absence, which may be related to the lower availability of nutrients in early spring in Barqueiro as compared to the Atlantic rias (8 mM of nitrate at the end of winter mixing versus 4 mM in Barqueiro).

Diatom biomass is clearly lower in the Ria of Barqueiro and the contribution of this group to phytoplankton biomass is higher in the upwelling rias (60% versus 40%, Table 4), highlighting the oligomeso-trophic character of the Ria of Barqueiro. This classification agrees with the trophic classification for marine systems, which have been generally associated with the concept defined by Nixon (1995), and is based on the supply of organic carbon. Following this simple approach and using the primary production as an indicator, the Ria of Barqueiro corresponds to a mesotrophic system with a primary production of 280 gC m⁻² y⁻¹. In contrast (Fig. 7), the Atlantic rias of Laxe (1250 gC m⁻² y⁻¹; Varela et al., 2005) and Pontevedra (600 gC m⁻² y⁻¹; Varela et al., 2008), correspond to hypertrophic systems.

5. Conclusions

Seasonal variations of hydrographical conditions in the Cantabrian Galician ria of Barqueiro have shown an annual cycle that is biogeochemically and phytoplanktonically very different from the typical cycle of the Atlantic Galician rias, where the influence of upwelling is noticeable. The absence of upwelling events inside the Cantabrian Ria of Barqueiro together with a low continental supply in spring causes a low input and nutrient availability for the phytoplankton bloom. Therefore, the phytoplankton assemblages over an annual period, with a (moderate) spring bloom and low biomass the rest of year, bear a greater resemblance to those common to the temperate zones. This pattern is

completely different from the highly productive Atlantic rias according to the information supplied by the phytoplanktonic component, abundances, biomasses, and relative contributions of different groups in the Ria of Barqueiro. Hence this Ria exemplified an oligo-mesotrophic ria-type, which had not been previously studied and described, in contrast to the paradigm of natural eutrophication of the Galician rias, following the well-known (Atlantic) upwelling ria-type of Galicia.

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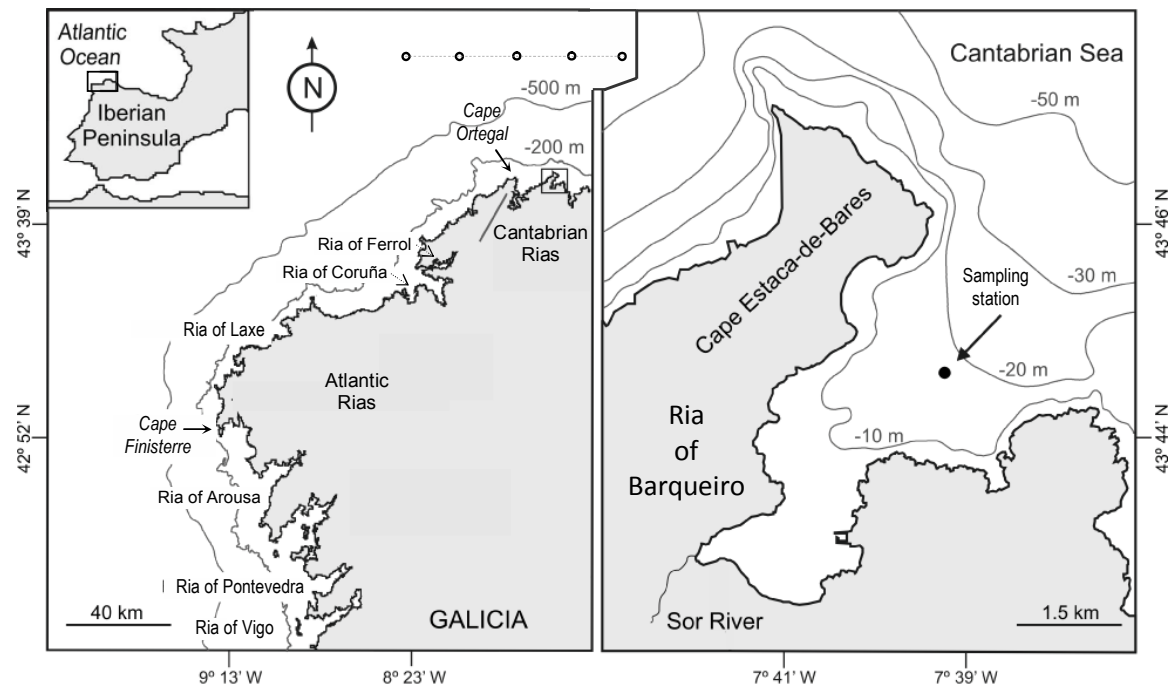


Figure 1. Map and bathymetry of the Galician Cantabrian Ria of Barqueiro and the six main Atlantic Galician rias in the Northwest of the Iberian Peninsula. Black circle on the right frame shows the sampling station sampled monthly in January 2008-09. Five empty circles in the right corner of Galician map correspond to the control points for upwelling index calculation.

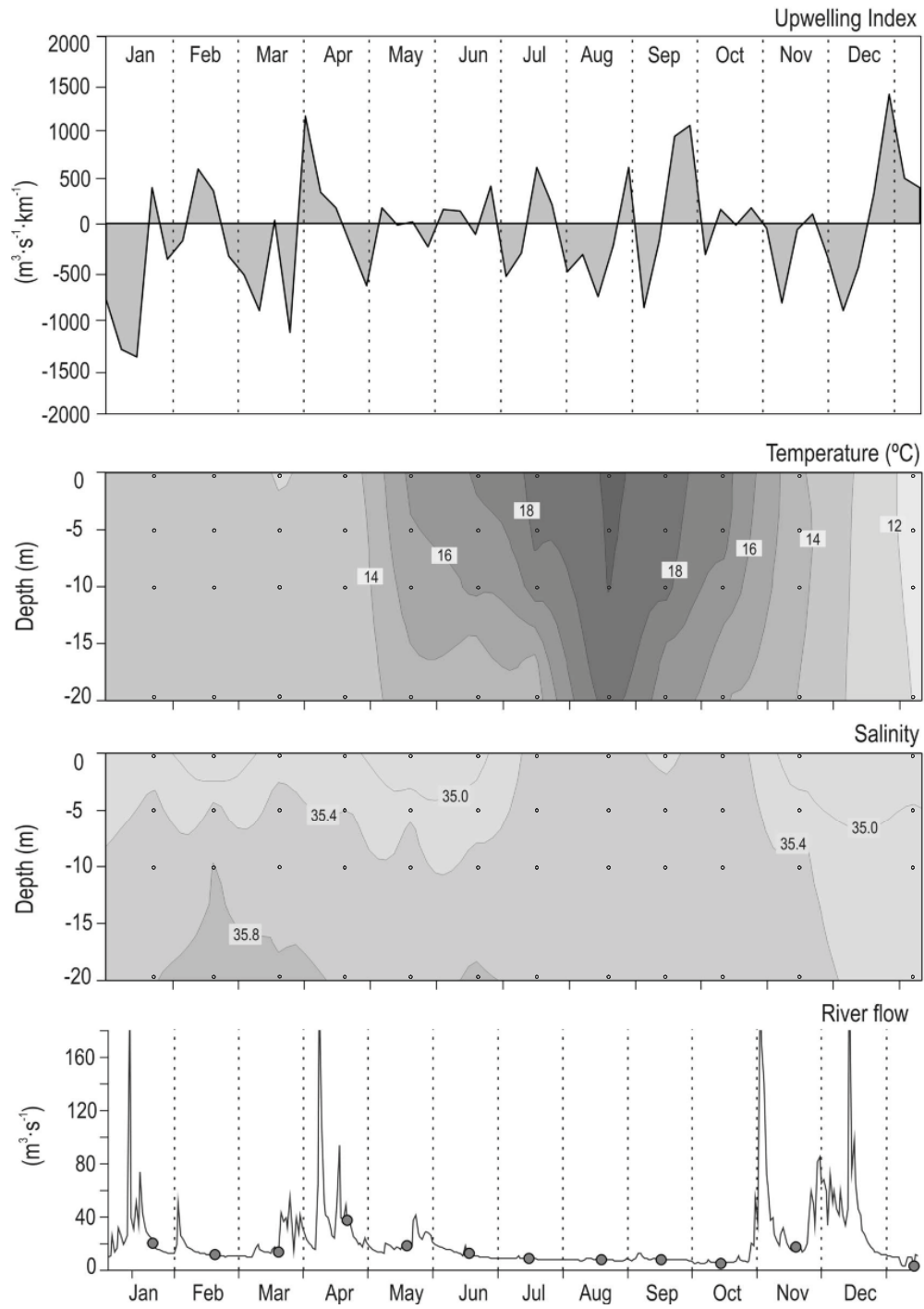


Figure 2. Temporal evolution of the Upwelling Index ($\text{m}^3 \cdot \text{s}^{-1} \cdot \text{km}^{-1}$) for the Cantabrian Galician rias (UI_N), water column temperature and salinity in the Ria of Barqueiro, and the Sor River flow ($\text{m}^3 \cdot \text{s}^{-1}$) during the annual study period. Gray dots correspond to the sampling dates.

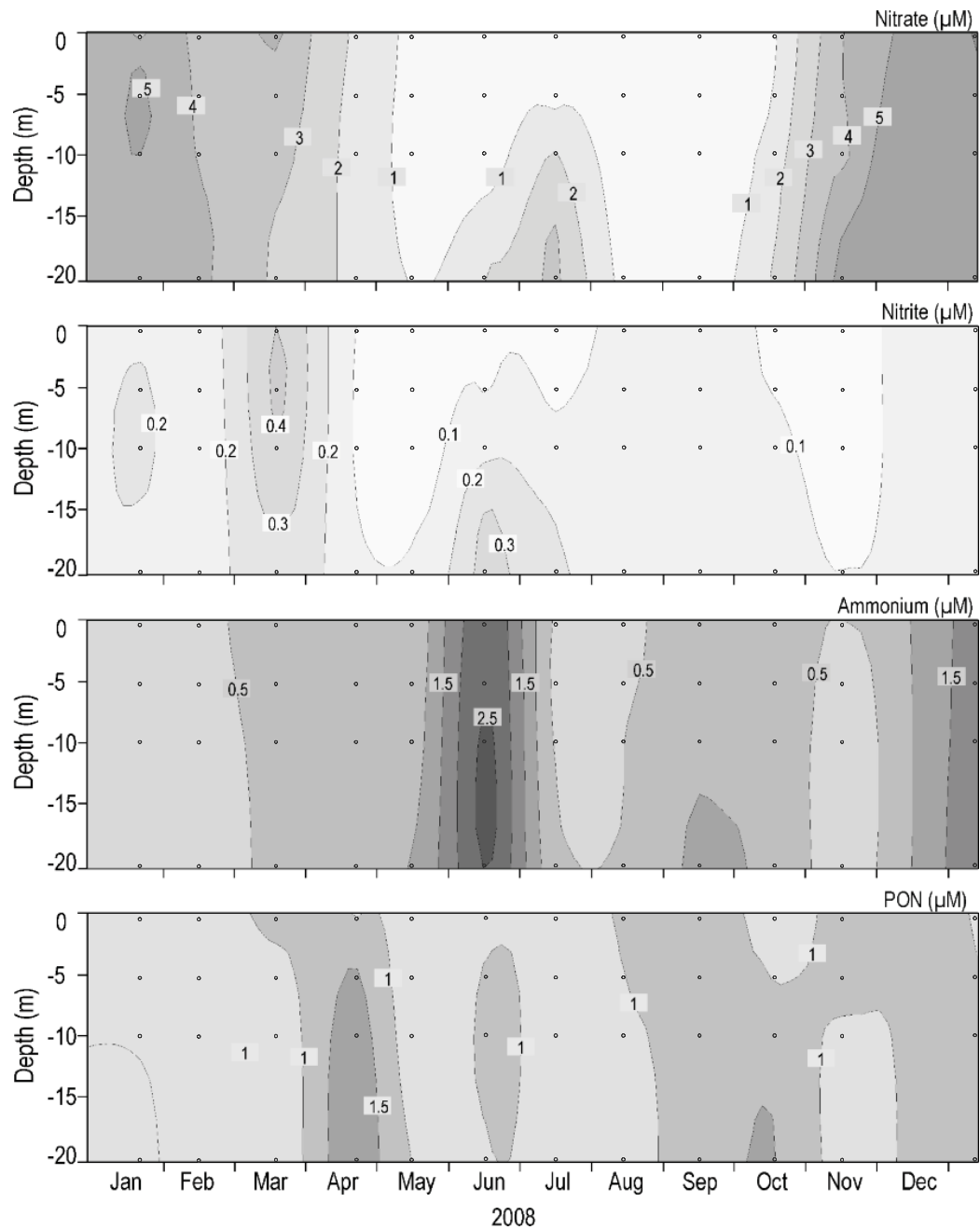


Figure 3. Contour plots of nitrate, nitrite, ammonium, and particulate organic nitrogen (PON) concentrations in the Ria of Barqueiro during the annual study period. All values are expressed in μM .

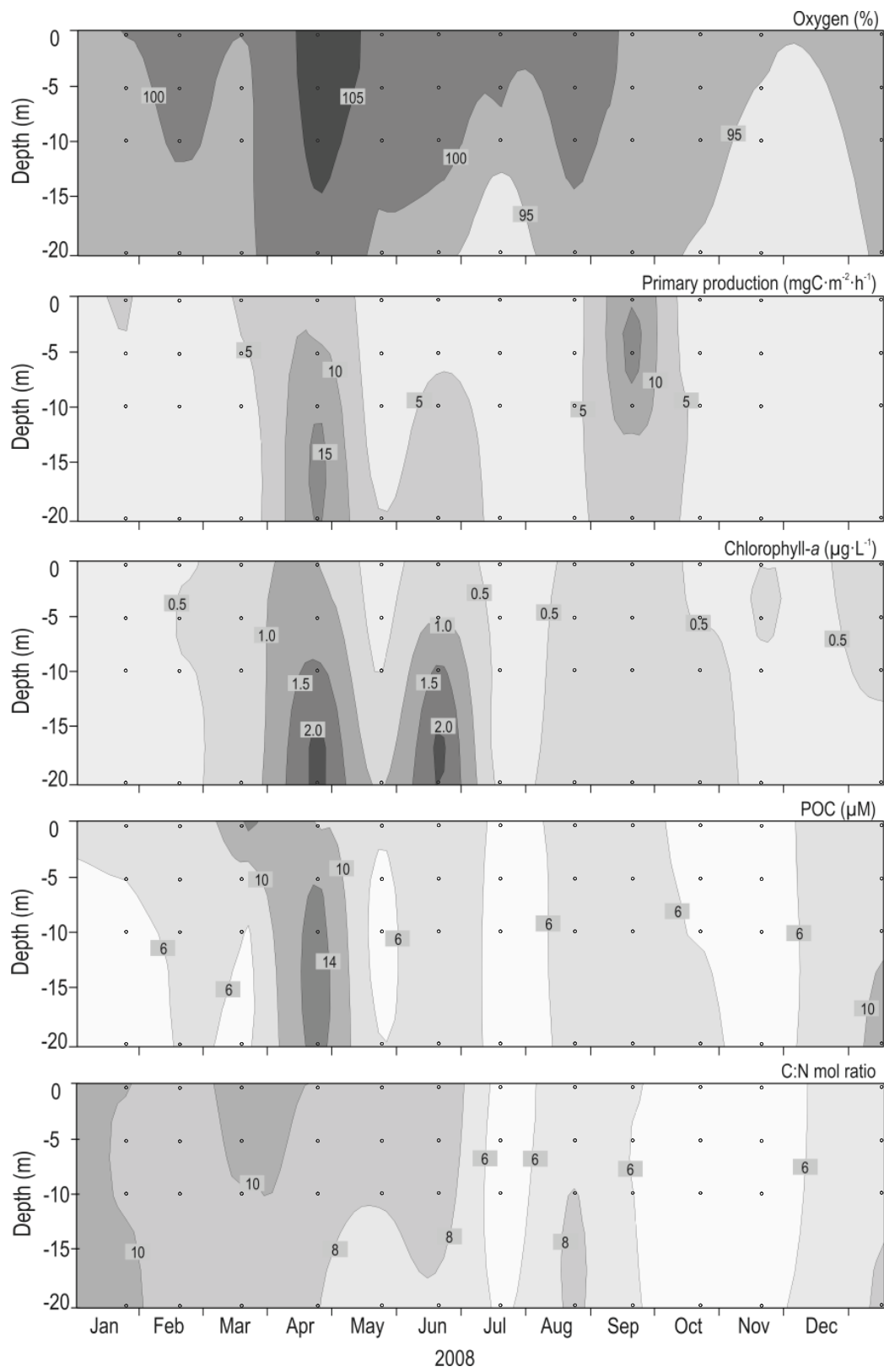


Figure 4. Contour plots of oxygen saturation (%), primary production (mgC·m⁻²·h⁻¹), chlorophyll-a (µg·L⁻¹) and particulate organic carbon (POC) concentrations (µM), and C/N mol ratio in the Ria of Barqueiro during the annual study period.

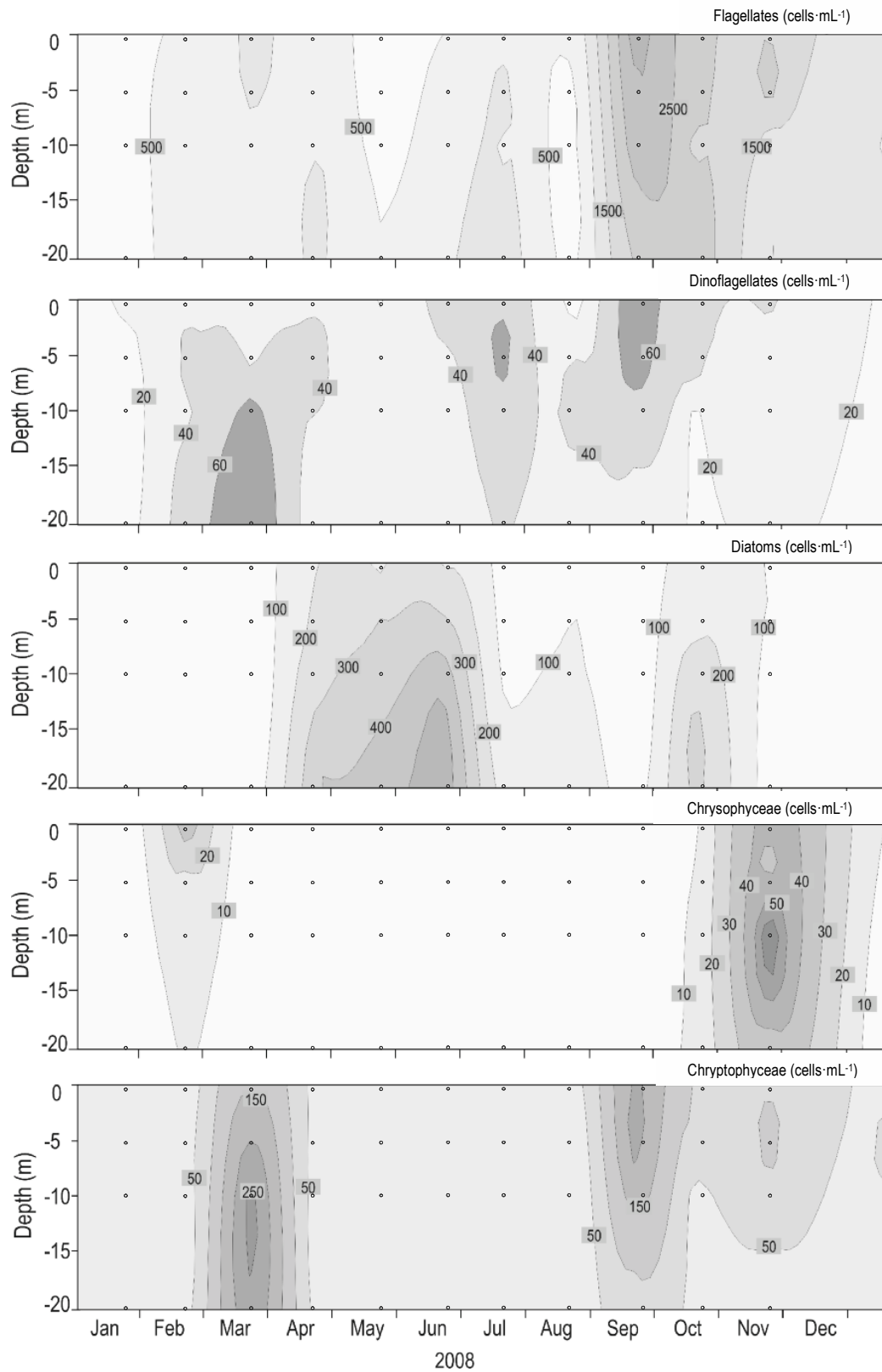


Figure 5. Contour plots of phytoplankton abundances (cells·mL⁻¹) in the Ria of Barqueiro during the annual study period.

Discriminant Functions

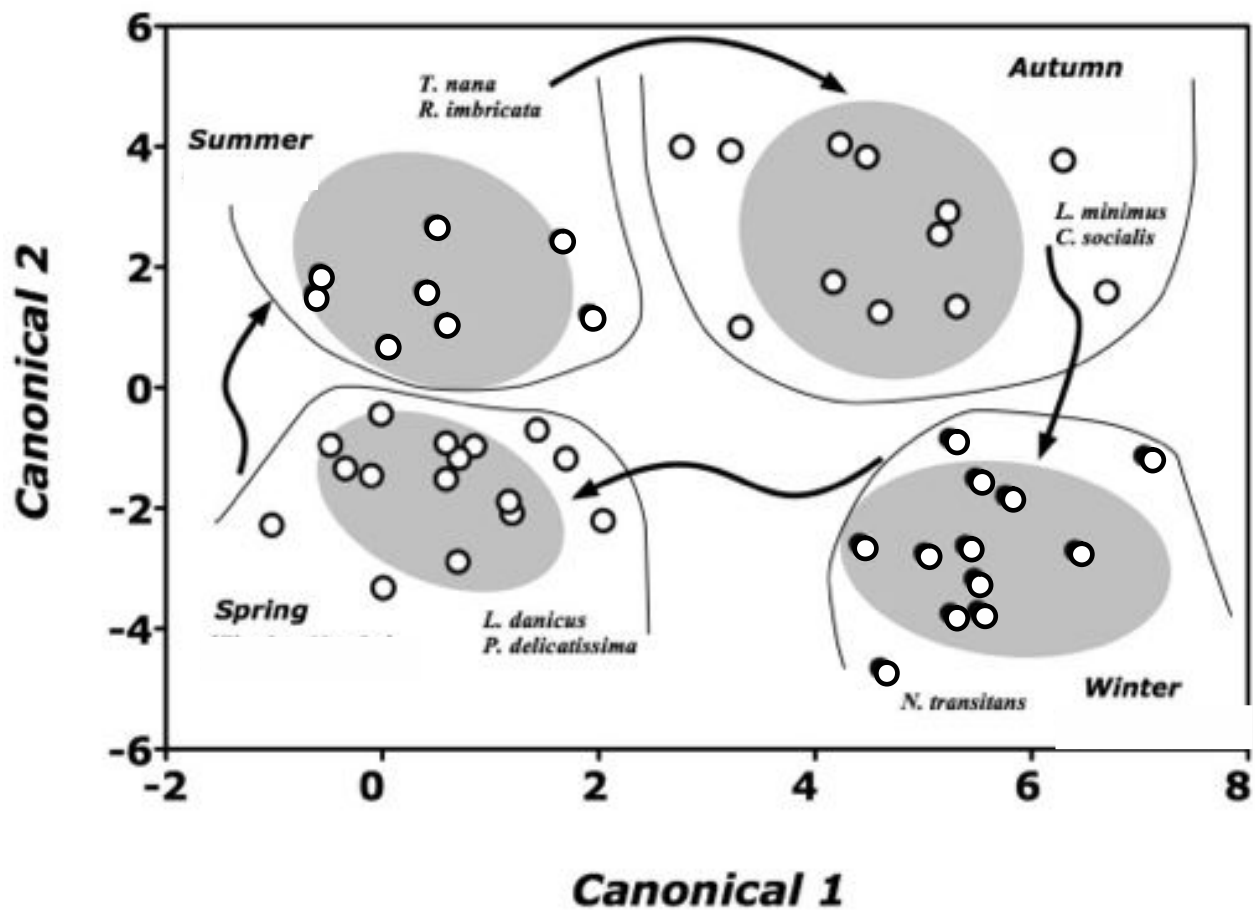


Figure 6. Projection of monthly samples (empty circles) on the first two discriminant (canonical) functions. For each oceanographic period the main phytoplankton species are given for the Ria of Barqueiro. Arrows show the evolution of annual succession. Ellipses represent a 95% confidence region to contain the true mean of the group.

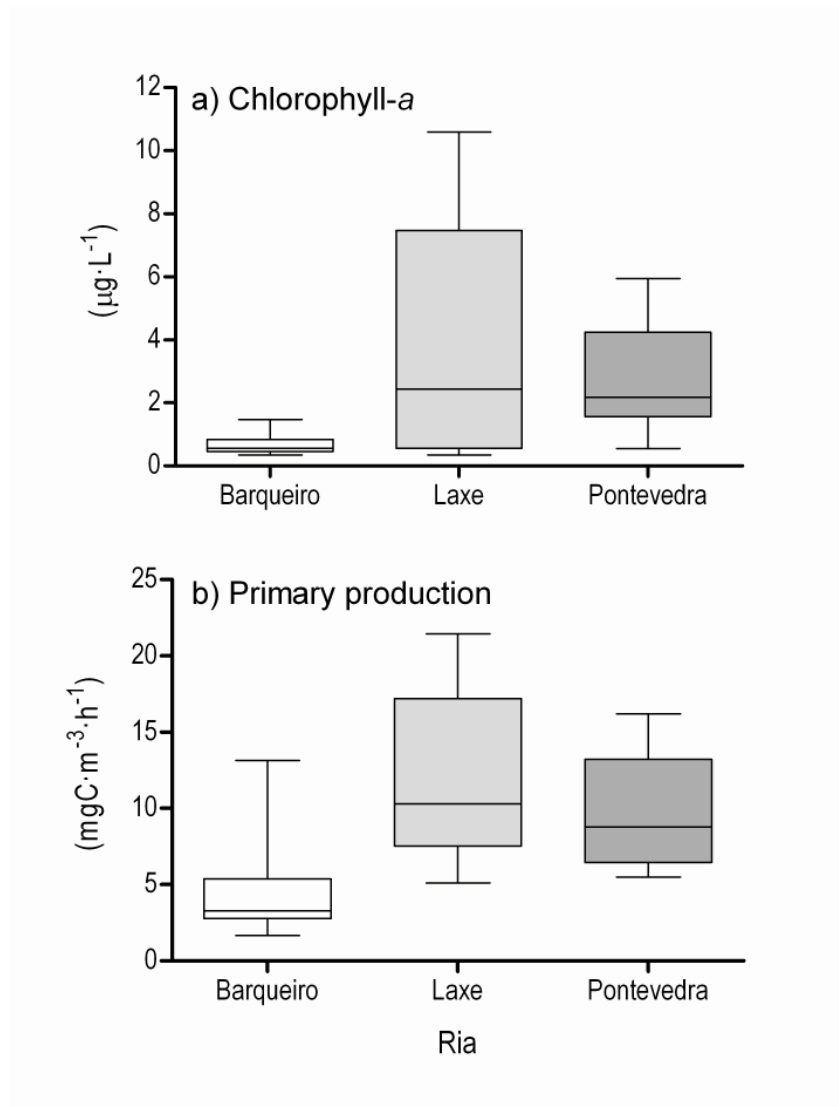


Figure 7. Box and whisker plots of Chlorophyll-*a* (a) and primary production (b) in the Ria of Barqueiro from the present study in comparison with the Atlantic Galician Rias of Laxe (Varela et al., 2005) and Pontevedra (Varela et al., 2008). Horizontal lines correspond to the median and the edges of the box on the 25th and 75th percentiles respectively.

Table 1. Physical, chemical and biological parameters of the water column found in the oceanographic periods identified in the Ria of Barqueiro during the annual study period. Values are given as the mean \pm standard deviation. Significant differences (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, one-way ANOVA) between oceanographic periods are shown for each variable.

Parameter	Oceanographic period				Unit
	Spring (month: 4, 5, 6)	Summer Stratification (7, 8, 9)	Autumn (10, 11)	Winter (1, 2, 3, 12)	
Salinity	35.3 \pm 0.4	35.6 \pm 0.1	35.4 \pm 0.5	35.4 \pm 0.5	-
Temperature **	15.0 \pm 1.5	18.0 \pm 1.2	15.3 \pm 1.5	13.0 \pm 0.8	°C
Oxygen saturation **	103.9 \pm 3.11	99.0 \pm 3.1	95.8 \pm 3.0	99.0 \pm 1.7	%
Nitrate **	0.96 \pm 0.77	0.56 \pm 0.77	2.77 \pm 2.05	4.48 \pm 1.10	μ M
Nitrite **	0.10 \pm 0.09	0.16 \pm 0.04	0.10 \pm 0.03	0.22 \pm 0.10	μ M
Ammonium *	1.44 \pm 0.85	0.59 \pm 0.22	0.59 \pm 0.37	0.74 \pm 0.68	μ M
Phosphate **	0.09 \pm 0.09	0.10 \pm 0.05	0.22 \pm 0.05	0.27 \pm 0.11	μ M
Silicate *	1.72 \pm 0.94	2.09 \pm 0.71	3.27 \pm 1.18	2.47 \pm 0.72	μ M
DOC***	83 \pm 8	80 \pm 8	64 \pm 5	72 \pm 10	μ M
DON***	5.3 \pm 1.1	9.6 \pm 4.2	6.3 \pm 2.3	4.3 \pm 1.4	μ M
Chlorophyll-a *	1.07 \pm 0.68	0.68 \pm 0.15	0.52 \pm 0.15	0.56 \pm 0.19	μ g·L ⁻¹
Primary production *	1.3 \pm 0.9	8.2 \pm 3.1	0.2 \pm 0.1	0.3 \pm 0.2	(gC·m ⁻² ·d ⁻¹)

Table 2. Characteristic phytoplankton species and their abundances (cells·mL⁻¹) identified for the oceanographic periods in the Ria of Barqueiro.

Group / Species	Oceanographic Period			
	Spring	Summer	Autumn	Winter
Dinophyceae				
<i>Amphidinium flagellans</i>	0.00	1.13	0.00	0.78
<i>Ceratium furca</i>	0.05	0.22	0.00	0.00
Dinoflagellates < 30 µm	17.85	19.98	30.60	12.54
Dinoflagellates > 30 µm	1.00	1.81	1.00	0.46
<i>Gyrodinium acuminata</i>	0.54	0.00	0.54	0.33
<i>Heterocapsa niei</i>	4.82	3.49	6.52	7.63
<i>Katodinium glaucum</i>	2.40	1.05	0.70	0.00
<i>Lingulodinium polyedra</i>	0.00	5.69	0.00	0.03
<i>Prorocentrum balticum</i>	2.03	0.43	0.73	0.11
<i>Prorocentrum minimum</i>	0.43	2.05	0.00	2.43
<i>Protoperidinium bipes</i>	0.43	0.00	0.17	0.13
<i>Scrippsiella trochoidea</i>	1.85	1.71	0.66	1.27
Bacillariophyceae				
<i>Asterionellopsis glacialis</i>	13.00	0.00	0.04	0.09
<i>Asteromphalus sarcofagus</i>	0.00	0.00	0.58	0.03
<i>Chaetoceros spp (total)</i>	28.88	14.81	38.71	6.99
<i>Cocconeis spp.</i>	0.00	4.61	0.00	0.30
<i>Corethron pelagicum</i>	0.08	0.21	0.58	0.49
<i>Detonula pumila</i>	3.68	0.85	0.13	0.00
Diatoms (Centric) < 30 µm	2.42	0.21	4.54	1.91
Diatoms (Pennate) < 30 µm	1.28	7.23	8.68	2.04
Diatoms (Pennate) > 30 µm	0.63	1.70	1.80	0.98
<i>Guinardia delicatula</i>	15.00	3.87	3.82	0.00
<i>Lauderia annulata</i>	36.17	0.00	1.79	0.35
<i>Leptocylindrus danicus</i>	94.38	1.51	9.69	0.39
<i>Leptocylindrus minimus</i>	1.70	2.88	16.80	0.00
<i>Navicula transitans</i>	0.21	0.34	0.42	1.78
<i>Nitzschia longissima</i> (large)	0.50	4.43	3.17	2.30
<i>Nitzschia longissima</i> (small)	0.85	0.73	4.39	4.50
<i>Pseudo-nitzschia</i> group <i>delicatissima</i>	106.52	4.85	1.25	0.91
<i>Pseudo-nitzschia</i> group <i>pungens</i>	3.67	1.44	1.69	0.23
<i>Rhizosolenia imbricata</i>	0.17	2.89	0.21	0.04
<i>Rhizosolenia setigera</i>	0.29	0.62	0.58	0.93
<i>Thalassionema nitzschioides</i>	0.71	0.09	2.18	1.81
<i>Thalassiosira angulata</i>	0.96	4.36	1.33	0.69
<i>Thalassiosira levanderi</i>	4.96	1.21	1.37	4.54
Cryptophyceae				
<i>Cryptomonas, Rhodomonas & Chroomonas</i>	13.83	9.99	106.53	79.90
Chrysophyceae				
<i>Distephanus speculum</i>	0.21	0.00	0.17	5.03
<i>Solenicola setigera</i>	0.00	0.38	18.11	0.00
Flagellates				
Small flagellates (2-20 µm)	407.85	412.26	1417.77	512.45

Table 3. Abundances and corresponding biomass of species forming blooms in the Ria of Barqueiro (Cantabrian Galician Ria) and the six main Atlantic Galician Rias, i.e Ferrol (Bode et al. 2005), Coruña (Varela et al., 2001; Varela and Prego, 2003), Laxe (Varela et al., 2005), Pontevedra (Varela et al., 2008), Arousa (Varela et al., 2005) and Vigo (unpublished data).

Group & specie / Rias	Abundance (cells·mL ⁻¹)		Biomass (µgC·L ⁻¹)	
	Barqueiro	Atlantic	Barqueiro	Atlantic
Dinophyceae				
<i>Hetercapsa niei</i>	17	129	1	8
<i>Scrippsiella trochoidea</i>	5	32	3	16
Diatoms				
<i>Asterionellopsis glacialis</i>	23	169	2	17
<i>Chaetoceros</i> spp.	77	6671	12	1026
<i>Detonula pumila</i>	20	97	5	23
<i>Guinardia delicatula</i>	60	100	17	29
<i>Lauderia annulata</i>	51	42	62	95
<i>Leptocylindrus danicus</i>	119	3552	8	241
<i>Pseudonitzschia</i> spp. (all species)	129	847	13	85
<i>Skeletonema costatum</i>	3	3344	0	86
<i>Thalassiosira</i> spp.	12	491	8	339
Flagellates	110	4807	97	192

