

ORIGINAL RESEARCH ARTICLE

# Annual cycle of phytoplankton community through the water column: Study applied to the implementation of bivalve offshore aquaculture in the southeastern Bay of Biscay

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Environmental variables

**Abstract** This study describes, for the first time, the annual variability of phytoplankton community in different layers of the water column in open waters off the Basque coast (southeastern Bay of Biscay). Phytoplankton composition, abundance and biomass, together with size-fractionated chlorophyll *a*, nutrients, and optical and hydrographic conditions were measured in an experimental bivalve culture area from May 2014 to June 2015. Water column conditions showed the typical dynamics previously described for temperate areas, characterised by winter homogeneity and summer stratification. Phytoplankton temporal variability was studied at depths of 3, 17 and 33 m, and was found to be related to those processes. In particular, temperature and nutrients (mostly nitrate and silicate) were the environmental variables which significantly explained most of the variability of chlorophyll concentration, whereas river flow was the main driver of abundance variability. Total chlorophyll was generally low ( $0.6 \mu\text{g L}^{-1}$  on average). Of the 194 registered taxa, 47.4% belonged to dinoflagellates and 35.1% to diatoms. In addition, diatoms showed the highest biomass values, and haptophytes represented the greatest

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contribution to cell-abundance. This fact, despite the low chlorophyll values indicating low phytoplankton biomass, could favour mussel growth given the high fatty acid content reported for diatoms and haptophytes.

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## 1. Introduction

Phytoplankton constitute an important component of the diet of suspension feeding bivalves (Grant, 1996; MacDonald and Ward, 1994; Petersen et al., 2008; Shumway and Cucci, 1987). In fact, microalgae have long been used as food resource for mollusc bivalves at all growth stages (Brown, 2002). This interaction of mollusc bivalves with phytoplankton as a food source has been studied extensively. For instance, it is known that the quantity and size of the phytoplankton can influence the recruitment of oysters, as well as the survival of bivalve larvae (Bourlès et al., 2009; Robert and Trintignac, 1997). Moreover, phytoplankton blooms have been directly related to the increase of mussel growth and condition index (*i.e.*, the ratio between the dry weight of the meat and the shell) (Blanton et al., 1987; Hickman et al., 1991; van der Veer, 1989). However, not all phytoplankton species are equal in terms of nutritional quality for bivalves. Several bivalves (including mussels) have shown a preferential utilisation of phytoplankton species which depends on both their food value and cell size (Cucci et al., 1985; Kiørboe and Møhlenberg, 1981; Møhlenberg and Riisgård, 1978; Rouillon and Navarro, 2003). In this sense, lipids are the main source of energy for larvae and lipid content of phytoplankton varies depending on the species or group (Marshall et al., 2010; Volkman et al., 1991, 1989). Feeding experiments on *Mytilus galloprovincialis* carried out by Petterson et al. (2010) showed that alterations in phytoplankton species composition can produce variations in mortality and settlement rates. Also, in field studies, Wall et al. (2013) found that the growth rates of bivalves were more related to the density of certain cellular types than to the total phytoplankton biomass. Therefore, the study of phytoplankton community composition is essential from the standpoint of bivalve nutrition in shellfish production areas.

Currently, there is an increasing interest in developing offshore aquaculture in regions where sheltered coastal areas are scarce or sustain activities incompatible with aquaculture (Azpeitia et al., 2016). This interest prompted the installation of an experimental bivalve farm in open waters off the Basque coast (southeastern Bay of Biscay). However, temporal variability of phytoplankton nutritional attributes and their relationships with environmental conditions needed further investigation. It is widely recognised that both top-down regulation, such as grazing (Burkill et al., 1987), and bottom-up processes driven by meteorological and hydrographic factors play a major role in the control and dynamics of phytoplankton populations (Nogueira et al., 2000; Smayda, 1998).

The Bay of Biscay is located at mid-latitude of the North-east Atlantic Ocean and thus, here, the annual cycle corresponds to that of temperate sea areas. Winter is characterised by water column mixing, which is generated by a combination of cooling, turbulence and downwelling. This mixing process modifies the properties of the upper waters and leads to great nutrient input from deep waters to the surface. In spring, solar irradiance heats the surface resulting in an increase in the temperature of these waters and a relative stabilisation. However, the stratification of the water column depends also on the relaxation of wind, turbulence and downwelling. Summer is characterised by stratification resulting from greater solar irradiance. Finally, during autumn the surface waters cool down and the southerly and westerly winds prevail, resulting in the mixing of the water column (Fontán et al., 2008; Valencia et al., 2004).

Many studies worldwide have highlighted the seasonal periodicity of phytoplankton assemblages linked to seasonal variations in physical forcing of mixing dynamics, temperature and light regime (Agirbas et al., 2017; Diehl, 2002; Diehl et al., 2002; Leterme et al., 2014; Vajravelu et al., 2017). In the Bay of Biscay in particular, according to the seasonal cycle of hydrographic conditions, phytoplankton biomass shows two main periods related to two main events: winter mixing and summer stratification (Valdés and Moral, 1998; Varela, 1996). On the one hand, the nutrient input caused by the winter mixing leads to favourable conditions for the proliferation of the phytoplankton community and, thus, biomass peaks are usually recorded during late winter and spring. On the other hand, heating of the surface waters during summer leads to a stratified water column. The thermocline acts as a physical barrier that prevents the supply of nutrients, and phytoplankton production and biomass show the lowest values (Calvo-Díaz et al., 2008; Fernández and Bode, 1991; Varela, 1996).

Although previous studies on phytoplankton communities had been carried out in the southern Bay of Biscay (*e.g.*, Bode and Fernández, 1992; Fernández and Bode, 1994; Varela, 1996) and, in particular, in open waters off the Basque coast (Estrada, 1982; Garmendia et al., 2011; Muñiz et al., 2017), further research was needed. The relevance of the present study is based on the inclusion of novel issues, such as the importance of phytoplankton community composition as a food resource for bivalves in waters off the Basque coast, which was not addressed before, and the variability through the water column, since most of the previous studies were limited to surface waters.

In this context, our study aims to characterize phytoplankton composition and annual variability within an experimental aquaculture farm, in relation with the good growth rates observed in mussels. Recent studies developed in that experi-

mental site indicate that mussels present good growth rates, biometry and nutritional quality (Azpeitia et al., 2016, 2017). Although chlorophyll values in the area are known to be relatively low (Estrada, 1982; Garmendia et al., 2011; Revilla et al., 2009), we hypothesise that the composition and contribution of the different major taxonomic groups could be favourable for bivalve growth. To this end, we examined phytoplankton community composition, abundance and biomass, as well as environmental conditions, through the whole water column from May 2014 to June 2015. Since the period of study covered more than one year, a complete seasonal cycle was investigated.

## 2. Material and methods

### 2.1. Study area

The Basque coast extends 100 km along the Cantabrian Sea (southeastern Bay of Biscay) (Fig. 1). The climate of the area is rainy, temperate and oceanic, with moderate winters and warm summers (Fontán et al., 2009). The Basque coast can be described as a littoral coast exposed to waves, mostly formed of cliffs and influenced by 12 short rivers. Although no large coastal plumes are formed (Diez et al., 2000), this freshwater supply modifies the chemical composition of the shelf waters and often leads to increased nutrient levels in inner shelf waters (Ferrer et al., 2009; Valencia et al., 2004).

Field samplings were carried out at a station (43°21,411'N; 2°26,918'W) immediately outside an experimental bivalve farm located at 2 nautical miles off the Basque coast, at a depth of approximately 45 m. The experimental farm used a longline system, based on a subsurface structure, from which bivalve ropes and lanterns were suspended. In particular, the installation consisted of three long lines, occupying a total area of 1 ha. Each longline sustained 100 vertical hanging ropes. The organisms cultured at the farm during the study were mainly mussels (*Mytilus galloprovincialis*) and, to a lesser extent, oysters (*Crassostrea gigas* and *Ostrea edulis*).

### 2.2. Sampling/laboratory strategy and data acquisition

Samplings took place from May 2014 to June 2015. CTD (conductivity, temperature and depth device) casts and

Secchi disk measurements were usually performed twice per month, whereas water samples were collected monthly, except for February when sampling could not be carried out due to meteorological conditions.

In the field, a Seabird25 CTD was employed for the measurement of temperature, salinity, chlorophyll *a* and photosynthetically active radiation (PAR) at every meter of the water column. The Secchi disk depth was measured as an indicator of the water transparency. Water samples were collected using Niskin bottles at six discrete depths through the water column: 3, 10, 17, 24, 33 and 42 m.

Water samples were used for the analysis of nutrients and fractionated chlorophyll *a*, as well as phytoplankton identification and counting. Inorganic nutrients (ammonium, nitrite, nitrate, silicate and phosphate) were measured using a continuous-flow autoanalyser (Bran + Luebbe Autoanalyzer 3, Norderstedt, Germany), according to colorimetric methods described in Grasshoff et al. (1983).

In order to obtain the concentrations of the different chlorophyll *a* fractions, sequential filtrations were performed. Three size fractions were differentiated: smaller than 3  $\mu\text{m}$ , between 3 and 20  $\mu\text{m}$ , and larger than 20  $\mu\text{m}$ , to quantify the chlorophyll contained in the pico-, nano- and microphytoplankton. Whatman Nuclepore track-etched membrane filters (pore size 3 and 20  $\mu\text{m}$ ) and Whatman GF/F glass microfiber filters were used, diameter 47 mm. Firstly, approximately 4.5 L of water was filtered through the polycarbonate 20  $\mu\text{m}$  filter to retain the largest fraction. Then, the filtrate was passed through the polycarbonate 3  $\mu\text{m}$  pore size filter to obtain the 3–20  $\mu\text{m}$  fraction. Finally, the smallest fraction (<3  $\mu\text{m}$ ) was retained using a Whatman GF/F filter. The nominal pore size of GF/F filters is 0.7  $\mu\text{m}$ , but the effective pore size of the glass-fibre filters is substantially smaller (Sheldon, 1972) and these are routinely used for picophytoplankton (Morán et al., 1999). Pigments were extracted in 10 ml of 90% acetone for 48 h in dark and cold conditions. The absorbance of the extract was measured using a UV–vis spectrophotometer (UV-2401PC Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The chlorophyll concentration was estimated according to the equations of Jeffrey and Humphrey (1975). The sum of the three fractions was used to determine if the total chlorophyll concentration was above 0.5  $\mu\text{g L}^{-1}$ ; this was considered the threshold below which bivalves do not filter (Dolmer, 2000; Riisgård, 2001; Riisgård et al., 2011).

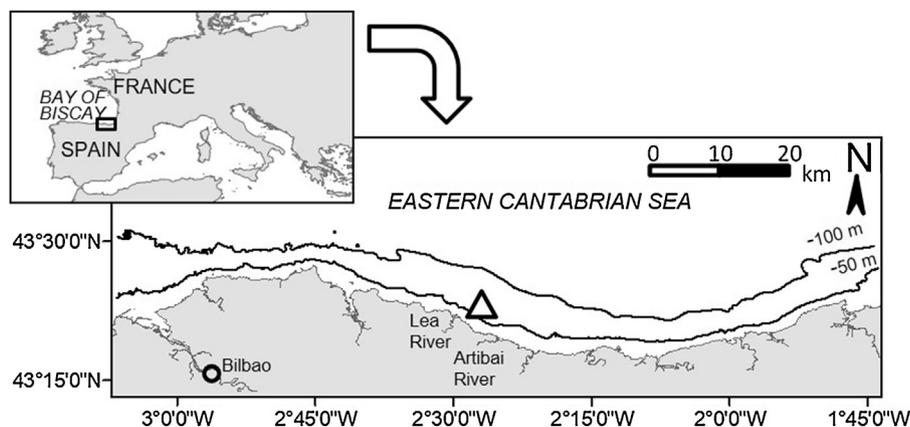


Figure 1 Map of the study area, located in the southeastern Bay of Biscay. The triangle shows the location of the experimental bivalve farm.

Phytoplankton identification and counting were conducted for three depths: 3, 17 and 33 m. Samples were preserved immediately after collection with acidic Lugol's solution (0.4% v/v) and maintained in 125-mL borosilicate bottles under dark and cool conditions (4°C) until analysis. Taxonomic identification and cell counting were performed on subsamples of 50 mL, following the Utermöhl method (Edler and Elbrächter, 2010; Hasle, 1978; Utermöhl, 1958) under a Nikon diaphot TMD inverted microscope. Depending on the organism size, 100× or 400× magnification was used; the detection limit of microscope counts for microplankton organisms was 20 cells L<sup>-1</sup>. Small nanophytoplankton cells that could not be assigned to any taxonomic group were assigned to a group named “unidentified forms <10 μm”. The minimum cell size that could be detected was 2–3 μm; therefore, picophytoplankton could not be identified and counted.

Three variables were used to describe hydrographic conditions: light extinction coefficient, depth of the photic zone and river flow. Light extinction coefficient (*k*) was estimated from the PAR measured by the CTD using the equation derived from the Beer–Lambert law:

$$I_z = I_f \cdot e^{-kz},$$

where  $I_z$  [E m<sup>-2</sup> d<sup>-1</sup>] is the radiation received at a specific depth,  $I_f$  is the radiation right below the surface, and  $z$  is the specific depth [m].

The *k* was then used to calculate the depth of the photic layer using the following equation: photic zone [m] = 4.605/*k*. Information on the flow rate of one of the rivers closest to the experimental site, Artibai river (Fig. 1), was obtained from a regional website (“Diputación Foral de Bizkaia”, <http://www.bizkaia.eus>). Information on the other river surrounding the farm, Lea river, was not included due to missing data on the time series. To account for a delay in the influence of river flow on the water column conditions, flow rates were averaged for the seven days prior to the sampling day.

### 2.3. Data analysis

The variability of temperature and salinity was represented using a temperature-salinity (TS) diagram. The temporal variation of chlorophyll *a* through the water column (up to 45 m depth) was presented as a contour map.

Regarding phytoplankton data, the species list was standardised prior to statistical analysis according to AlgaeBase (Guiry and Guiry, 2015). The phytoplankton community was analysed according to cell concentration [cell L<sup>-1</sup>] and biomass [μg C L<sup>-1</sup>]. In order to calculate the latter, the biovolume of each taxon was first calculated from its equivalent spherical diameter (ESD) using the equation of the sphere's volume. Information on phytoplankton cell size was collected from two sources: (i) the ESD measured in phytoplankton species from the north-west Spanish coast by investigators from other institutions (M. Huete from the Spanish Institute of Oceanography – A Coruña Centre, and M. Varela, L. Mene and J. Lorenzo from the University of Vigo) and (ii) the report by Olenina et al. (2006). Then, biomass was determined using the equation reported by Montagnes et al. (1994) for marine phytoplankton:  $Biomass = 0.109 \times Volume^{0.991}$ , where *Biomass* is expressed in pg C cell<sup>-1</sup> and *Volume* is expressed in μm<sup>3</sup>. For the data analyses, the specific results on abundance and biomass were combined to obtain

total data for the following groups: chlorophytes, kleptoplastidic ciliates (*Mesodinium* spp.), cryptophytes, diatoms, dinoflagellates, euglenophytes, haptophytes, ochrophytes (chrysophyceans, dictyochophyceans, raphidophyceans and xanthophyceans), heterotrophic nanoflagellates (including the taxa *Ebria tripartita*, *Katablepharis remigera*, *Leucocryptos* sp. and *Telonema* sp., traditionally considered in phytoplankton studies) and unidentified forms <10 μm. For the description of phytoplankton abundance and biomass, some of these groups were merged into a group called “others”. This group was primarily comprised of unidentified forms, but also included the following minority groups (*i.e.* those contributing less than 6.5% to total abundance and biomass): chlorophytes, euglenophytes, ochrophytes and heterotrophic nanoflagellates.

For the study of relationships between the environment and phytoplankton community, exploratory analysis was conducted by means of biplots representing environmental variables against phytoplankton. Correlation matrices (Pearson correlation coefficient, alpha = 0.05) were also performed. Two separate analyses were undertaken: the first one for abundance of phytoplankton groups and the second one for chlorophyll *a* fractions, as a proxy for phytoplankton biomass. The group “unidentified forms” was excluded from the correlation analysis due to its heterogeneity.

Among the environmental variables, only those that *a priori* could be considered most explanatory of phytoplankton variability were included in the analysis, namely Secchi disk depth, light extinction coefficient, temperature, salinity, Artibai river flow and nutrient concentration (ammonium, nitrite, nitrate, phosphate and silicate). Environmental variables were previously transformed in order to attain a distribution close to normal.

Phytoplankton data were processed as follows: prior to analysis, phytoplankton rare taxa, defined here as those occurring in less than 10% of the samples, were removed to avoid noise data (Austin and Greig-Smith, 1968). A total of 78 of the 194 taxa were excluded from the analysis. Phytoplankton abundance data were log-transformed (after adding one to avoid taking the log of zero values) and relationships with environmental variables were studied at depths of 3, 17 and 33 m.

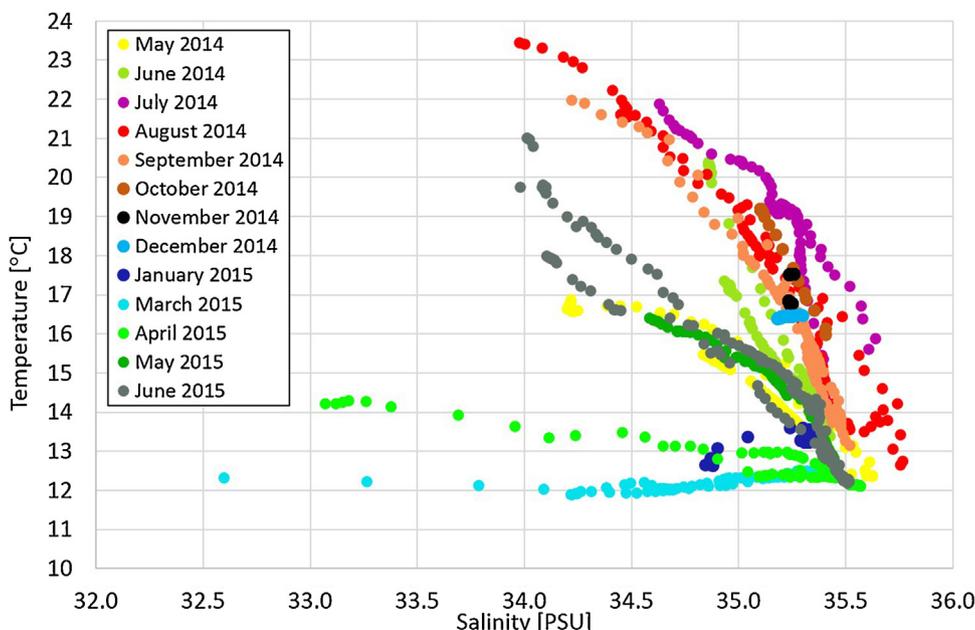
Finally, chlorophyll *a* was also log-transformed prior to analysis and relationships between the three size fractions of chlorophyll and environmental variables were studied at depths of 3, 10, 17, 24, 33 and 42 m.

In ecological research, when multiple statistical tests are undertaken, each at the same significance level (alpha), the probability of achieving at least one significant result is greater than that significance level. In this context, to avoid a “Type I” error, one strategy is to correct the alpha level when performing multiple tests. The most well-known correction is called Bonferroni correction; in this study, Bonferroni sequential correction, described by Holm (1979), was applied. Statgraphics Centurion XVI software was used for the correlation matrices.

## 3. Results

### 3.1. Hydrographic, physico-chemical conditions and bulk chlorophyll *a*

The TS diagram shows the prevalence of thermohaline stratification due to spring warming and the presence of waters



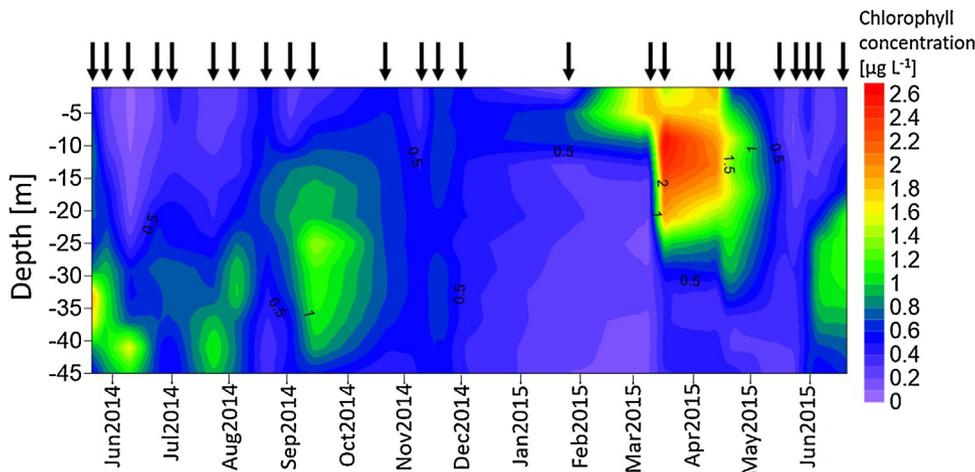
**Figure 2** Temperature-Salinity diagram of the waters off the Basque coast, in the SE Bay of Biscay, from May 2014 to June 2015.

of continental origin in May 2014 (Fig. 2). The thermal stratification prevailed from June to October in relation to the progression of the summertime warming. Moreover, more or less extended haline stratification was present throughout this period. In November, a reduction of the vertical gradients of temperature and salinity was observed induced by vertical mixing and cooling. December was characterised by thermohaline homogeneity of the water column and, more importantly, by high water column temperatures (above 16°C) associated with previous warm conditions. Conversely, in January 2015, the entire water column cooled due to extremely cold winter months. This change, together with high precipitation, resulted in the prevalence of haline stratification and thermal inversion in January. The haline stratification was especially enhanced in March and April. Again, the thermal stratification was observed in May and

June, induced by an extremely warm spring in 2015. Again, relatively strong haline stratification could be observed in spring 2015.

Overall, thermohaline stratification could be observed throughout the period, with a few exceptions in November–December 2014 and January–April 2015 where homogeneity and haline stratification of the water column prevailed, respectively. Additional information on river flows is included in Fig. 1 of the Supplementary Material.

Chlorophyll *a* (obtained from the fluorescence measured by the CTD) showed several peaks during the studied period (Fig. 3). At the end of May and beginning of June 2014, two deep chlorophyll peaks were observed at depths of 34 and 41 m, respectively, with values between 1.6 and 2.0  $\mu\text{g L}^{-1}$ . Three other sub-surface chlorophyll increases were then detected at the end of July, beginning of August and mid-



**Figure 3** Contour map of chlorophyll *a* in the waters off the Basque coast from the surface to 45 m in depth. The period from May 2014 to June 2015 is represented. Arrows on the top of the plot point the dates of the samplings.

September, ranging from 1.1 to 1.4  $\mu\text{g L}^{-1}$ . These were followed by a period with low values ( $<0.8 \mu\text{g L}^{-1}$ ) from October to March. The maximum value reported was detected in March at approximately 12 m depth, reaching 2.6  $\mu\text{g L}^{-1}$ . In April, a surface peak was observed (2  $\mu\text{g L}^{-1}$ ). Subsequently, chlorophyll concentrations decreased reaching the lowest surface values during spring 2015, although higher concentrations were detected around 20–35 m depth in June.

Table 1 shows the mean values and standard deviations of the parameters relating to the physico-chemical conditions of the study area at the different depths studied and for the whole water column. Secchi disk depth mean value was 11 m. Mean light extinction coefficient ( $k$ ) was 0.1  $\text{m}^{-1}$ . Photic layer depth had a mean value of 43.7 m. Mean temperature values for each depth ranged from 17.4 to 14.3°C, showing a decreasing trend from the surface to the deeper waters. In contrast, salinity increased towards the deeper water, with mean values ranging from 34.5 to 35.4. The mean chlorophyll concentrations measured by the CTD were very similar between the six depths, approximately 0.6–0.7  $\mu\text{g L}^{-1}$ . The concentration of several inorganic nutrients did not present great dissimilarities between the mean values of the different sampled depths, showing ranges of 1.4–1.8  $\mu\text{M}$  (ammonium), 0.3–0.4  $\mu\text{M}$  (nitrite), 0.2–0.3  $\mu\text{M}$  (phosphate) and 0.9–1.5  $\mu\text{M}$  (silicate). However, nitrate concentration varied more through the water column, with mean values close to 1  $\mu\text{M}$  within the shallower and intermediate layers (3, 10, 17 and 24 m) to a maximum of 3.0  $\mu\text{M}$  at 42 m depth (additional information on nutrient concentrations is shown in Fig. 2 of the Supplementary Material).

### 3.2. Phytoplankton composition, abundance and biomass

With regard to phytoplankton richness, a total of 194 phytoplankton taxa were identified during these surveys. Dinoflagellates and diatoms represented the most diverse groups, comprising 47.4% and 35.1% of the total taxa described, respectively.

Phytoplankton total abundance ranged from  $3.4 \times 10^4$  cells  $\text{L}^{-1}$  to  $5.1 \times 10^6$  cells  $\text{L}^{-1}$ . Differences were found in relation to the different taxonomic groups. Putting aside the group of “unidentified forms”, which in several samplings was the most abundant due to its heterogeneity, haptophytes were the most abundant group in 46% of the samples, followed by dinoflagellates (26%), cryptophytes (15%) and diatoms (13%). Table 2 shows the most abundant taxon in each phytoplankton group at the three sampled depths and during the two main seasons (*i.e.*, winter and summer).

The phytoplankton community differed in composition as well as in total cell density between the three sampled depths (Fig. 4). Firstly, at 3 m depth, where the highest abundance values were found, showed a maximum of approximately  $5 \times 10^6$  cells  $\text{L}^{-1}$  in May 2014 (Fig. 4a), which was characterised by a large proportion of the group called “others” (mainly, unidentified forms  $<10 \mu\text{m}$ ). During June and July, the abundance at 3 m depth dropped to just over half of that registered in May, followed by a period of low densities from August 2014 to January 2015, ranging from  $1.8 \times 10^5$  to  $5.0 \times 10^5$  cells  $\text{L}^{-1}$ . The end of the studied period was characterised by a first peak dominated by diatoms, contributing to more than 50% of the total abundance in March 2015, followed by an increase of the haptophyte community representing 60% of the total abundance in April 2015 (maximum abundance of  $2.8 \times 10^6$  cells  $\text{L}^{-1}$ ).

Similarly, at 17 m depth the highest cell densities were found at the beginning of the study period, from May to July 2014 (Fig. 4b). However, here maximum values were much lower compared to those at the 3 m depth, with the highest value of  $1.3 \times 10^6$  cells  $\text{L}^{-1}$  occurring in July. This peak was dominated by the group labelled as “others”. Two more increases in abundance were detected in October 2014 and April 2015, with very low values during the intervening period. The three peaks observed at 17 m depth involved an important contribution from the haptophytes, ranging from 40% to 47% of the total abundance. Dinoflagellates gradually raised their contribution within the three peaks.

The greatest depth (33 m) produced the lowest total abundance values, with a maximum of approximately  $8.4 \times 10^5$  cells  $\text{L}^{-1}$  (Fig. 4c). The cell density increases

**Table 1** Description (mean values and standard deviations) of the water column conditions in a bivalve culture experimental site off the Basque coast for the period May 2014–June 2015. Water-column weighted mean values, as well as the values for the six discrete sampled depths, are shown.  $k$ : light extinction coefficient; Chl  $a$  CTD: chlorophyll  $a$  obtained from the fluorescence measured by the CTD.

Variable	Mean $\pm$ SD						
	Water column	3 m	10 m	17 m	24 m	33 m	42 m
Secchi disk depth [m]	11.0 $\pm$ 3.5	–	–	–	–	–	–
$k$ [ $\text{m}^{-1}$ ]	0.1 $\pm$ 0.0	–	–	–	–	–	–
Photic layer depth [m]	43.7 $\pm$ 9.3	–	–	–	–	–	–
Temperature [°C]	15.6 $\pm$ 2.6	17.4 $\pm$ 3.4	16.8 $\pm$ 2.7	16.1 $\pm$ 2.4	15.4 $\pm$ 2.1	14.7 $\pm$ 2.0	14.3 $\pm$ 1.7
Salinity	35.1 $\pm$ 0.4	34.5 $\pm$ 0.5	34.9 $\pm$ 0.3	35.1 $\pm$ 0.2	35.2 $\pm$ 0.1	35.3 $\pm$ 0.1	35.4 $\pm$ 0.1
Chl $a$ CTD [ $\mu\text{g L}^{-1}$ ]	0.6 $\pm$ 0.4	0.6 $\pm$ 0.5	0.6 $\pm$ 0.6	0.7 $\pm$ 0.5	0.7 $\pm$ 0.3	0.7 $\pm$ 0.4	0.6 $\pm$ 0.3
Ammonium [ $\mu\text{M}$ ]	1.5 $\pm$ 0.6	1.4 $\pm$ 0.7	1.4 $\pm$ 0.7	1.8 $\pm$ 0.9	1.7 $\pm$ 1.2	1.4 $\pm$ 0.5	1.4 $\pm$ 0.8
Nitrite [ $\mu\text{M}$ ]	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	0.3 $\pm$ 0.2	0.4 $\pm$ 0.3	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2
Nitrate [ $\mu\text{M}$ ]	1.4 $\pm$ 1.5	1.0 $\pm$ 1.7	0.9 $\pm$ 1.6	0.9 $\pm$ 1.3	1.1 $\pm$ 1.4	1.9 $\pm$ 2.2	3.0 $\pm$ 2.6
Phosphate [ $\mu\text{M}$ ]	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1					
Silicate [ $\mu\text{M}$ ]	1.1 $\pm$ 0.6	1.5 $\pm$ 1.1	1.2 $\pm$ 0.9	1.0 $\pm$ 0.6	0.9 $\pm$ 0.5	1.1 $\pm$ 0.7	1.5 $\pm$ 0.8

**Table 2** List of the most abundant taxon in each phytoplankton group at three different sampled depths and during the two main seasons (*i.e.*, winter and summer). The winter season includes two surveys (January and March 2015) and the summer season includes four surveys (July, August and September 2014 and June 2015).

Season	Depth [m]	Group	Taxon	Abundance [cells L <sup>-1</sup> ]
Winter	3	Cryptophytes	<i>Teleaulax</i> spp.	$1.2 \times 10^5$
		Dinoflagellates	<i>Heterocapsa</i> sp.	$3.1 \times 10^5$
		Diatoms	<i>Thalassiosira</i> sp.	$1.3 \times 10^6$
		Haptophytes	Prymnesiales	$1.4 \times 10^5$
		Ciliates	<i>Mesodinium</i> sp.	$4.2 \times 10^3$
		Others	Unidentified forms	$1.9 \times 10^5$
	17	Cryptophytes	Cryptophycophyta	$1.9 \times 10^4$
		Dinoflagellates	<i>Heterocapsa</i> sp.	$1.2 \times 10^4$
		Diatoms	Pennales 10-50 $\mu\text{m}$	$4.2 \times 10^3$
		Haptophytes	Prymnesiales	$8.5 \times 10^3$
		Ciliates	<i>Mesodinium</i> sp.	$2.2 \times 10^2$
		Others	Unidentified forms	$2.2 \times 10^4$
	33	Cryptophytes	Cryptophycophyta	$9.6 \times 10^3$
		Dinoflagellates	Gymnodiniales <20 $\mu\text{m}$	$7.8 \times 10^3$
		Diatoms	Pennales <10 $\mu\text{m}$	$3.5 \times 10^3$
		Haptophytes	Prymnesiales	$9.6 \times 10^3$
		Ciliates	<i>Mesodinium</i> sp.	80
		Others	Unidentified forms	$1.5 \times 10^4$
Summer	3	Cryptophytes	<i>Plagioselmis</i> sp.	$4.9 \times 10^4$
		Dinoflagellates	Gymnodiniales <20 $\mu\text{m}$	$6.6 \times 10^4$
		Diatoms	<i>Pseudo-nitzschia galaxiae</i>	$2.5 \times 10^5$
		Haptophytes	Prymnesiales	$9.8 \times 10^5$
		Ciliates	<i>Mesodinium</i> sp.	$1.1 \times 10^3$
		Others	Unidentified forms	$7.4 \times 10^5$
	17	Cryptophytes	<i>Plagioselmis</i> sp.	$2.5 \times 10^4$
		Dinoflagellates	Gymnodiniales <20 $\mu\text{m}$	$8.9 \times 10^4$
		Diatoms	<i>Chaetoceros</i> sp.	$6.8 \times 10^4$
		Haptophytes	Prymnesiales	$5.4 \times 10^5$
		Ciliates	<i>Mesodinium</i> sp.	40
		Others	Unidentified forms	$6.1 \times 10^5$
	33	Cryptophytes	<i>Plagioselmis</i> sp.	$3.6 \times 10^4$
		Dinoflagellates	Gymnodiniales <20 $\mu\text{m}$	$8.5 \times 10^4$
		Diatoms	Pennales <10 $\mu\text{m}$	$4.7 \times 10^4$
		Haptophytes	Prymnesiales	$3.3 \times 10^5$
		Ciliates	<i>Mesodinium</i> sp.	$9.6 \times 10^3$
		Others	Unidentified forms	$3.1 \times 10^5$

observed in July and October 2014 were concurrent with the first two peaks observed at the 17 m depth. Very low abundances were registered from December 2014 to May 2015, between  $1.1 \times 10^5$  and  $1.4 \times 10^5$  cells L<sup>-1</sup>, followed by a six-fold increase in June 2015. As with the intermediate depth (17 m), dinoflagellate abundance slightly increased during the peaks.

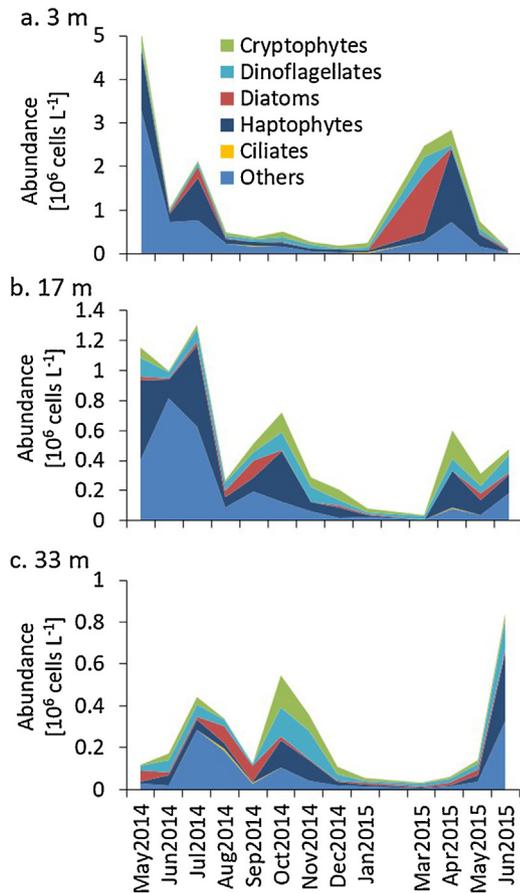
The comparison of biomass variability with abundance variability showed that most of the peaks or increases were simultaneous in time and space (Fig. 5). However, the greatest difference was the relative contribution of each group.

The highest biomass values were observed at 3 m, with a maximum of  $435 \mu\text{g C L}^{-1}$ . The contribution of the different phytoplankton groups to the peaks of May and July 2014 was similar compared to abundance values, being dominated by small unidentified forms and haptophytes. Nevertheless, from December 2014 to March 2015 diatoms dominated

the community, representing between 54% and 78% of the total biomass (Fig. 5a).

At the intermediate depth (17 m), biomass values were notably lower than at the 3 m depth, ranging from 9 to  $104 \mu\text{g C L}^{-1}$  (Fig. 5b). Similar to the shallower depth studied, diatoms were the dominant group from December 2014 to March 2015 (44–79% of the total biomass). The occasional and significant contribution of ciliates (represented by the genus *Mesodinium*) during the peak of April 2015 was also notable, representing 30% of the total biomass. Dinoflagellates gained importance during the biomass increases, especially in September when they represented 33% of the total biomass.

Finally, the range of biomass values at the 33 m depth was similar to that at 17 m, with the exception of the occurrence of a larger peak which reached  $153 \mu\text{g C L}^{-1}$  in May 2014 (Fig. 5c). Diatoms dominated the community in May and June



**Figure 4** Contribution of each of the major phytoplankton groups to the total abundance per sample at three different depths (3, 17 and 33 m). The group “others” consisted of chlorophytes, euglenophytes, ochrophytes, heterotrophic nanoflagellates and unidentified forms. Note that plots have different scaled y axes.

2014 and from January to May 2015, representing 74–95% of the total biomass. In August 2014, ciliates contributed 44% of the total biomass.

### 3.3. Size-fractionated chlorophyll *a*

The relative contribution of the three chlorophyll size fractions was studied at six depths (Fig. 6). Overall, the picophytoplankton made the greatest contribution. However, an increase in the nanophytoplankton was observed towards the greatest depths (33 and 42 m). Results for May 2014 at the 33 m depth were remarkable, with 58% of the total chlorophyll provided by the microphytoplankton (Fig. 6e).

Total chlorophyll *a* concentrations, estimated from the sum of the three size fractions studied, showed values lower than  $1 \mu\text{g L}^{-1}$  in most of the samples. The highest concentrations were observed in March 2015, with approximately  $6 \mu\text{g L}^{-1}$  at the 3 m depth and  $2.5 \mu\text{g L}^{-1}$  at the 10 m depth, although values were still low in deeper samples. One month later, in April 2015, secondary peaks were found at depths of 3 to 24 m. Similar peaks were also detected in late spring 2014 at depths of 33 m and 42 m.

Total chlorophyll concentration was above the  $0.5 \mu\text{g L}^{-1}$  threshold in 62% of the samples. The depth of 42 m showed the highest proportion of values below that value (67% of the samples). Overall, chlorophyll concentrations below  $0.5 \mu\text{g L}^{-1}$  were found during the summer.

### 3.4. Relationship between environmental variables and phytoplankton community

Several strong linear relationships were found between some environmental variables and both phytoplankton abundance and chlorophyll *a* measured in the laboratory (as a proxy for phytoplankton biomass).

Firstly, the relationships between environment and abundance of phytoplankton groups at each depth were studied (Table 3). Biplots for each significant correlation are shown in Fig. 3 of the Supplementary Material.

At 3 m, total abundance of phytoplankton was not significantly correlated with any environmental variable. Some of the minor groups, such as chlorophytes and heterotrophic nanoflagellates, showed inverse correlations with different environmental variables. Ciliates (*Mesodinium* spp.) appeared to reach higher abundance at higher values of light extinction coefficient.

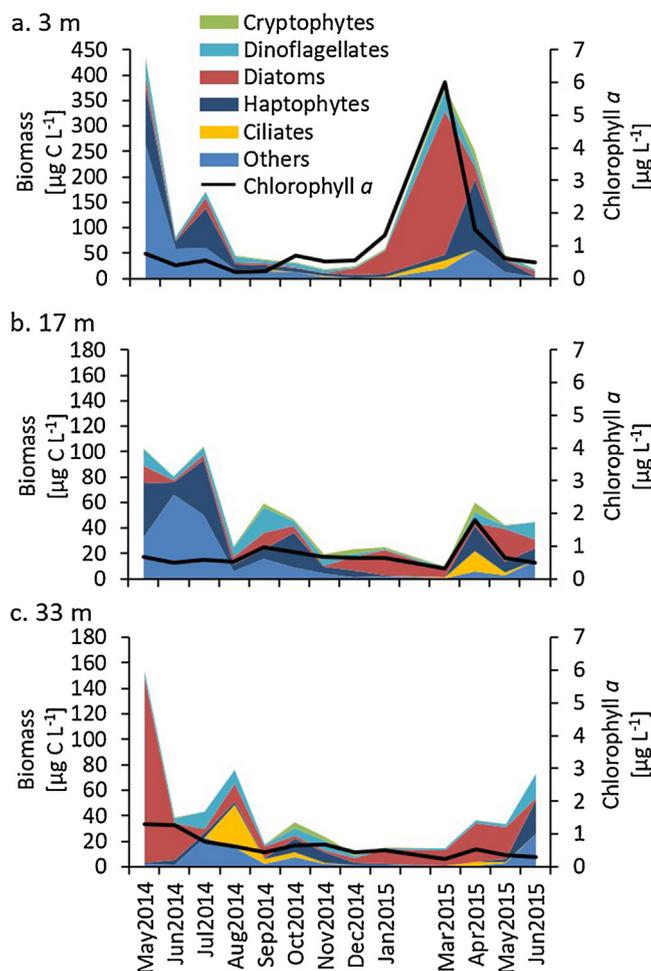
Overall, at 17 m depth, some nutrients were the main variables that explained phytoplankton abundance. Ammonium concentration significantly explained the variability of chlorophytes and dinoflagellates, showing a direct correlation. Nitrate showed a strong inverse relationship with total abundance of phytoplankton and, in particular, with dinoflagellates and haptophytes. Finally, silicate partly explained the variability of heterotrophic nanoflagellate abundance (inverse correlation). In addition, Artibai river flow showed inverse correlation with total abundance.

Finally, the greatest number of significant linear correlations was found at a depth of 33 m. However, some of these correlations should be viewed with caution since there were several ‘zero’ values in the dependent variable. Similar to the pattern observed at the 17 m depth, Artibai river flow showed inverse correlation with total abundance of phytoplankton and, in particular, with diatom abundance. Cryptophytes showed greater abundance at higher temperature and lower salinity.

Similarly, relationships between environment and different chlorophyll size fractions were ascertained at six depths: 3, 10, 17, 24, 33 and 42 m (Table 4). Biplots for each significant correlation are shown in Fig. 4 to Fig. 9 of the Supplementary Material.

At the 3 m depth, temperature, nitrate and silicate concentration were the variables explaining most of the variability of the different chlorophyll fractions: higher chlorophyll values were found at lower temperatures and higher nitrate concentrations. Higher concentrations of the chlorophyll fraction of 3–20  $\mu\text{m}$  were found at lower Secchi disk depths and at higher silicate concentrations. Similar results were obtained at the 10 m depth: higher chlorophyll values were observed at lower temperatures and at higher nitrate and silicate concentrations. In addition, the chlorophyll fraction of 3–20  $\mu\text{m}$  was associated with lower Secchi disk depths, whereas the larger fraction (>20  $\mu\text{m}$ ) was directly related to Artibai river flow.

At the 17 m depth, variability of chlorophyll was explained to a lesser extent by environmental variables



**Figure 5** Contribution of each of the major phytoplankton groups to the total biomass per sample at three different depths. The group “others” consisted of chlorophytes, euglenophytes, ochrophytes, heterotrophic nanoflagellates and unidentified forms. The black line represents total chlorophyll  $a$  concentration [ $\mu\text{g L}^{-1}$ ] obtained by means of chemical analysis (right axis).

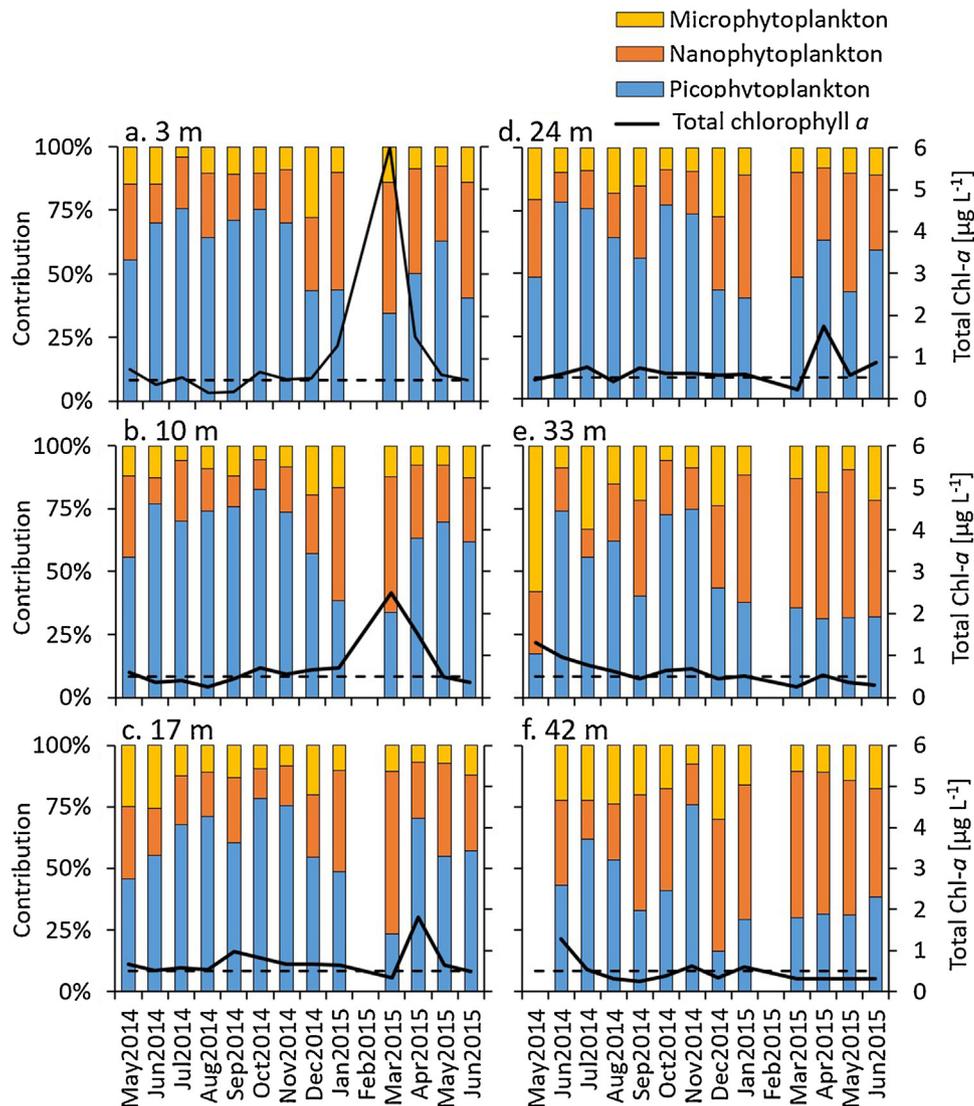
compared to the shallower depths. Only the chlorophyll fraction of 3–20  $\mu\text{m}$  showed significant correlation with the environment, with higher values at lower temperatures and higher silicate concentrations. At the 24 m depth, silicate was the only variable explaining chlorophyll variability: the 3–20  $\mu\text{m}$  fraction was directly correlated with silicate concentration.

At the 33 m depth, temperature, nitrate and silicate concentrations significantly explained the variability of the small chlorophyll fraction, but with the opposite pattern to that observed at 3, 10, 17 and 24 m: higher chlorophyll values were found at higher temperatures and lower nutrient concentrations. The large fraction ( $>20 \mu\text{m}$ ) was directly related to salinity. Finally, at the 42 m depth, the small ( $<3 \mu\text{m}$ ) and large ( $>20 \mu\text{m}$ ) chlorophyll fractions were inversely correlated with silicate concentration. Higher concentrations of the intermediate chlorophyll fraction (3–20  $\mu\text{m}$ ) were found at lower Secchi disk depths. In contrast, the large fraction presented lower values as the light extinction coefficient increased.

#### 4. Discussion

The study area showed the typical hydrographic conditions of temperate coastal zones (Mann and Lazier, 1991), previously described for the Bay of Biscay (Valdés and Moral, 1998; Valencia et al., 2004; Varela, 1996). The sea surface appeared stratified during summer months, due to heating by solar irradiation (Varela, 1996). Late autumn and winter were mostly characterised by vertical mixing, which might be generated by a combination of cooling, turbulence and downwelling processes (Valencia and Franco, 2004; Valencia et al., 2004). The low surface salinity values observed in the present study were explained by river discharges mostly during late winter and spring.

Mixing processes are usually accompanied by changes in light and nutrient availability and, thus, growth performance of phytoplankton species within the water column is partly defined by vertical mixing (Diehl, 2002; Huisman et al., 2004). In this study, a well-mixed homogeneous water column was observed in November, December and January, when



**Figure 6** Chlorophyll *a* size fraction (<3  $\mu\text{m}$ , 3–20  $\mu\text{m}$  and >20  $\mu\text{m}$ ; *i.e.* pico-, nano- and micro-phytoplankton, respectively) contribution at the six depths for the period May 2014 to June 2015. Total chlorophyll *a* (sum of fractions) is shown on the right axis. The dotted line shows the chlorophyll threshold below which mussels do not filter (Dolmer, 2000; Riisgård, 2001; Riisgård et al., 2011). This threshold should be viewed with caution since it was not developed for open waters (see Section 4).

phytoplankton abundance and biomass showed the lowest values or a decreasing trend. As described by Fernandez and Bode (1991), during this period, although an upward flux of nutrients from deep water layers occurs as a consequence of the mixing, phytoplankton biomass is expected to be low due to limited light. From January onwards, surface phytoplankton abundance and biomass, as well as chlorophyll concentration, started to increase. This increase notably coincided with nutrient input, reaching a maximum in March–April. In particular, these peaks in surface waters were characterised by a high contribution of diatoms, as shown in other late winter blooms previously described in the southern Bay of Biscay (Guillaud et al., 2008; Labry et al., 2001). This fact also agrees with Margalef (1978), who found that strong vertical mixing favours the dominance of diatoms. According to Margalef’s mandala, dinoflagellates are expected to be favoured in stratified water columns, where they show com-

petitive advantage over other groups based on their ability to swim to zones rich in light and nutrients (Glibert, 2016; Margalef, 1978; Smayda and Reynolds, 2003). Here, a slight increase in the contribution of dinoflagellates was detected during August–September 2014, when the water column was stratified.

Among the studied environmental variables, temperature and nutrients (mostly nitrate and silicate) seemed to be the variables that explained most of chlorophyll annual variability. The results at depths of 3 m and 10 m coincided with the winter conditions, when deeper cold and nutrient-rich water is mixed with surface waters leading to the increase in phytoplankton biomass (Valdés and Moral, 1998; Varela, 1996). According to this, the observed chlorophyll peak at these depths in March 2015 might be explained by the contemporaneous increase in nitrate and silicate concentrations and low temperatures.

**Table 3** Significant correlations (alpha = 0.05, adjusted by sequential Bonferroni correction) between abundance of major phytoplankton groups and environmental variables at depths of 3 m, 17 m and 33 m. The Pearson coefficient ( $r$ ) and the probability ( $p$ ) are shown.  $k$ : light extinction coefficient estimated for the whole water column. Correlations with an \* should be viewed with caution since there were several 'zero' values in the dependent variable.

Depth	Phytoplankton abundance	Environmental variable	$r$	$p$
3 m	Chlorophytes	Temperature	-0.553	0.0497
	Ciliates	$k$	0.686	0.0096 *
	Heterotrophic nanoflagellates	Nitrate	-0.745	0.0035
		Phosphate	-0.638	0.0190
17 m	Chlorophytes	Ammonium	0.621	0.0236
	Dinoflagellates	Ammonium	0.658	0.0145
		Nitrate	-0.628	0.0214
		Nitrate	-0.651	0.0159
	Haptophytes	Silicate	-0.559	0.0471
	Heterotrophic nanofl.	Artibai flow	-0.684	0.0099
	Total abundance	Nitrate	-0.749	0.0032
33 m	Chlorophytes	Phosphate	0.749	0.0032 *
	Cryptophytes	Temperature	0.688	0.0093
		Salinity	-0.755	0.0028
		Artibai flow	-0.687	0.0095
	Diatoms	Nitrite	-0.686	0.0095 *
	Euglenophytes	$k$	-0.644	0.0175 *
	Heterotrophic nanoflagellates	Ammonium	0.705	0.0071 *
		Secchi disk depth	0.589	0.0340 *
	Ochrophytes	Artibai flow	-0.570	0.0419
	Total abundance			

In contrast, different results were obtained for phytoplankton abundance. Neither temperature nor silicate explained the variability in total abundance. Among the significant correlations between environmental variables and abundance of phytoplankton groups, the fewest number of correlations was found at the 3 m depth. In fact, previously it had been found that environmental variables explained little about phytoplankton group variability (usually <16%, except in winter when this was 24%) in surface waters off the Basque coast, although the explained variability was higher at the species level (Muñiz et al., 2018). At 17 m, a reduced total abundance coincided with higher river flow and nitrate concentrations. Nitrate has been found to be linked to river discharges into the Basque coastal waters (Borja et al., 2016). This situation would reflect winter conditions, when river flows are high and phytoplankton abundance is low. Indeed, the low abundance of dinoflagellates during winter conditions (and its inverse relation with nitrate) is consistent again with the reported preference of this group for summer stratified waters. Variability in the abundance of dinoflagellates at 17 m was also explained by ammonium concentrations. This observed direct relationship is in accordance with the well-established concept that ammonium is the preferred nitrogen source for marine phytoplankton, with the exception of diatoms, that have shown higher nitrate uptake rates (Heil et al., 2007; Walsh and Dugdale, 1971). Specifically, in the case of dinoflagellates, Li et al. (2010) found the higher acquisition of reduced forms of nitrogen, such as ammonium.

As mentioned before, the variability explained by the environment was different for chlorophyll concentration and for phytoplankton abundance. Although chlorophyll  $a$  has long been used as a proxy for phytoplankton biomass, it is

well known that chlorophyll  $a$  concentration, phytoplankton biomass (in carbon units) and cell abundance are three different attributes of the phytoplankton community (Domingues et al., 2008). Therefore, different results can be expected from each of them. In the present study, marked differences were found between chlorophyll concentrations and biomass (determined from biovolumes and cell densities). It should be considered that there is an associated error when biomass is calculated from the ESD and the abundance. In addition, the ratio of carbon biomass to chlorophyll in the cell is highly variable, both at intra- and inter-specific levels, and also depending on environmental conditions, mainly light and nutrients (Domingues et al., 2008; Ríos et al., 1998; Taylor et al., 1997).

Overall, chlorophyll values were low compared to adjacent areas, such as the Atlantic French coast with median values from 1.2 to 3.2  $\mu\text{g L}^{-1}$  (Fariñas et al., 2015); the euhaline zone of Basque estuaries with median values about 2  $\mu\text{g L}^{-1}$  from spring to autumn (Garmendia et al., 2011), or the Galician Rias with values up to 20  $\mu\text{g L}^{-1}$  (Varela et al., 2008). For two stations off the Basque coast located at a depth of nearly 50 m, similar to the one studied here, Estrada (1982) found similar results to the ones described above: overall, chlorophyll values ranged between 0 and 1  $\mu\text{g L}^{-1}$  during the year, showing occasional peaks in the winter. In the present study, during most of the year phytoplankton biomass was dominated by picophytoplankton. However, at the time of maximum biomass, a relative decrease in the contribution of the smallest fraction compared to the larger ones could be noticed. This is in accordance with the findings by Calvo-Díaz et al. (2008) reported for the central Cantabrian Sea.

Similarly, for an eventual estimation of the capacity of the Basque coast for bivalve aquaculture, it is also relevant the

**Table 4** Significant correlations (alpha = 0.05, adjusted by sequential Bonferroni correction) between chlorophyll *a* size fractions analysed at the laboratory and environmental variables at depths of 3, 10, 17, 24, 33 and 42 m. The Pearson coefficient (*r*) and the probability (*p*) are shown. *k*: light extinction coefficient estimated for the whole water column.

Depth	Chlorophyll <i>a</i>	Environmental variable	<i>r</i>	<i>p</i>
3 m	Chl <3 μm	Temperature	−0.833	0.0004
		Nitrate	0.621	0.0236
	Chl 3–20 μm	Secchi disk depth	−0.759	0.0026
		Temperature	−0.864	0.0001
		Nitrate	0.783	0.0015
	Chl >20 μm	Silicate	0.839	0.0003
		Temperature	−0.814	0.0007
		Nitrate	0.806	0.0009
	10 m	Chl <3 μm	Silicate	0.696
Temperature			−0.654	0.0152
Secchi disk depth			−0.796	0.0011
Chl 3–20 μm		Temperature	−0.870	0.0001
		Nitrate	0.785	0.0015
		Silicate	0.899	0.0000
Chl >20 μm		Temperature	−0.864	0.0001
		Artibai flow	0.687	0.0095
		Nitrate	0.830	0.0004
17 m	Chl 3–20 μm	Silicate	0.813	0.0007
		Temperature	−0.800	0.0010
		Silicate	0.735	0.0042
24 m	Chl 3–20 μm	Silicate	0.714	0.0061
33 m	Chl <3 μm	Temperature	0.677	0.0111
		Nitrate	−0.718	0.0057
		Silicate	−0.895	0.0000
	Chl >20 μm	Salinity	0.633	0.0201
		Silicate	−0.755	0.0045
42 m	Chl <3 μm	Secchi disk depth	−0.747	0.0052
	Chl 3–20 μm	<i>k</i>	−0.662	0.0189
	Chl >20 μm	Silicate	−0.648	0.0226

information about the phytoplankton primary production in this area, and how it compares with other coastal areas (e.g., Figueiras et al., 2002). Maps of primary production depicted from recent satellite-based data are available at <http://www.bio-oracle.org/explore-data.php> (Assis et al., 2018; Tyberghein et al., 2012). These maps show relatively low values in the surface waters of the Basque coast in comparison with other coastal areas within the Bay of Biscay, which is in accordance with the low values of chlorophyll “*a*” found in this study, and previously reported by other authors. As regards for direct measurements of primary production in the marine environment of the Basque Country, these have been undertaken mostly in estuaries and only one study can be cited for open marine waters (Orive et al., 2004; Valencia et al., 1989). An additional study can be considered representative of these waters, as it was conducted in the Cap Ferret Canyon, about 150 km off the Basque coast, on the French shelf (Laborde et al., 1999). The values obtained in surface waters of these two locations resulted lower than those measured in the nearby estuarine environments of Plencia and Urdaibai (Basque Country) and Arcachon Bay (France) (Table 5). However, primary production rates per unit area measured in both studies were of similar magnitude

that those obtained, some years later, by Labry et al. (2002) in a northward location influenced by the discharges of the Gironde river, one of the two largest rivers on the French Atlantic coast, and also by Glé et al. (2008) in the mesotrophic Arcachon Bay. The photic layer-integrated production rates can result more similar among those systems due to the occurrence of sub-surface peaks in the offshore waters, as those described by Laborde et al. (1999). In any case, the highest photic layer integrated rates that have been cited for open waters off the Basque coast and for the Cap Ferret Canyon waters are much lower than the maxima cited in the Galician rias, an area of the Iberian coast influenced by upwelling (Bode and Varela, 1998; Cermeño et al., 2006; Figueiras et al., 2002; Tilstone et al., 1999).

In relation to mussel filtration, not all of the seston is available as food for these bivalves. Although controversy still exists, it has been reported by some authors that mussels do not filter below a chlorophyll threshold of around 0.5 μg L<sup>−1</sup> (Dolmer, 2000; Riisgård, 2001). This threshold should be viewed with caution since it was not developed for open waters. Although on some occasions chlorophyll concentrations were below this limit, the annual average value was slightly above this value. Nevertheless, despite chlorophyll

**Table 5** Phytoplankton primary production rates cited for some locations selected within the Bay of Biscay and the northwest coast of the Iberian Peninsula. Most methods involved the  $^{14}\text{C}$  incorporation technique. The incubation period has been indicated, as it can affect primary production estimates (Regaudie-de-Gioux et al., 2014).

Geographical area	Environment	Location	Study period	Surface [ $\text{mg C m}^{-3} \text{ d}^{-1}$ ]	Photic zone integrated rates [ $\text{mg C m}^{-2} \text{ d}^{-1}$ ]	Method and incubation time	Reference
Bay of Biscay	Open marine waters	Basque coast (43°25'N, 2°W)	April 1986 to April 1987	<1–47 <sup>a</sup>	<50–600 <sup>a</sup>	$^{14}\text{C}$ (2 h)	Valencia et al. (1989)
		Cap Ferret Canyon (44°45'N, 2°W)	Spring, summer and autumn (1989–1991)	4–144	262–1537	$^{14}\text{C}$ (2 h)	Laborde et al. (1999)
		Oceanic waters off the Gironde	Early winter to late spring (1998)	–	15–990	$^{14}\text{C}$ (8–14 h)	Labry et al. (2002)
		Gironde plume	Early winter to late spring (1998)	–	28–1329	$^{14}\text{C}$ (8–14 h)	Labry et al. (2002)
	Euhaline and polyhaline waters in partially enclosed systems	Plencia Bay	February to June (1985)	0.8–153 <sup>a</sup>	–	$^{14}\text{C}$ (3 h)	Elósegui et al. (1987)
		Urdaibai estuary (lower station)	Annual cycle (1996–1997)	38–387 <sup>b</sup>	–	Dark-Light method (24 h)	Revilla et al. (2000)
Northwest Iberian Peninsula	Upwelling-influenced systems	Arcachon Bay	Annual cycle (2003)	<50–497	<175–1740 <sup>c</sup>	$^{14}\text{C}$ (24 h)	Glé et al. (2008)
		Rías Baixas (Vigo)	Annual cycle (2001–2002)	16.5–1100 <sup>a</sup>	224–9950 <sup>a</sup>	$^{14}\text{C}$ (2 h)	Cermeño et al. (2006)
		Rías Baixas (Vigo)	Spring, summer and autumn (1993)	–	65–3690	$^{14}\text{C}$ (2 h)	Tilstone et al. (1999)
		Rías Altas (Ares, Ferrol, La Coruña Bay)	March, August and December (1993)	–	Maximum > 3000	$^{14}\text{C}$ ( $\geq 2$ h)	Bode and Varela (1998)

<sup>a</sup> Original data were expressed in hour units. For comparative purposes, daily rates have been derived assuming a constant photoperiod of 10 h (Cermeño et al., 2006).

<sup>b</sup> Gross oxygen production has been transformed to particulate carbon production using a photosynthetic quotient of 2.2 obtained by Iriarte et al. (1997) in the Urdaibai estuary.

<sup>c</sup> Rates per surface area have been calculated using 3.5 m, the approximate mean depth of Arcachon Bay (Glé et al., 2008).

concentrations being not very high in comparison to other areas where bivalve aquaculture has traditionally developed (Figueiras et al., 2002; Varela et al., 2008), it has previously been reported that mussels from the experimental site off the Basque coast show good growth and biochemical performance, with similar mean chlorophyll values to the ones described here (Azpeitia et al., 2016, 2017).

In addition, the dominance of the diatoms during spring peaks in biomass, together with the relevant contribution of dinoflagellates to the sub-surface abundance and biomass, suggests favourable conditions for mussel culture, since some of the important fatty acids for bivalve growth (EPA, Eicosapentaenoic acid; and DHA, Docosahexaenoic acid) are known to be synthesised by these two groups (e.g., Azpeitia et al. (2016)). Experiments on mussel nutrition, in terms of carbon biomass, have also shown highest retention of diatoms and dinoflagellates, together with ciliates, compared to other phytoplankton groups (Trottet et al., 2008). Moreover, direct correlations have been reported between diatoms and bivalve growth (Beukema and Cadée, 1991; Pernet et al., 2012; Wall et al., 2013; Weiss et al., 2007). Thompson et al. (1993) found that diets containing high levels of saturated fats were more nutritious for oyster larvae. The observed high contribution of haptophytes also suggests favourable conditions for bivalve growth, since they have been reported to contain, on average, the highest proportion of saturated fats (33%), followed by diatoms (27%) (Volkman et al., 1991, 1989). In this study, one genus of ciliates (*Mesodinium* spp.) and four taxa of heterotrophic nanoflagellates (*Ebria tripartita*, *Katablepharis remigera*, *Leucocryptos* sp. and *Telonema* sp.) were taken into account. However, for future studies it would be of interest to account for all the nanoheterotrophs and ciliates, given their significant role as a food source for mussels (Trottet et al., 2008).

Some of the observed results from the water column, such as the higher phytoplankton abundance and biomass registered at shallower depths in comparison to the greater depths, suggest that bivalves would grow better in shallower waters. Furthermore, abundance and biomass of diatoms, dinoflagellates and haptophytes (i.e. the groups with the highest fatty acid content) were lower at the 33 m depth. In contrast, some subsurface chlorophyll maxima were found during the summer. Also, as previously mentioned, the chlorophyll size fractions above 3  $\mu\text{m}$  (corresponding to nano- and micro-phytoplankton) appeared to increase slightly towards the greatest depths that were sampled. These size fractions are the ones of interest for the correct growth of bivalves as, although there is still considerable controversy, the majority of the studies indicate that the minimum particle size for efficient retention is 4  $\mu\text{m}$  (Jørgensen, 1990; Møhlenberg and Riisgård, 1978; Riisgård, 1988). Azpeitia et al. (2016) analysed mussels from the same experimental site to compare whether there were differences between two culture depths. They found significant differences between mussels cultured at 5 m and at 15 m in terms of dry weight, length, shell shape and density, but not for any of the biochemical parameters analysed, such as fatty acid content. Nevertheless, they concluded that these differences might not lessen the quality of the final product.

In summary, the water column conditions in open waters off the Basque coast were characterised by the classical seasonal cycle of temperate areas at mid-latitudes of the

Northeast Atlantic. These hydrographic and environmental conditions influenced to a great extent the vertical distribution and temporal variability of the phytoplankton community. The overall phytoplankton community found through the water column in the experimental site seemed to be suitable for bivalve aquaculture, based on the dominance of diatoms, dinoflagellates and haptophytes, and a chlorophyll concentration that was above the reported threshold for bivalve filtration in most of the samples collected. Composition and contribution of the major groups were in accordance with the reported requirements for mussel growth. Although chlorophyll values were found to be relatively low during some periods, this may not be a problem for the good performance of mussels, as other authors who found very similar average chlorophyll values have previously reported good growth and biochemical composition in mussels from the experimental site.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.oceano.2018.08.001>.

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