






ORIGINAL RESEARCH

## Seasonal variability of phytoplankton community structure in a coastal station of the Argentine continental shelf based on a chemotaxonomic approach

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**ABSTRACT.** The composition, abundance and size structure of the phytoplankton community at a coastal time series station (38° 28' S-57° 41' W, EPEA, Argentina) was characterized by applying the chemotaxonomic approach. The seasonal variability of pigment diversity determined by high-performance liquid chromatography (HPLC, n = 171), nutrient concentration (n = 934), and temperature, salinity and degree of stratification of the water column was identified (n = 190 CTD profiles). The CHEMTAX program was used to estimate phytoplankton abundance in terms of contribution to chlorophyll *a* concentration of the different phytoplankton pigmentary types (PPTs). Two different pigment indices were compared to estimate phytoplankton community size fractions throughout the year, giving contrasting results. Water column was mostly mixed, with minimum temperatures between July and September and maximum between January-March (range: 8-23 °C). Nitrate was the limiting nutrient, with minimal concentrations at the end of summer. Its range varied between 0.010-13.330 μM, while silicate ranged between 0.016-10.670 μM without major seasonal variations, and phosphate between 0.120-2.180 μM. Fucoxanthin, chlorophyll *c*2, 19'-hexanoyl-oxy-fucoxanthin, diadinoxanthin, chlorophyll *b*, chlorophyll *c*3, peridinin and, alloxanthin, were the most frequent phytoplankton pigments. The PPT DINO-1 (dinoflagellates with peridinin), haptophytes types: HAPTO-6, HAPTO-7 and HAPTO-P showed a seasonal cycle with peaks of abundance in autumn and spring, while the diatoms DIATO-1 was high during the whole year and DIATO-2 mainly during winter. A pigment profile of a group of prymnesiophytes possessing MVChl\_c3 was described. It was evident that at this site PPTs having potentially toxic species bloom under different hydrological conditions. DINO-1 is likely to bloom in April (autumn), with temperatures close to 18 °C and weak stratification conditions, while October bloom (spring) occurs with lower temperatures of 10-12 °C. DINO-4 was noted during January and February (summer), when temperature was > 18 °C, salinity < 33.7, and the water column showed maximum stratification. In contrast, the maximum abundances of DIATO-2 occurred between August and September (winter), under completely mixed conditions, high nitrate concentration and low temperature of 10 °C. This work constitutes the first description of the variability of the abundance of the main PPTs in a coastal a time series station in the southwestern Atlantic Ocean shelf throughout the annual cycle, demonstrating the power of chemotaxonomy and CHEMTAX to perform descriptive analysis of a large number of samples.

**Key words:** CHEMTAX, phytoplankton pigments, nutrients, dinoflagellates, harmful algal blooms, southwestern Atlantic.

## Variabilidad estacional de la estructura de la comunidad fitoplanctónica en una estación costera de la plataforma continental argentina basada en un enfoque quimiotaconómico

**RESUMEN.** Se caracterizó la composición, abundancia y estructura de tamaño de la comunidad fitoplanctónica en una estación costera (38° 28' S-57° 41' W, EPEA, Argentina) de serie de tiempo aplicando el enfoque quimiotaconómico. Se identificó la variabilidad estacional de la diversidad de pigmentos determinada por cromatografía líquida de alta resolución (HPLC,  $n = 171$ ), la concentración de nutrientes ( $n = 934$ ) y la temperatura, salinidad y grado de estratificación de la columna de agua ( $n = 190$  perfiles CTD). Se utilizó el programa CHEMTAX para estimar la abundancia de fitoplancton en términos de contribución a la concentración de clorofila *a* de los diferentes tipos pigmentarios de fitoplancton (PPTs). Se compararon dos índices pigmentarios diferentes para estimar las fracciones de tamaño de la comunidad fitoplanctónica a lo largo del año, obteniéndose resultados contrastantes. La columna de agua estuvo mayoritariamente mezclada, con temperaturas mínimas entre julio y septiembre, y máximas entre enero y marzo (rango: 8-23 °C). El nitrato fue el nutriente limitante, con concentraciones mínimas al final del verano. Su rango varió entre 0.010-13.330  $\mu\text{M}$ , mientras que el silicato varió entre 0.016-10.670  $\mu\text{M}$  sin grandes variaciones estacionales, y el fosfato entre 0.120-2.180  $\mu\text{M}$ . La fucoxantina, clorofila *c2*, 19'-hexanoil-oxi-fucoxantina, diadinoxantina, clorofila *b*, clorofila *c3*, peridina y aloxantina fueron los pigmentos fitoplanctónicos más frecuentes. Los PPT DINO-1 (dinoflagelados con peridina), y los tipos de haptofitas HAPTO-6, HAPTO-7 y HAPTO-P mostraron un ciclo estacional con picos de abundancia en otoño y primavera, mientras que la abundancia de diatomeas DIATO-1 fue alta durante todo el año, y las DIATO-2 principalmente durante el invierno. Se describió un perfil de pigmentos de un grupo de primnesiófitas que poseen MVChl\_c3. Se evidenció que en este sitio los PPTs que tienen especies potencialmente tóxicas florecen bajo diferentes condiciones hidrológicas. Es probable que DINO-1 florezca en abril (otoño) con temperaturas cercanas a 18 °C y condiciones de estratificación débil, mientras que la floración de octubre (primavera) ocurre con temperaturas más bajas de 10-12 °C. DINO-4 se observó durante enero y febrero (verano), cuando la temperatura fue > 18 °C, la salinidad < 33,7 y la columna de agua mostró una estratificación máxima. En cambio, las máximas abundancias de DIATO-2 se dieron entre agosto y septiembre (invierno), en condiciones de mezcla completa, alta concentración de nitratos y baja temperatura de 10 °C. Este trabajo constituye la primera descripción de la variabilidad de la abundancia de los principales PPTs en una estación de series de tiempo costera en la plataforma del Atlántico Sudoccidental a lo largo del ciclo anual, demostrando el poder de la quimiotaconómica y CHEMTAX para realizar análisis descriptivos de un gran número de muestras.

**Palabras clave:** CHEMTAX, pigmentos fitoplanctónicos, nutrientes, dinoflagelados, floraciones algales nocivas, Atlántico Sudoccidental.

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## INTRODUCTION

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The composition of phytoplankton communities varies in time and space, spanning several taxa and orders of magnitude in cell size (Jeffrey et al. 2011). Knowing the size structure of the phytoplankton community is key to understanding the dynamics of marine food webs or oceanic processes such as carbon flow or nutrient cycling (Bouman et al. 2005; Cai et al. 2019). At the same time, knowing the succession of species that make up the community is relevant, since different taxa have toxin-producing species representing a potential danger to marine fauna, fishery resources and public health (Montoya et al. 2018; Montoya 2019; Cadaillón et al. 2024).

The concentration of chlorophyll *a* (Chl\_a) in seawater is the most used indicator of phytoplankton biomass, although it does not provide information on the community composition. On the other hand, chemotaxonomy based on the separation, identification and quantification of phytoplankton pigments diversity by high performance liquid chromatography (HPLC) is a powerful tool to study phytoplankton communities with high precision and reproducibility, overcoming some of the limitations of microscopy (e.g. underestimation of taxa not resistant to fixation or lacking identifying traits or the inability to identify cells smaller than 5  $\mu\text{m}$ ).

The theoretical foundation of chemotaxonomy is the existence of marker pigments assignable to different taxonomic levels (Jeffrey et al. 1997). Marker pigments have a restricted distribution to

an algal class, such as the carotenoid peridinin to a certain group of dinoflagellates, although in general it is a combination of pigments establishing the marker capacity. Therefore, pigment assemblages together with their typical relative concentrations allow the definition of different ‘phytoplankton pigmentary types’ (PPTs) and their applicability of pigment diversity to the study of phytoplankton taxonomy. Based on these principles, Mackey et al. (1996) developed ‘CHEMTAX’, a factor analysis to determine the relative abundance of different PPTs present in a set of samples. This analysis requires hypotheses about which PPTs are present in the samples and prior knowledge of the pigment:Chl<sub>a</sub> ratios of their marker pigments. The CHEMTAX has been widely used in different environments such as the Southern Ocean (Rodríguez et al. 2002), Pacific waters (Armbrecht et al. 2015; Larios-Muñiz et al. 2022) and the Atlantic Ocean (Carreto et al. 2003, 2008, 2016, 2018; Goela et al. 2015; Nunes et al. 2019), revealing the structure of the phytoplankton community with a qualitative and quantitative approach.

In parallel, marker pigments can be used to determine the size structure of the phytoplankton community. Considering that some taxa are represented mostly in one of the three size classes (picoplankton < 2 µm, nanoplankton 2–20 µm and microplankton > 20 µm) proposed by Sieburth et al. (1978), Vidussi et al. (2001) proposed a method to estimate the relative proportion of these three classes based on 7 diagnostic pigments (DPs). Later, Uitz et al. (2006) modified Vidussi equations by applying weighted coefficients to the same DPs, obtaining equations to calculate the contribution to the total Chl<sub>a</sub> concentration of each size class in a given sample. The ‘Uitz indices’ have been used in numerous studies and have been precursors of other similar indices applicable at a regional scale (Hirata et al. 2008; Brewin et al. 2010; Devred et al. 2011). Recently, Chase et al. (2020) proposed new equations using the same DPs but revising assumptions and reducing uncertainty in assigning PPTs to size classes, using flow cytometry for this purpose.

First studies aimed at understanding the plankton community and ecological conditions on the Argentine continental shelf, more precisely on the coast of the province of Buenos Aires, date back to the 1970s (Carreto et al. 1973) or even to some previous taxonomic studies. Thus, the dominance, from west to east, of microflagellates, diatoms and haptophytes was documented in a section in front of Mar del Plata city as the influence of Malvinas Current on the platform increases (Lange 1985). Studies on the abundance and distribution of toxic dinoflagellates gained greater importance in the region after the first toxic outbreak of paralytic shellfish poisoning (PSP) detected in the Argentine sea during the spring of 1980, causing the death of two fishermen due to ingestion of poisoned mussels (Carreto et al. 1981). At that time, a monitoring plan was established at a fixed station off Mar del Plata with the objective of investigating the levels of PSP toxicity in mussel (*Mytilus edulis*) populations as well as the relative abundance of the dinoflagellate *Alexandrium tamarense/catenella* complex. Towards the late 1990s, the mussel bank off Mar del Plata disappeared, but periodic sampling continued at the same site, capitalizing on the time series of previous ecological observations. The time series project was renamed and has been known as Estación Permanente de Estudios Ambientales (EPEA) since 2000. The EPEA station is located in the transition zone between the coastal and shelf systems on the Argentine continental shelf. Previous studies have partially reported the environmental characteristics of this fixed station (Carreto et al. 2004; Lutz et al. 2006; Viñas et al. 2013; Ruiz 2018; Ruiz et al. 2020), as well as the summer succession of pico and ultraphytoplankton (fraction between 2 and 5 µm) (Silva et al. 2009; Silva 2011). Under certain circumstances, harmful algal blooms (HABs) occur on the coast of the Argentine province of Buenos Aires, particularly species that produce paralytic, amnesic, and diarrhetic shellfish toxins (Carreto et al. 2004; Fabro et al. 2017; Montoya et al. 2020). In this region, the increase in intensity and geographical spread

of blooms of *A. tamarensis/catenella* complex and *Gymnodinium catenatum*, both species associated with PSP, have been documented (Méndez and Carreto 2018; Montoya et al. 2018). In addition, diarrhetic toxins from shellfish associated with *Dinophysis* sp. have been recorded in Villa Gesell, Buenos Aires province (Montoya et al. 2008, 2020; Sar et al. 2012) and in Uruguay (Méndez and Medina 2004; Méndez and Carreto 2018), causing bans on the extraction and marketing of bivalves. Furthermore, amnesic shellfish toxins have been produced by some toxic diatoms of the genus *Pseudo-nitzschia* in the same region (Krock et al. 2018; Méndez and Carreto 2018; Montoya et al. 2020).

In this work, the annual cycle of hydrological conditions and the composition, abundance and size structure of the phytoplankton community were characterized applying the chemotaxonomic approach (pigment diversity and CHEMTAX) at this fixed station of ecological observations on the Argentine continental shelf. Main environmental factors driving the seasonal succession of different phytoplankton groups were discussed, with emphasis on dinoflagellates and diatom groups having toxigenic species.

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## MATERIALS AND METHODS

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### Sampling at the study site

The study site (EPEA, 38° 28' S-57° 41' W) is located at 13 nmi from the coast, in the proximity of the 50 m isobath (Figure 1). Periodic sampling began in 1994 and, from 2000 onwards, studies were expanded with the incorporation of new variables such as the diversity of phytoplankton pigments, among others. Typically, water sampling was performed with 4-liter Niskin bottles associated with a CTD/carousel water sampler system at discrete depths. Sampling depths were 0 m, 5 m, the depth of maximum fluorescence, and a depth below it just near the bottom. Data from the CTD

were processed, qualified and incorporated into the BaRDO Regional Oceanographic Data Base of INIDEP (Instituto Nacional de Investigación y Desarrollo Pesquero) (BaRDO 2023) in order to obtain profiles of temperature, salinity and the Simpson parameter ( $\Phi$ ,  $\phi$ ) (Simpson et al. 1981). This parameter was used as a measure equivalent to the variation of the potential energy required to mix the water column, and used as an indicator of the degree of water column stratification. Given the large number of records representative of the entire annual cycle, Simpson values under the first quartile (Q1) were considered vertically homogeneous and values over the third quartile (Q3) were considered stratified waters.

### Determination of nutrient concentration

The macronutrients nitrate, nitrite, phosphate and silicate were analyzed using two different autoanalyzers: autoAnalyzer II Technicon (years 1994-2018) and SKALAR San++ (year 2019). The method used for the analysis of nitrate and nitrite corresponded to a modification of the original method of Armstrong et al. (1967) (Skalar Methods, Catnr. 461-032). Phosphate was determined by a modification of the original procedure of Murphy and Riley (1962) (Skalar Methods, Catnr. 503-010w/r), while silicate analysis followed the method described by Grasshoff et al. (1983) (Skalar Methods, Catnr. 563-051). Since 2013, concentration calculations have been done using Certified Reference Material (CRM), manufactured by Kan-so Technos Co., Ltd., Katano, Osaka, Japan 3.

### Determination of the concentration of phytoplankton pigments by HPLC

A total of 171 samples from different depths (0-47 m) were analyzed in the period 2000-2005 ( $n = 64$ ) and 2012-2019 ( $n = 107$ ). The method of Garrido and Zapata (1997) was used for samples from 2000 to 2001, while the method of Zapata et al. (2000) was used for samples from 2002

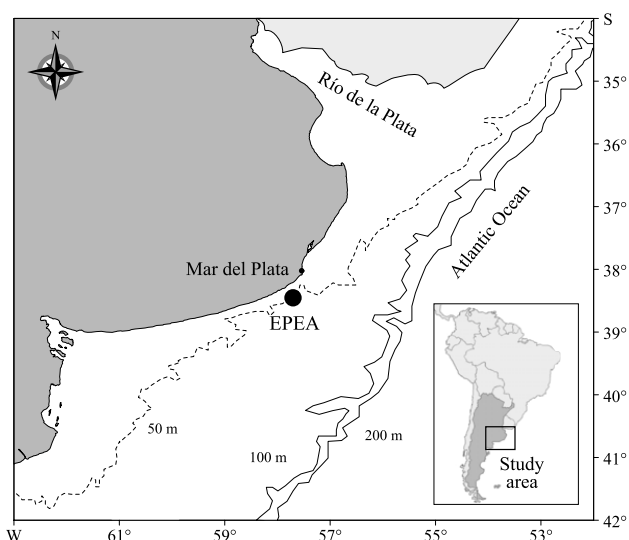


Figure 1. Study location. Former ‘Estación de Marea Roja’ currently known as ‘Estación Permanente de Estudios Ambientales (EPEA)’, 38° 28’ S-57° 41’ W.

onwards. Both methods were proved to resolve and separate chlorophyll *c*1, chlorophyll *c*2 and Mg-3,8-divinyl-pheoporphyryin a5 mono-methyl ester (MgDVP). The chromatographic method was changed after the improvement that the authors made on their previous one, since the later provides a better resolution of the divinyl and monovinyl forms of chlorophyll *a* in natural sea water samples. However, at this study site there *Prochlorococcus marinus* was not present and therefore divinyl chlorophyll *a* was never detected (see Results section, Table 3). Filters were kept in liquid nitrogen or ultra-freezer until being analyzed by HPLC. For pigments quantification, high purity standards acquired from the VKI (The International Agency for 14C Determination, Denmark) or isolated from cultures of *Emiliana huxleyi* (Haptophyta) clone CCMP 370 and the dinoflagellate *A. tamarensel catenella* complex clone MDQ 1096 were used. Those detected pigments for which the extinction coefficients were not known were quantified using the extinction coefficients of pigments containing the chromophore with the greatest similarity (e.g. chlorophyll *c*3 and non-polar chlorophylls *c* were quantified as chlorophyll equivalents *c*2).

### Chemotaxonomic analysis of the phytoplankton community (CHEMTAX)

The CHEMTAX program version 1.95 based on Microsoft Excel provided by the Australian Antarctic Data Center (<https://data.aad.gov.au>) was used. This program allowed firstly to optimize the pigment:Chl\_ *a* ratios selected for the given set of samples, and secondly, to determine the biomass of each PPT as the proportion of Chl\_ *a* concentration contributed to the sample. Samples with Chl\_ *a* < 0.3 mgm<sup>-3</sup> were excluded from the analysis since very low pigment concentration values increase the estimated error. For calculations, CHEMTAX program assumed that pigment:Chl\_ *a* ratios were constant within each phytoplankton pigment type in the entire set of samples. To satisfy this assumption, the final data set (n = 165) was divided so that environmental and therefore physiological conditions of phytoplankton were homogeneous within each subset. For this, a cluster analysis was carried out taking as classifying variables the Simpson’s parameter, temperature and nitrate concentration as the limiting nutrient. Two groups were obtained: one corresponding to the warm-stratified period (n



= 26) and another to the cold-homogeneous period ( $n = 139$ ). For each subset (winter-summer) an initial matrix of pigment:Chl\_a relationships was generated and the program was executed.

### Construction of PPT matrices and pigment relationships

The selection of PPTs included in the initial CHEMTAX matrices was based on pigments determined in samples, published microscopy information for the picoplankton fraction of the EPEA time series (Silva et al. 2009; Silva 2011) and several institutional campaign reports or unpublished data for other size fractions ('grey literature', not available to the international scientific community). The nomenclature proposed by Llewellyn et al. (2011) was used to name PPTs and pigments. Pigment:Chl\_a ratios used in the initial CHEMTAX matrices were obtained from cultures of isolated species at the EPEA station (Montoya et al. 2015), pigments analysis of monospecific blooms samples from the area (Ruiz et al. 2023) and from the literature (Table 1).

Initial ratio values were identical for winter and summer matrices, except for the PPT CYANO-2 (cyanobacteria with Zea, example: genus *Synnechococcus* sp.), which is known to show higher Zea:Chl\_a ratio values in summer.

Three groups of haptophytes were considered for the summer matrix: HAPTO-6 (e.g. *E. huxleyi*), HAPTO-7 (e.g. *Crysochromulina* sp.) and a group defined as 'HAPTO-P' to separate the contribution of a particular bloom of *Primnesium* sp. with high concentrations of MVChl\_c3 (Negri 2005; Ruiz et al. 2023; see Discussion section). The MVChl\_c3 differs from Chl\_c3 in that it has a single vinyl group, which gives it slightly more polarity and an additional absorption peak at 630 nm. These characteristics result in a shorter retention time and a different absorption spectrum, allowing these pigments to be separated using the chromatographic method of Zapata et al. (2000). Thus, MVChl\_c3 as marker pigment of HAPTO-P

was only included in the summer matrix and, in contrast, only one group of haptophytes was included in the winter matrix, HAPTO-6. Also, PPTs PRASINO-3 (Prasinophyceae with Chl\_b and Pras, e.g. *Pycnococcus provasolii*), CRYPTO-1 (Cryptophyceae with Allo e.g.: genus *Hemiselmis* sp. and *Plagioselmis* sp.) and PELAGO-1 (algae with the carotenoid But\_fuco, which includes silicoflagellates, dictioceae and pelagophytes) were included in both matrices. Two PPTs dinoflagellates containing DINO-1 (dinoflagellates with Peri, e.g. *A. tamarensis/catenella* complex and *G. catenatum*, among others) and DINO-4 (dinoflagellates with Allo e.g. *Dinophysis acuminata*, *D. caudata* and *D. tripos*) were considered, the latest only for the summer matrix. Regarding the great variety of diatom species reported, DIATO-1 (e.g. *Chaetoceros didymus*) and DIATO-2 (e.g. *Rhizosolenia setigera* and *Pseudo-nitzschia* sp.) PPTs were included, but DIATO-2 only in winter.

### Analysis of phytoplankton size structure based on DPs

To estimate phytoplankton size fractions, we compared equations of Uitz et al. (2006) with those proposed by Chase et al. (2020). This model is distinguished from that of Uitz in that it assigns half of the Chl\_b concentration to picoplankton and the other half to nanoplankton, half of the Fuco concentration to microplankton and the other half to nanoplankton, 75% of the Peri concentration to nanoplankton and only 25% to microplankton (Table 2).

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## RESULTS

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### Annual cycle of environmental properties

Water column temperature oscillated between 8 °C and 23 °C, with minimum temperatures occurring in late July, August and September, and



Table 2. Formula used to calculate phytoplankton size fractions micro ( $F_{micro}$ ), nano ( $F_{nano}$ ) and picoplankton ( $F_{pico}$ ) based on two different pigment indexes.  $DP_w$  = weighted sum of diagnostic pigments concentration ( $\text{mgm}^{-3}$ );  $DP_w = \sum_{i=1}^7 w_i P_i$ , where  $w$  = weighted weight (dimensionless) and  $P$  = pigment concentration ( $\text{mgm}^{-3}$ ).

Pigment ( $P_i$ )	Uitz indexes ( $w_i$ )	Uitz equations	Chase equations
Fuco ( $P_1$ )	1,41		
Peri ( $P_2$ )	1,41	$F_{micro} = \frac{\sum_{i=1}^2 w_i P_i}{DP_w}$	$F_{micro} = \frac{0,5 \cdot w_1 P_1 + 0,25 \cdot w_2 P_2}{DP_w}$
Hex_fuco ( $P_3$ )	1,27		
But_fuco ( $P_4$ )	0,35	$F_{nano} = \frac{\sum_{i=3}^5 w_i P_i}{DP_w}$	$F_{nano} = \frac{0,5 \cdot w_1 P_1 + 0,75 \cdot w_2 P_2 + \sum_{i=3}^5 w_i P_i + 0,5 \cdot w_6 P_6}{DP_w}$
Allo ( $P_5$ )	0,60		
Chl_b ( $P_6$ )	1,01	$F_{pico} = \frac{\sum_{i=6}^7 w_i P_i}{DP_w}$	$F_{pico} = \frac{0,5 \cdot w_6 P_6 + w_7 P_7}{DP_w}$
Zea ( $P_7$ )	0,86		

maximum between January and early March, with greater seasonal amplitude on the surface than at the bottom (Figure 2). The water column was homogeneous during the cold period (June-September,  $\varphi < Q1$ ), and slowly stratified from the beginning of spring in October until reaching the maximum values of the Simpson parameter in summer, between January and March ( $\varphi > Q3$ ) (Figure 2). Typically, salinity ranged between 33.3-33.7, although low salinity values  $< 33.5$  were registered sporadically (April-May 1998: minimum 27.25) and high values (salinity  $> 33.90$ ) were observed along the entire annual cycle. No seasonality was observed for low salinity values, while for high salinity values the highest occurrence was observed during autumn-early winter months (Figure 3).

Nitrate concentration ranged mainly between 0.010 and 5.000  $\mu\text{M}$ , although values  $> 5.000 \mu\text{M}$  were observed particularly at greater depths during the summer (December-January). Similarly, silicate also showed higher values at greater depths, ranging between 0.016 and 10.670  $\mu\text{M}$ . Regarding the nitrite ion, concentration values were lower than those of nitrate, as expected, and ranged between 0.010 and 1.910  $\mu\text{M}$ . On the contrary, phosphate did not show a large variation with depth and its

values ranged between 0.120 and 2.180  $\mu\text{M}$ . The concentration of nitrates showed a marked cyclical seasonal variation with maximum concentrations during the winter (July-August), as did that of phosphate, although this was less pronounced (Figure 4). The concentration of other macronutrients studied did not show a particular mode of evolution throughout the year, presenting variable concentrations within defined limits. It should be noted that the highest nitrate concentration values corresponded to samples obtained from greater depths during December and January, which were associated with lowest temperatures recorded throughout the entire time series. In these stations, but at lower depths, the concentration of nitrates was lower with values similar to the rest of the time series and associated with higher temperatures, which demonstrates the early establishment of the thermocline in summer (Figure 4).

### Phytoplankton pigment diversity

A total of 34 pigments were identified at the EPEA station, some of them in most of the samples and others rarely or in very low concentration (Table 3). The maximum Chl\_a concentration



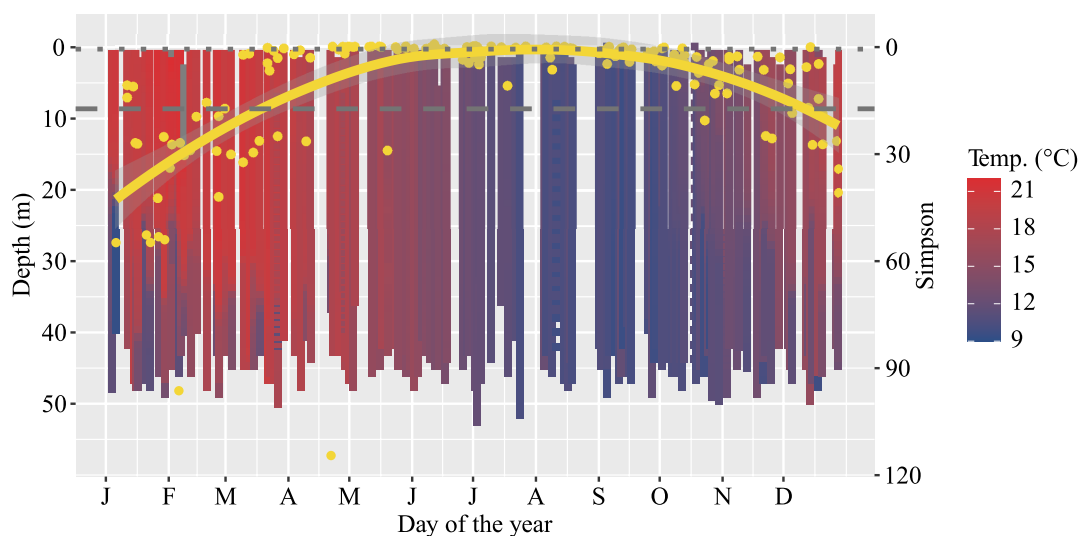


Figure 2. Water temperature as a function of depth and day of the year at EPEA station time series based on CTD data (period 1994-2019,  $n = 190$  CTD profiles). In yellow, the Simpson parameter values (points), and superimposed on them, a local weighted regression (yellow line). Dotted and dashed grey horizontal lines indicate Simpson's quartiles Q1 and Q3, respectively ( $Q1 = 1.028 \text{ Jm}^{-3}$  and  $Q3 = 34.529 \text{ Jm}^{-3}$ ). Letters on the horizontal axis indicate the beginning of the corresponding month. Temperature scale is shown in the right color bar, with reddish indicating warmer and blueish colder values.

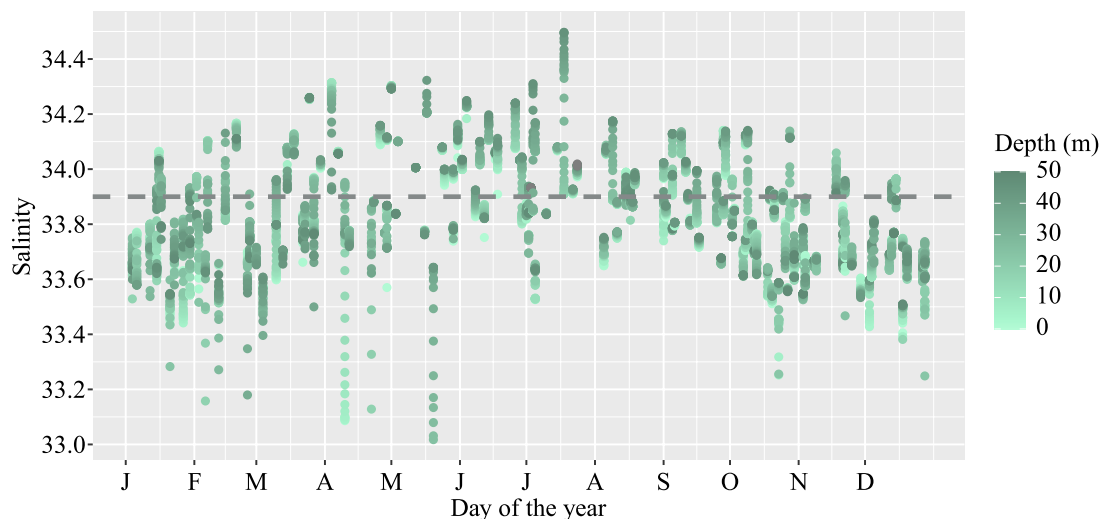


Figure 3. Salinity as a function of day of the year at EPEA station time series based on CTD data (period 1994-2019,  $n = 190$  CTD profiles). Depth (m) is expressed in the  $z$  dimension (right color bar; maximum depth at EPEA = 48 m). Letters on the horizontal axis indicate the beginning of the corresponding month.

value determined by HPLC ( $8.301 \text{ mgm}^{-3}$ ) was associated with the maximum concentration of Fuco ( $5.404 \text{ mgm}^{-3}$ ; the main diatom marker) and also with the maximum of Allo (marker pigment

for cryptophytes and dinoflagellates of the DINO-4 PPT) during April 2019, in what appeared to be a bloom dominated by more than one phytoplankton group. In contrast, the second highest

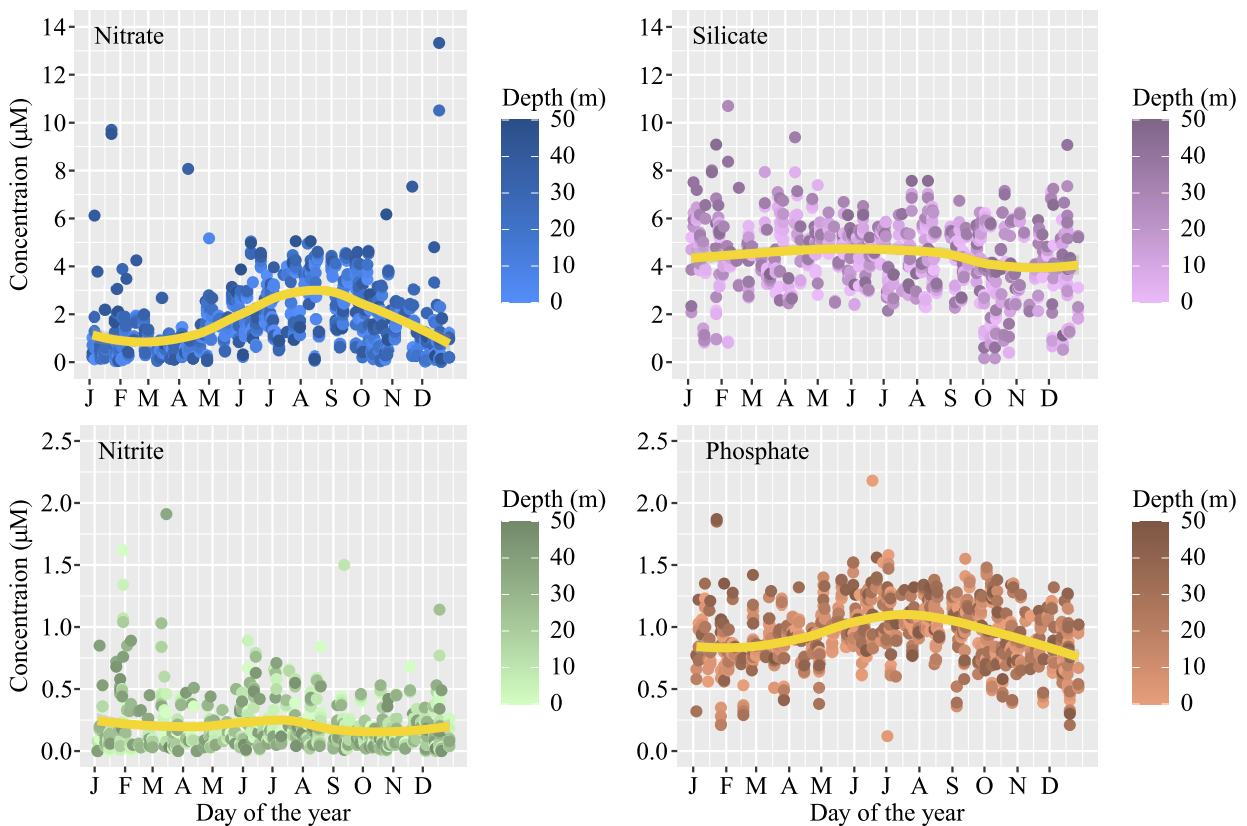


Figure 4. Concentration of main macronutrients determined at EPEA station in the period 1994-2019 ( $n = 934$ ). Sampling depth (m) is expressed in the  $z$  dimension (right color bar; maximum depth at EPEA = 48 m). Orange line corresponds to a local weighted regression. Letters on the horizontal axis indicate the beginning of the corresponding month.

recorded concentration of Fuco ( $4.498 \text{ mgm}^{-3}$ ) occurred in a late spring bloom in November 2005, associated with high concentrations of MVChl\_c3 ( $0.510 \text{ mgm}^{-3}$ ) and low concentrations of Hex\_fuco ( $0.060 \text{ mgm}^{-3}$ ). Regarding Peri, the unequivocal marker pigment for dinoflagellates of the DINO-1 PPT, the maximum recorded concentration ( $3.236 \text{ mgm}^{-3}$ ) occurred in October 2019 and it was followed in magnitude by the record of April 2019 ( $0.563 \text{ mgm}^{-3}$ ). Chl\_b, present in green algae, and Pras, present in prasinophytes, were found at very high frequency (Table 3). Although pigment sampling at this study site was stopped between 2005 and 2012, these values were representative of the relative magnitude of bloom events at this coastal location. The Chl\_a

annual cycle showed higher concentration values in winter (Figure 5), and Fuco presented the same seasonal pattern. On the other hand, Peri concentration followed a bimodal cycle, increasing notably in autumn and spring. Hex\_fuco, marker pigment for haptophytes, also showed a bimodal pattern, although its concentration was on average 50% lower than that of Peri. For its part, both Zea (marker pigment for cyanobacteria) and But\_fuco increased their concentration during summer months (December, January and February), when the water column begins to get stratified. It should be noted that, except for special events, the Chl\_a concentration range throughout the year was small and, therefore, that of the other pigments even smaller.

Table 3. Average concentration ( $\text{mgm}^{-3}$ ) and frequency (N) of phytoplankton pigments detected in the study site in the period 2000-2005 and 2012-2019. Conventional name and international abbreviation used in this work were indicated. Pigments were arranged in descending order of frequency of appearance at the study site.

Pigment	Abbreviation	Mean concentration	Standard deviation	Minimum	Maximum	N
Chlorophyll <i>a</i>	Chl_a	1.012	0.916	0.157	<b>8.301</b>	171
Fucoxanthin	Fuco	0.430	0.632	0.038	<b>5.404</b>	170
Chlorophyll <i>c2</i>	Chl_c2	0.152	0.206	0.009	1.732	167
19'-hexanoyloxyfucoxanthin	Hex_fuco	0.07	0.049	0.004	0.294	161
Diadinoxanthin	Diadino	0.087	0.136	0.007	1.553	160
Chlorophyll <i>b</i>	Chl_b	0.080	0.052	0.004	0.297	159
Chlorophyll <i>c3</i>	Chl_c3	0.044	0.047	0.003	0.367	159
Peridinin	Peri	0.108	0.275	0.006	<b>3.236</b>	149
Alloxanthin	Allo	0.038	0.070	0.001	<b>0.782</b>	137
19'-butanoyloxyfucoxanthin	But_fuco	0.027	0.033	0.001	0.247	132
Magnesium divinylpheoporphyrin	MgDVP	0.015	0.022	0.002	0.230	128
Prasinoxanthin	Pras	0.021	0.017	0.002	0.118	124
Neoxanthin	Neo	0.015	0.009	0.002	0.049	120
Chlorophyll <i>c1</i>	Chl_c1	0.031	0.042	0.001	0.283	119
$\beta\beta$ -carotene	b_Car	0.025	0.039	0.002	0.301	114
Zeaxanthin	Zea	0.028	0.070	0.001	<b>0.720</b>	112
Violaxanthin	Viola	0.015	0.009	0.001	0.049	108
4-keto-19'-hexanoyloxyfucoxanthin	Hex_kfuco	0.016	0.012	0.001	0.055	98
Non-polar chlorophyll of <i>Emiliana huxleyi</i>	Chl_c2_MGDG [18:4/14:0]	0.013	0.024	0.001	0.200	92
Chlorophyll <i>a</i> allomer	Chla_allomer	0.066	0.153	0.001	1.020	90
$\beta\epsilon$ carotene	a_Car	0.010	0.008	0.001	0.035	79
Diatoxanthin	Diato	0.026	0.078	0.001	0.653	76
Chlorophyll <i>a</i> epimer	Chla_epimer	0.020	0.022	0.003	0.137	59
Lutein	Lut	0.010	0.008	0.001	0.041	57
Non-polar chlorophyll of <i>Chrysocromulina</i> sp.	Chl_c2_MGDG [14:0/14:0]	0.007	0.014	0.001	0.071	48
Chlorophyllide <i>a</i>	Chlide	0.061	0.254	0.001	1.622	40
Dinoxanthin	Dino	0.014	0.021	0.002	0.128	40
Antheraxanthin	Anth	0.034	0.093	0.002	0.480	26
Monovinyl chlorophyll <i>c3</i>	MVChl_c3	0.047	0.110	0.001	0.510	26
Methylchlorophyllide <i>a</i>	MeChlide	0.073	0.144	0.002	0.479	10
Pheophytin	Phe	0.136	0.173	0.011	0.463	6
Perididinol	perididinol	0.025	0.010	0.011	0.033	4
DehydroLutein	dehidroLuteina	0.012	0.004	0.008	0.015	3
Uriolide	Uri	0.015	0.006	0.009	0.021	3

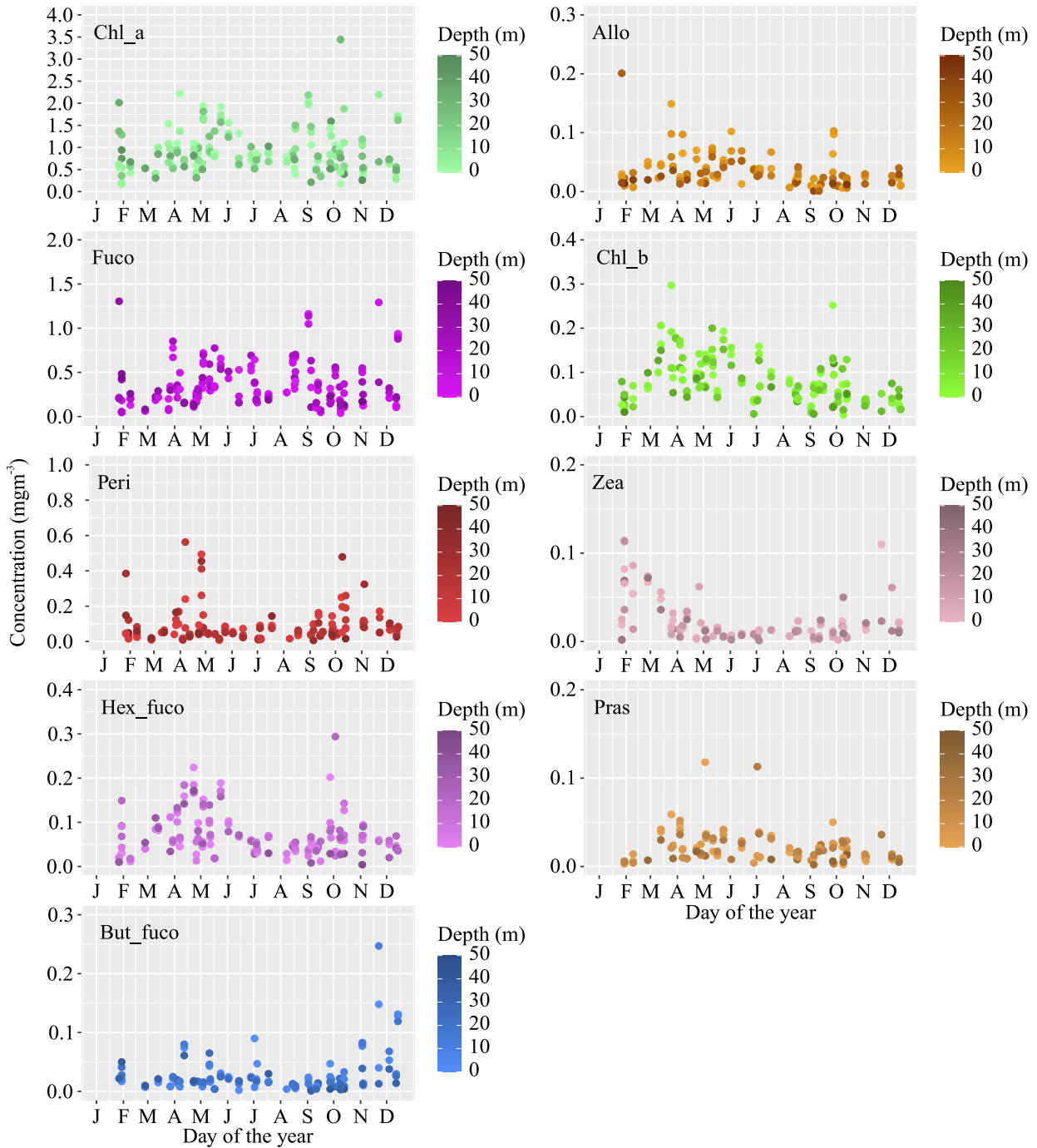


Figure 5. Annual cycle of main phytoplankton pigments concentration found at EPEA station. Note differences in the concentration scale of each pigment. Maximum values corresponding to infrequent bloom events were excluded for greater appreciation of the variability throughout the year (see Table 3). Letters on the horizontal axis indicate the beginning of the corresponding month.

### Phytoplankton community size structure

The models of Uitz et al. (2006) and Chase et al. (2020) estimated different percentages of the phytoplankton size fractions (micro, nano and picoplankton) throughout the annual cycle based on the analysis of DPs (Figure 6). Uitz's model estimated that microplankton makes up on average 80% of the community throughout the year, with a slight decrease towards summer, while Chase's model estimated an average of 40% for this fraction. According to Chase, nanoplankton was the dominant fraction throughout the annual cycle (average 60%), which was more than double of that estimated by the Uitz model (20%). Both models agreed that picoplankton reached its maximum in January-February (making up to 40%) and hovers around 10-20% during the rest of the year.

### Annual cycle of phytoplankton pigmentary types estimated by CHEMTAX

Only two CHEMTAX cycles were enough to optimize the pigment:Chl<sub>a</sub> ratios in each matrix until

reaching acceptable residual levels of pigments according to the standards established in the method (Mackey et al. 1996). The Chl<sub>a</sub> concentration contributed by each PPT in each sample was then estimated by CHEMTAX and the relative contribution of each PPT over the annual cycle was calculated (Figure 7). During winter months (June-August), biomass was dominated by diatoms, contributing more than 50% of Chl<sub>a</sub>, with the greatest contribution from PPT DIATO-2, i.e. diatoms containing Chl<sub>c3</sub>. DIATO-2 abundance was relatively higher at greater depths than DIATO-1. As spring progresses, diatoms decline and dinoflagellates with peridinin, the PPT DINO-1, increases their representation, even reaching levels of up to 90% of total Chl<sub>a</sub>. Altogether, different types of haptophytes (HAPTO-6, HAPTO-7 and HAPTO-P) also thrived at this time of year, sustaining levels close to 20% of the total Chl<sub>a</sub> throughout the summer. In the summer period, other groups such as CYANO-2, formed by cyanobacteria of the type *Synechococcus* sp. increased their biomass, especially in December-January, although their contribution occasionally exceeds 10% of the total Chl<sub>a</sub>, and

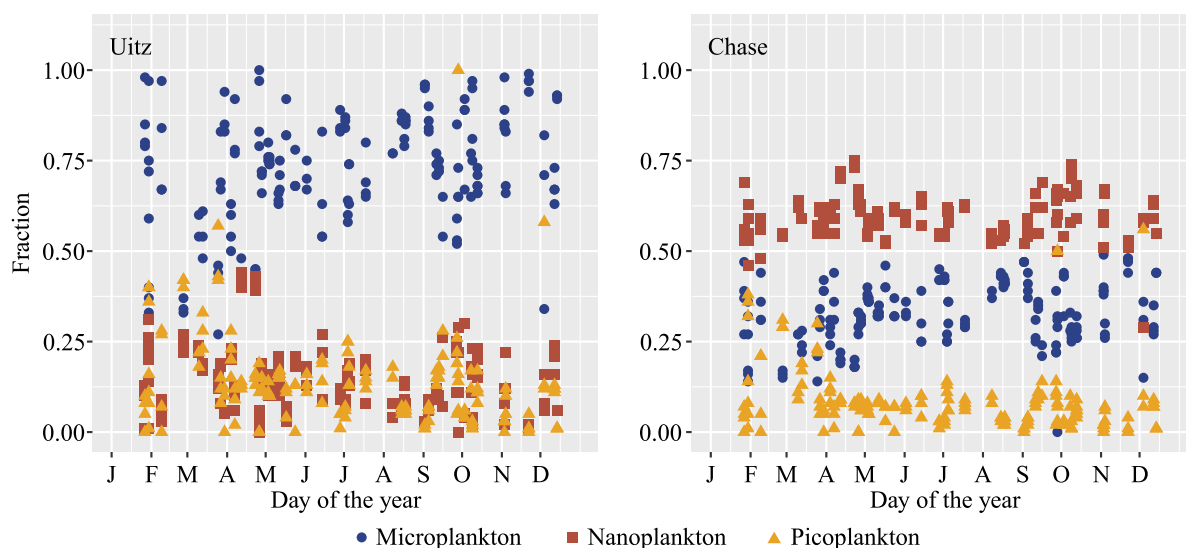


Figure 6. Annual cycle of phytoplankton size fractions estimated indirectly from pigment indices and the equations proposed by Uitz et al. (2006) and Chase et al. (2020). Each fraction is expressed as a contributed percentage of total Chl<sub>a</sub> concentration in each sample. Letters on the horizontal axis indicate the beginning of the corresponding month.

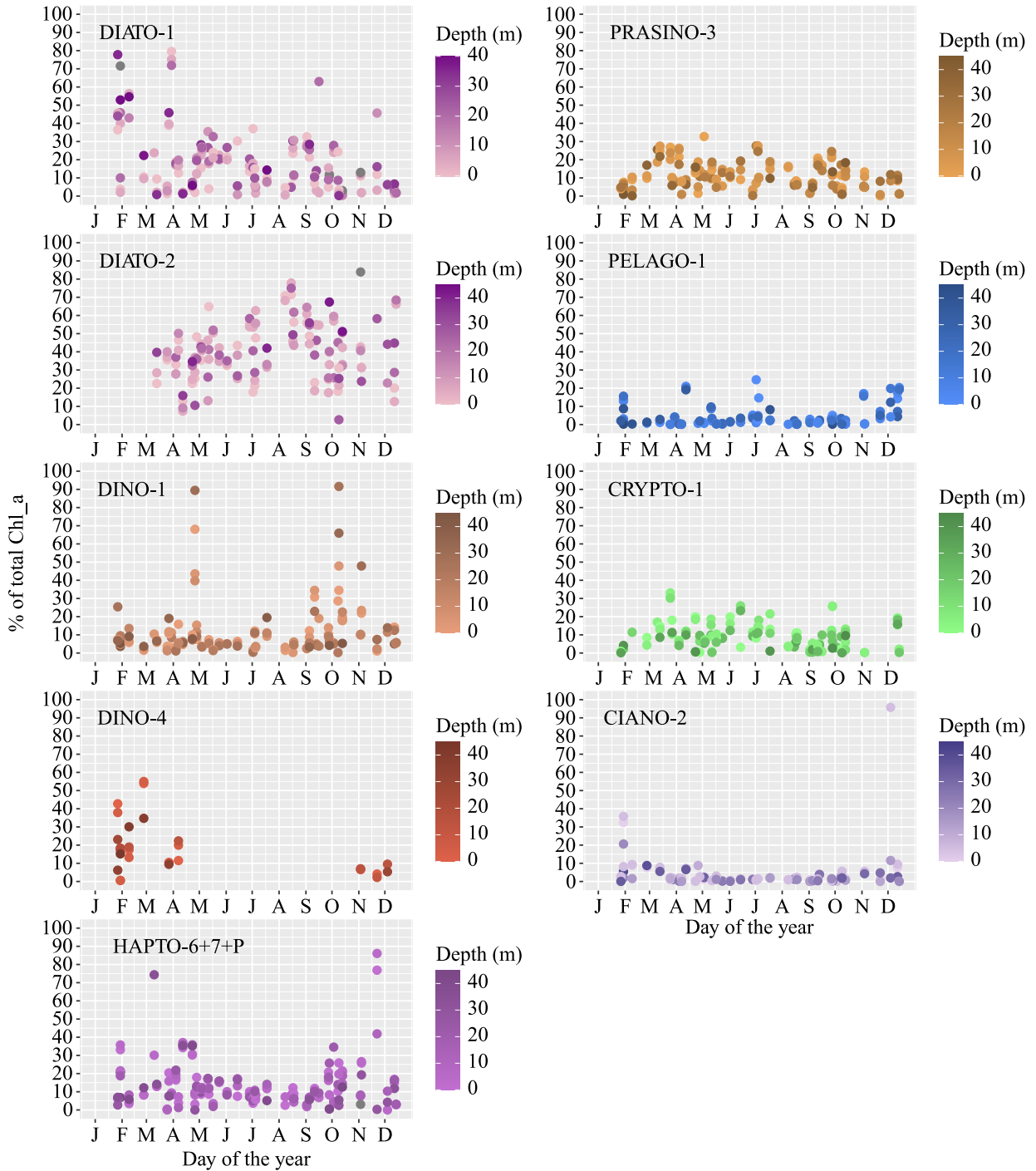


Figure 7. Annual cycle of different phytoplankton groups defined as phytoplankton pigmentary types (PPTs) expressed as relative contribution to the total Chl<sub>a</sub> concentration in each sample. Letters on the horizontal axis indicate the beginning of the corresponding month.



dinoflagellates with Allo, such as DINO-4, can represent up to 40% of the total Chl<sub>a</sub>. Other groups such as PRASINO-3, CRYPTO-1 and PELAGO-1 were present throughout the year, contributing on average 25% of the total biomass.

The CHEMTAX analysis revealed the relative abundance of three PPTs to which potentially toxin-producing phytoplankton species belong, which were maximum under different hydrological conditions (Figure 8). The PPT DINO-1 (represented by the paralyzing shellfish toxin producers *G. catenatum* and *A. tamarense/catenella* complex), presented an autumn bloom during April when the concentration of nitrate begins to increase after summer consumption, which is characterized by temperatures close to 18 °C, salinity in the range of 34.0-34.2, and weak stratification conditions. In contrast, DINO-1 spring bloom takes place during October at lower temperatures of 10-12 °C and slightly lower salinity of 33.8-34.0, under conditions where the column begins to stratify and nitrate concentration is not limiting. On the contrary, the abundance of PPT DINO-4, group housing the genus *Dinophysis* sp. to which several species producing lipophilic toxins belongs, was highest during the summer months of January and February when temperature exceeded 18 °C, salinity was > 33.7 and the water

column presented maximum stratification. Finally, DIATO-2 (PPT containing potentially DA producer species such as *Pseudo-nitzschia* sp.), had maximum abundances at the end of winter (August and September) under conditions where the column was completely mixed, nitrate concentration was high, average temperature was 10 °C and salinity was between 33.8-34.0.

## DISCUSSION

The composition and size structure of the phytoplankton community has been characterized through the chemotaxonomic approach in a coastal site throughout the annual cycle in relation to environmental conditions.

Located at 27 nmi from Mar del Plata city, the study site has a marked seasonal pattern of thermocline formation and rupture, with incident irradiance being one of its main drivers (Carreto et al. 2004; Lutz et al. 2006; Viñas et al. 2013; Ruiz 2018; Ruiz et al. 2020). Stratification breakdown occurs more slowly than the thermocline establishment. The EPEA station is bathed by a single water mass, the Subantarctic Shelf Waters (ASAP), which is

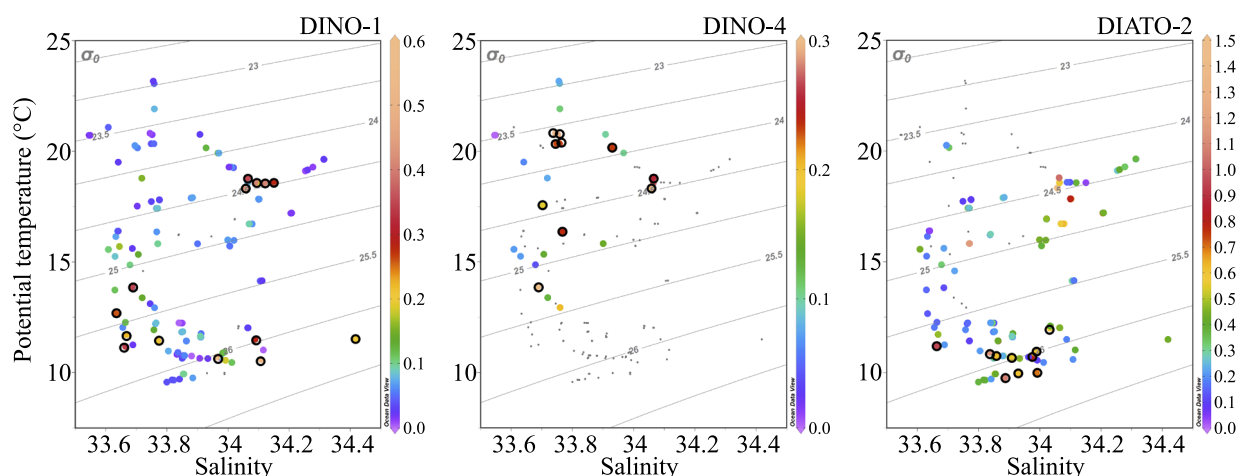


Figure 8. Relative abundance of PPTs having toxin-producing species recorded at the EPEA station during the study period, expressed as the contribution to the total concentration of Chl<sub>a</sub> (mgm<sup>-3</sup>) in the temperature-salinity diagrams.

modified as it travels along the continental shelf (Guerrero and Piola 1997). At the study site, salinity values  $> 33.9$  were observed during fall-early winter, denoting the influence of high salinity coastal waters originating in the summer in the San Matías Gulf due to excess evaporation over precipitation and advected to these latitudes. Further, the influence of low-salinity diluted waters was observed in different years, driving salinity values at this latitude to  $< 33.4$ , even at depth. Apart from these two phenomena, no other phenomenon establishing seasonality in salinity was observed at the EPEA station. Nutrient enrichment is provided by ASAP, as well as vertical mixing taking place mainly during July-September. Therefore, nitrate concentration follows this cycle with higher values during winter and lower in summer, mainly due to spring consumption of phytoplankton. Silicate and phosphate concentrations vary little throughout the year and do not appear to limit phytoplankton growth.

It has been shown that the period between June and October coincides with the lowest Simpson parameter values, coldest temperatures and higher nitrate concentrations. Also, the depth of the euphotic zone is minimum during these months at this study site (Lutz et al. 2006; Ruiz 2018; Ruiz et al. 2020). These conditions seem to support the development of diatoms groups (DIATO-1 and DIATO-2), which dominated phytoplankton biomass in terms of Chl\_a concentration contribution. The predominance of diatoms is typical of temperate seas, similar to what was previously described for this site (Carreto et al. 2004; Negri and Silva 2011). Despite the relatively low light availability, diatom growth could be favored by strong vertical mixing driving cells towards the illuminated layer with a frequency high enough to allow growth. The total biomass of phytoplankton decreases in October as the thermocline is established and nutrient consumption progresses. In turn, the relative contribution of various PPTs increases, resulting in a more diverse community. On average, the contribution of DIATO-1 and DIATO-2 to phytoplankton biomass start to decrease, while that of other groups be-

comes more conspicuous, mainly DINO-1, DINO-4 and haptophyte groups. It is known that under conditions of high irradiance and low nutrient availability, smaller cells outperform larger ones by having a higher surface/volume ratio, giving them greater efficiency in nutrient uptake. Consequently, a change in community size structure toward summer can be suspected.

Two pigment-based size indices have been used to examine the size structure of the phytoplankton community. The main difference between the two is that the Chase index considers the contribution of diatoms and dinoflagellates to the nanoplankton fraction (Chase et al. 2020). According to differences in their equations, these indices showed different results regarding micro- and nanoplankton fractions, but, contrary to expectations, none showed seasonal variability. However, it can be assumed that the phytoplankton community presents a change in size structure considering the observed increase in the concentration of typical pigments of nanoplankton species (Hex\_fuco, Zea, Chl\_b, But\_fuco, peri and Fuco) during the stratified period. This, in turn, was reflected in the relatively higher abundance during this period of the haptophyte PPTs, PELAGO-1, CYANO-2, DINO-1, and CRYPTO-1. Likewise, previous studies demonstrated that some bio-optical parameters associated with small cells also showed relative increases during the stratified period at the study site (Ruiz et al. 2020). The relative abundance of PPTs to which cyanobacteria and cryptophytes belong was higher in summer, as previously observed at the EPEA station (Silva et al. 2009; Silva 2011). The HAPTO-6, HAPTO-7 and HAPTO-8 and PELAGO-1 groups, among others, made a greater contribution to the phytoplankton biomass during the stratified period. When considering that the average 60 % estimated by the model of Chase et al (2020) for the nanoplankton fraction is composed primarily of diatoms, dinoflagellates, haptophytes, and pelagophytes, it appears to be more approximate than that of Uitz et al. (2006) to estimate the size fractions using DPs. This is because haptophytes correspond to the nano

and picoplankton fraction. However, these models will need to be validated with direct estimates of cell size obtained through microscopy or new optical analysis techniques such as flow imaging.

Some blooms events were registered at the EPEA station during the study. A multispecies algal bloom occurred in autumn 2019, with diatoms (DIATO-2 and DIATO-1) accounting for nearly 70% of the Chl<sub>a</sub> produced, followed by DINO-4 which contributed 22% of Chl<sub>a</sub> and DINO-1 with 5%. Fuco was mainly contributed by diatoms of the genus *Chaetocelos* sp., including *C. curvisetus*, *C. decipiens* and *C. danicus*, while Allo was due to *D. tripos*, a species that produced PTX2 and sec PTX2 (Negri 2019). Regarding DINO-1 dinoflagellates, the spring bloom in October 2019 was dominated by an unarmored dinoflagellate of the genus *Gymnodinium* sp. (Ruiz et al. 2023), and the autumn bloom of 2000 was dominated by the *A. tamarensis/catenella* complex with paralyzing toxin detected in mussels collected at the site (Carreto et al. 2004). Only once an extraordinary growth of *Synechococcus* sp. was observed in early summer (Ruiz et al. 2020), which explains the high levels of Zea concentration registered at the surface layer. Regarding the late spring bloom in November 2005, associated with high MVChl<sub>c3</sub> and low Hex<sub>fuco</sub> concentrations, Negri (2005) reported the dominance of an indeterminate species of *Prymnesium* sp. (Prymnesiophyceae), a haptophyte that caused a bright green surface discoloration with concentrations of at least  $12 \times 10^6$  cells l<sup>-1</sup> and characterized by a ‘... total absence of diatoms...’ (Negri 2005). Zapata et al. (2004) described eight different haptophyte PPTs based on their pigment suits and pigment:Chl<sub>a</sub> ratios. According to this study, Prymnesiophyceae belongs to HAPTO-4 (lacking Hex-fuco and MVChl<sub>c3</sub>) and *E. huxleyi*-type coccolithophorids belong to HAPTO-6 (with MVChl<sub>c3</sub> and Hex-fuco, among other pigments). In this event, a high concentration of MVChl<sub>c3</sub> was determined, which is a characteristic pigment of HAPTO-6 but absent in HAPTO-4. For this reason, the PPT ‘HAPTO-P’ was defined to resolve the contribution to the to-

tal Chl<sub>a</sub> concentration of samples from this event. Evidently, this is a type of prymnesiophyte having MVChl<sub>c3</sub>, since in the presence of HAPTO-6 in the sample was ruled out based on the taxonomic determination (Negri 2005) and also due to the Hex<sub>fuco</sub>:Chl<sub>a</sub> ratio observed, which was much lower than expected for the group ( $0.010 < 0.629$ ). To our knowledge, this is the first record of a prymnesiophyte possessing MVChl<sub>c3</sub>.

The annual cycle of two different groups of dinoflagellates at EPEA station was revealed by the CHEMTAX. On the one hand, PPT DINO-1, which groups species with carotenoid Peri as an unequivocal marker, and on the other, DINO-4, a group including dinoflagellates with Allo. Both PPTs host, on the one hand, the main harmful species described for the coast of Buenos Aires province, *A. tamarensis/catenella* complex and *G. catenatum*, producers of PSP, and on the other, the genus *Dinophysis* sp., producer of lipophilic toxins, respectively. Dinoflagellates contribution to total Chl<sub>a</sub> was considerably lower than that of diatoms, partly due to their lower abundance and also to the presence of smaller cell-sized nanoplanktonic dinoflagellates (pers. comm. R. Silva).

As in previous works (Carreto et al. 2004), a bimodal cycle was revealed for the PPT DINO-1, an autumn bloom just before the beginning of the of greatest vertical mixing period, and another in spring coinciding with the beginning of the heating and stratification of the water column. Environmental conditions are very different at these two moments of the annual cycle. The autumn bloom at the EPEA station may be dominated by *G. catenatum*, whereas in the spring bloom *A. tamarensis/catenella* complex (Méndez and Carreto 2018) dominates, although this should be fully confirmed by more in-depth taxonomy studies across the entire time series. Previously, between 1994 and 1997, Akselman et al. (1998) associated the autumn PSP toxicity peak in *M. edulis* mussels with *G. catenatum* at a fixed station off Mar del Plata, also observing the absence of *A. tamarensis/catenella* complex for this time of the year. Méndez

and Carreto (2018) described the ecology of these two species for the Río de la Plata front. Long-term studies have recorded several *G. catenatum* blooms on the Uruguayan coast with temperatures between 21.8 °C and 24.0 °C in late summer-autumn. These blooms eventually spread to higher latitudes along the Argentine coast. That is, *G. catenatum* often blooms at lower latitudes than the EPEA associated with relatively warm temperatures. Meanwhile, the growth of *A. tamarense/catenella* complex may be favored by periods of calm winds and high solar irradiation, such as those prevailing in spring at the study site (Carreto et al. 1993). The germination of resting cysts results in the first planktonic forms of *A. tamarense/catenella* complex in this region at the end of winter. This process is controlled by endogenous biological mechanisms synchronized by certain environmental factors, resulting in high interannual variability. However, several other dinoflagellate species with Peri are known (i.e. belonging PPT DINO-1), such as *Azadinium* sp., a genus capable of synthesizing azaspiracid phycotoxins (AZAs). *Azadinium* sp. was recorded in a broad range of environmental conditions along the southwestern Atlantic, including this study site (Akselman and Negri 2012; Akselman et al. 2014). Also, an extraordinary bloom of an unarmored *Gymnodinium* sp. was registered in October 2021 in the Argentine continental shelf, with Peri concentration of up to 69.197 mgm<sup>-3</sup> on the surface (unpublished data).

Furthermore, the CHEMTAX effectively estimated the relative abundance of PPT DINO-4 (dinoflagellates with Allo). Interestingly, the highest abundance of DINO-4 was recorded throughout 2019. Negri (2019) reported the presence of *D. tripos* in summer close to the depth of the thermocline in the EPEA station. It has been documented that *Dinophysis* sp. growth is favored under conditions of high thermohaline stratification and persistent winds (Escalera et al. 2006, and references therein). On that occasion, cell abundance reached 36,700 cells l<sup>-1</sup> and toxins pectenotoxin PTX2 and secacid PTX2 linked to the presence of *D. tripos*

were recorded, while dinophysis toxins were not detected. It should be noted that PTXs have been removed from health standards for live bivalve mollusks since 2021 in accordance with current legislation, as the European Food Safety Authority (EFSA) recently concluded that there are no reports of adverse effects associated with PTXs in humans.

Theory behind CHEMTAX has been described extensively (Mackey et al. 1996). CHEMTAX main limitation lies in the fact that it is very sensitive to the initial ratio matrix. Small differences in initial pigment:Chl<sub>a</sub> ratio values are not sufficient to estimate with good approximation the abundance of PPTs sharing the same pigment arrangement, such as haptophytes and diatoms, or haptophytes and pelagophytes. However, CHEMTAX's performance improves by applying various strategies in its execution. On the one hand, the RSM decreases if unambiguous marker pigments are included in the matrix (for example, gyroxanthin diester to estimate *Karenia brevis*, or as described above, MVChl\_c3 to separate HAPTO-P), as well as if the pigments present in all groups are excluded, such as Chl\_c2. Another strategy is to separate the samples into different run cycles based on the presence/absence of certain marker pigments and assemble the ratio matrices accordingly (e.g. run samples lacking Chl\_c1 on a matrix that does not include DIATO-1). In this way, the probability that CHEMTAX assigns part of the total Chl<sub>a</sub> to a PPT absent in the samples is reduced to zero. Finally, starting from the best possible approximation of pigment:Chl<sub>a</sub> ratio values in each group improves the performance of the method. For example, pigment:Chl<sub>a</sub> ratios in different haptophyte groups vary in very high ranges according to different physiological conditions (Schlüter et al. 2000; Zapata et al. 2004). In the present study, pigment:Chl<sub>a</sub> ratios were determined from cultures or from *in situ* samples corresponding to blooms from the region (Ruiz et al. 2023), obtaining good results with CHEMTAX (low RMS in only two execution cycles). In summary, CHEMTAX should be regarded as a 'thick brush' tool for distribution

and biogeography studies that provides a very good approximation to the estimation of relative abundances, but not a way to discriminate down to the species level (except for a few particular cases).

Phytoplankton pigments constitute many more compounds than chlorophyll *a* that can be applied to study phytoplankton diversity or develop better primary production models. In recent years, several works have addressed the estimation of phytoplankton pigments from the remote sensing of ocean color. Without a doubt, the combination of the chemotaxonomic approach and the information from recently launched hyperspectral remote sensors will contribute to improve algorithm applicable to the study of the biogeography of different phytoplankton taxa, especially in sites where the early detection of potentially harmful blooms is desired. Although the toxicity of an event cannot be determined remotely, in the medium term the combination of these tools will be very useful to contribute to early warning systems of potentially harmful blooms for society and the ecosystem in general.

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## CONCLUSIONS

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The present work is the first description of the composition of the phytoplankton community applying the chemotaxonomic approach (HPLC + CHEMTAX pigments) at the EPEA station, currently the longest time series of ecological observations in the southwestern Atlantic Ocean. Chemotaxonomy is shown to be a powerful tool for rapid and reproducible estimation of the relative abundance of different classes of phytoplankton in a large number of samples, including groups of interest for having toxin-producing species. Without a doubt, this tool will allow studying the ecology of harmful or toxic phytoplankton groups and making contributions to the knowledge of their seasonal or interannual variation in time series as well as in other coastal marine environments.

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## Author contributions

M. Guillermina Ruiz: conceptualization; software; formal analysis; investigation; data curation; writing-original and final draft; visualization. M. Belén Mattera Coy: data analysis; methodology. Mario C. Carignan: sampling; formal analysis; investigation; software; data curation. Macarena Albornoz: sample analysis; data curation; formal analysis. Graciela N. Molinari: software; validation; formal analysis; investigation; supervision. Nora G. Montoya: conceptualization; methodology; formal analysis; investigation; resources; writing-review and editing; supervision; project administration.

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