# Seasonal and interannual variability of phytoplankton community structure in a Mediterranean coastal site

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ABSTRACT: We studied phytoplankton community structure in surface waters of the fixed coastal station of the Blanes Bay Microbial Observatory (NW Mediterranean Sea). A chemotaxonomic approach based on HPLC analysis of phytoplankton pigments, followed by CHEMTAX algorithm implementation, was applied to a set of monthly samples taken during a 14 yr period (2000-2014). Additional samples were taken for nutrient analyses, flow cytometric measurements and during part of the period for phytoplankton cell counts by optical microscopy. Overall, the most abundant groups in terms of chlorophyll a (chl a) were haptophytes, diatoms and prasinophytes. In general, diatoms were the most important components of the total chl a maxima (T\_Chl a). We observed a marked seasonality of T\_Chl a and several phytoplankton groups (prasinophytes, diatoms, haptophytes, cryptophytes and pelagophytes) with autumn-winter or winter-spring maxima and summer minima, coinciding with similar variation in major nutrient concentrations. Prochlorococcus presented a fall-winter maximum and a spring-summer minimum, while Synechococcus peaked in April and August, and dinoflagellates were relatively important in summer. Superimposed to this general pattern, prasinophytes and diatoms responded positively to episodic fertilization events associated with freshwater runoff caused by rain storms. Most phytoplankton groups presented a decreasing linear interannual trend that could be associated with a reduction in nutrient availability. A possible driver for this oligotrophication is the improvement of wastewater treatment in the region.

KEY WORDS: Mediterranean  $\cdot$  Blanes Bay  $\cdot$  Phytoplankton  $\cdot$  Community structure  $\cdot$  HPLC  $\cdot$  Pigments  $\cdot$  Time series

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## INTRODUCTION

Phytoplankton organisms constitute a crucial link in marine ecosystem dynamics. They contribute about half of the world's total primary production, are the main base of the food web of ocean communities and represent a key component of nutrient cycling and particle fluxes from surface to deep waters (Ryther 1969, Eppley & Peterson 1979). Furthermore, phytoplankton plays a key role in climate processes, contributing to the biological carbon pump and helping to remove the anthropogenic carbon liberated to the atmosphere (Sabine & Feely 2007).

Phytoplankton distributions are strongly influenced by abiotic factors, such as turbulence, temperature, irradiance and nutrient availability (Margalef 1978), and by interactions with other biological components of the food web. Classically, most studies on the composition of phytoplankton have been conducted by means of microscopic examination, which is time-consuming, requires a high level of taxonomic skill and is only adequate for the larger forms (>10  $\mu$ m). For groups in the picoplankton (<2  $\mu$ m) and small nanoplankton (<5 µm) size fractions, techniques such as flow cytometry (FC) and epifluorescence microscopy (Porter & Feig 1980, Marie et al. 2001) have enhanced knowledge and understanding of phytoplankton morphotypes, although with low taxonomic specificity. Recently, molecular techniques have provided new possibilities for the assessment of microbial biodiversity, but molecular information still needs to be related to the morphological counterpart (e.g. Amato et al. 2007).

Another alternative, the application of chemical taxonomy methods has been implemented and progressively improved over the past 20 yr. Accurate and relatively fast determination of chlorophyll *a* (chl *a*) and other phytoplankton pigments can be carried out by means of high performance liquid chromatography (HPLC), even for samples collected from very oligotrophic areas. In a given sample, chl *a* concentration provides an estimate of total phytoplankton biomass, while pigment composition patterns can be used to derive quantitative information on the spatio-temporal variability of the coarse taxonomic structure of the phytoplankton communities, including the contribution of small-sized cells that cannot be reliably identified by microscopy.

The presence and relative contribution of the different phytoplankton pigments depends on the composition of the community. Some pigments are unambiguous markers of certain phytoplankton groups; for example, prasinoxantin is only found in prasinophytes, peridinin in dinoflagellates, divinyl-chlorophyll *a* in *Prochlorococcus* and alloxantin in cryptophytes (Jeffrey et al. 1997, Mackey et al. 1996, Roy et al. 2011). Other pigments, however, occur in several classes of phytoplankton, such as fucoxanthin, found in diatoms, haptophytes and pelagophytes, among others (Roy et al. 2011).

The lack of unique markers for some groups and the presence of shared pigments in others makes it difficult to estimate the abundance of all algal groups using pigment composition. A useful approach to this problem is based on the implementation of the CHEMTAX software program (CHEMical TAXonomy: CHEMTAX), developed by Mackey et al. (1996) to estimate the contribution of different algal classes to the total chl *a*, based on the pigment data. The calculations start with one or several initial chl *a*:pigment ratio matrices for the selected phytoplankton groups (Latasa 2007). Next, the program performs iterations to optimize the proportion of chl *a* accounted for by the phytoplankton groups considered.

Phytoplankton composition varies in response to seasonal and other sources of environmental vari-

ability, and tracking community composition in space or along a temporal series provides crucial information about potential ecological effects of natural and anthropogenic perturbations, including climate change. HPLC pigment analysis and subsequent application of CHEMTAX allow the processing of the high number of samples needed in these studies (Millie et al. 1993). Although this approach estimates phytoplankton biomass in terms of pigments rather than carbon content, which may be the desired variable for some applications, a number of studies have shown the usefulness of chl *a* concentration as a predictor of phytoplankton carbon biomass estimated from cell counts and biovolume measurements (Schlüter et al. 2000, Lionard et al. 2008, Mendes et al. 2016).

In this study, we use HPLC to determine the concentration of phytoplankton pigments in samples collected at the time-series station of Blanes Bay (NW Mediterranean) between 2000 and 2014 (Gasol et al. 2012), and we apply CHEMTAX to derive the contribution of major phytoplankton chemotaxonomic groups to total chl a. Blanes Bay is a temperate, coastal environment with typical Mediterranean seasonality and strong physico-chemical and biological variability (Mura et al. 1996, Calbet et al. 2001, Schauer et al. 2003, Lucea et al. 2005, Alonso-Sáez et al. 2007, Guadayol et al. 2009, Gutiérrez-Rodríguez et al. 2011). The site is oligotrophic with an estimated annual integrated primary production of 48 g C m<sup>-2</sup> yr<sup>-1</sup> (Gasol et al. 2016). Nutrient availability is driven mainly by the mixing-stratification cycle of the water column (Estrada & Vaqué 2014) but is also influenced by terrestrial runoff from episodic rains that occur predominantly in spring and fall and from the contribution of treated wastewater that rises in the summer due to increased tourist activity (Guadayol et al. 2009). Nutrient concentrations may also be enhanced by advection of oceanic water (Arin et al. 2013) and, during strong wind events, by sediment resuspension and augmented nutrient diffusion from the sediments (Guadayol et al. 2009). Another factor that could play a role in supplying nutrients to the surface waters is aerosol deposition (Gallisai et al. 2014). In a context of marked vulnerability to climate change of the Mediterranean Sea (UNEP-MAP-RAC/SPA 2010, Martin-Vide 2016), these characteristics heighten the importance of gathering information on the temporal evolution of the Blanes Bay phytoplankton communities. Although previous studies have assessed different aspects of phytoplankton variability, mainly in relation to the seasonal cycle, an integrated view of the long-term (14 yr) seasonal and multiannual variability of phytoplankton composition using comparable methodology is still lacking. Specifically, our investigation aimed to (1) identify seasonal and interannual patterns in the variability of phytoplankton community structure of Blanes Bay and (2) investigate the relation between the environmental parameters of the study area and the variability of the phytoplankton groups.

#### MATERIALS AND METHODS

#### Study area and sampling

Sampling was conducted over a period of 14 yr (2000 to 2014) at the Blanes Bay Microbial Observatory (BBMO) station, located in the Bay of Blanes, in the Northwestern Mediterranean Coast (Fig. 1), approximately 60 km north of Barcelona. The BBMO station is a shallow site (~20 m depth) approximately 800 m offshore from the town of Blanes (41°40' N, 2°48'E). A total of 162 surface water samples were considered for analysis. The first one was taken in September 2000; from 2001 to 2014, samples were in general collected monthly, with some exceptions (see Table S1 in the Supplement at www.int-res.com/ articles/suppl/m592p057\_supp.pdf). Until 2007, water temperature and salinity were measured, respectively, with a mercury thermometer at surface and with a YSI 556 MPS Multi Probe system. After 2007, vertical profiles of these variables were obtained with a CTD model SAIV A/S SD204. Due to problems



Fig. 1. Position of the Blanes Bay Microbial Observatory station in Blanes Bay (NW Mediterranean Sea). The 25, 50, 75, 100 and 125 m isobaths are shown

in the calibration of the sensors, only salinities determined in 2007-2008 and 2010-2014 with the CTD were used to calculate monthly averages. Water samples for pigment determinations were collected by gently sinking 20 l polycarbonate acid-cleaned carboys with a 200 µm mesh net in the mouth into the water. This step is generally adopted to avoid the capture of larger zooplankton. Immediately after collection, the carboys were covered with black plastic bags and transferred to the laboratory within 2 h. Additional samples were taken for classic fluorometric determination of extracted total chlorophyll a (Fl\_Chl a), for nutrient analyses and for flow cytometric counts, and during some periods (June 2006 to June 2014) also for phytoplankton enumeration by inverted microscopy. Records of 24 h precipitation (see Fig. 2B) and wind velocity and direction were obtained from meteorological measurements (41° 39' N, 2°45′36″E) at Malgrat de Mar, ~6 km southwards from the sampling site). Wind data were taken at 2 m from the ground between September 2000 and 4 May 2005 (data set 2000-2005) and at 10 m afterwards (data set 2005-2014). Because of the bias introduced by this change, we dealt separately with the 2 sets of wind data and used only the second, i.e. the longest period for statistical analyses. A precipitation index (PI7) consisting of the total amount of precipitation during the 7 d ending 2 d before each sampling date was used to test the potential relationship between precipitation and chl a concentration anomalies. The reason for the 2 d gap between the precipitation index and the sampling day is the observation that,

> while phytoplankton response to nutrient discharges takes some time, the likely short-term effect of heavy rains is the dilution of the coastal phytoplankton communities (Estrada et al. 2008).

# Dissolved inorganic nutrient and Fl\_Chl *a* concentrations

Water for nitrate (NO<sub>3</sub>), nitrite (NO<sub>2</sub>), ammonium (NH<sub>4</sub>), silicate (SiO<sub>4</sub>) and phosphate (PO<sub>4</sub>) determination was stored in 100 ml polyethylene bottles and kept frozen ( $-20^{\circ}$ C) until analysis in the laboratory. Nutrient concentrations were measured colorimetrically with an Alliance Evolution II autoanalyser, following the procedures described in Hansen & Koroleff (1999). Precision was  $\pm 0.015 \ \mu mol \ kg^{-1}$  for NO<sub>2</sub>,  $\pm 0.02 \ \mu mol \ kg^{-1}$  for NO<sub>3</sub>,  $\pm 0.016 \ \mu mol \ kg^{-1}$  for NH<sub>4</sub>,  $\pm 0.0015 \ \mu mol \ kg^{-1}$  for SiO<sub>4</sub> and  $\pm 0.02 \ \mu mol \ kg^{-1}$  for PO<sub>4</sub>.

For fluorometric chl *a* (Fl\_Chl *a*) determination, 150 ml of water were filtered onto Whatman 25 mm diameter GF/F filters that were subsequently frozen at  $-20^{\circ}$ C. For analysis, the filters were ground in 90% acetone and left in the dark at 4°C for at least 24 h. The fluorescence of the extract was measured with a Turner Designs fluorometer as described by Yentsch & Menzel (1963). No phaeophytin 'correction' was applied.

### Phytoplankton abundance

Immediately after collection, 100 ml of water were placed in glass bottles and preserved with formaldehyde-hexamine solution (4%). The samples were kept in dark and cool (4°C) conditions until examination following the Uthermöhl (1958) method. For this purpose, 50 ml of each sample were left to settle for 24 h in a composite chamber, and the phytoplankton cells were enumerated using an inverted microscope, as described by Estrada et al. (2016). At least 2 transects of the chamber bottom were observed at 312× magnification to enumerate the most frequent phytoplankton forms (note that the method is not adequate for cells in the picoplankton size range). Additionally, the whole chamber bottom was examined at 125× magnification to enumerate the larger, less frequent cells. When possible, classification was done to the genus or species level, but many taxa could not be properly identified and were pooled in categories such as 'small flagellates' or 'small dinoflagellates'.

#### Abundance of cyanobacteria by flow cytometry

Water subsamples of 1.8 ml were fixed with 0.18 ml of a 10% paraformaldehyde and 0.5% glutaraldehyde mixture and stored at -80°C until analysis. The abundance of *Synechococcus* spp. and *Prochlorococcus* spp. was estimated by flow cytometry using a Becton Dickinson FACSCalibur flow cytometer with a laser emitting at 488 nm (Marie et al. 2001).

### Determination of phytoplankton pigments and total chlorophyll *a* (T\_Chl *a*) by HPLC

The pigment data set consisted of 162 samples (Table S1). A volume of water varying from 0.7 l to 1 l

(depending on the sampling season) was filtered onto Whatman GF/F (nominal pore size 0.7 µm; 25 mm diameter) glass fiber filters with low vacuum (0.4 bars) to prevent cells from breaking. Subsequently, the filters were folded, dried, wrapped in aluminum foil and stored frozen at -80°C until analysis. The method of Wright & Jeffrey (1997) was chosen for pigment extraction. The filters were placed in tubes with 2.5 ml of 90% acetone submerged in ice, and the pigments were extracted by sonication for 30 s. Subsequently, the filters were stored at -20°C. After 24 h, the samples were vortexed and filtered through Whatman GF/F glass fiber filters. Prior to 2008, the samples were analyzed at the Institute of Marine Sciences (CSIC) in Barcelona according to the method of Zapata et al. (2000), using a ThermoQuest chromatograph (hereafter System 1), which included a P2000 solvent module, an A/S 3000 autosampler, a UV-3000 absorbance detector (440 nm), a FL2000 fluorescence detector (excitation = 430 nm, emission = 662 nm), and an SN 4000 controller. After 2008, analyses were carried out at the Centro Oceanográfico de Gijón (IEO, Instituto Español de Oceanografía) following the procedure (hereafter System 2) of Latasa (2014), which increased the sensitivity and lowered the detection limit, using an Agilent series 1200 chromatographic system consisting of a G1311A quaternary pump, a G1367C autosampler with a 100 µl capillary loop, a G1316B column thermostat, and a G1315C diode array detector. A significant change added to the System 2 analyses was the use of trans- $\beta$ -apo-8'-carotenal, as an internal standard, dissolved in the acetone used for sample extraction. In total, 28 pigments (see Table S2 in the Supplement) were detected at 440 and 665 nm (System 1) or 474 and 664 nm (System 2) and identified by retention time and online diode array detector. Total monovinyl-chlorophyll a (TMV\_Chl a) concentration was estimated as the sum of monovinyl-chlorophyll a (MV\_Chl a), chlorophyllide a, and chlorophyll a allomers and epimers. T\_Chl a was calculated as TMV\_Chl a + divinyl-chlorophyll a $(DV_Chl a)$ .

#### **CHEMTAX** analysis

The CHEMTAX computer program v.1.95 (Mackey et al. 1996, Latasa 2007) was used to calculate the relative contribution of different phytoplankton groups to total chlorophyll *a* biomass, based on marker pigments. Essentially, the program uses an initial matrix of pigment:chl *a* ratios for all the algal groups considered and optimizes the ratio matrix to generate the fraction of the total chl a accounted for by each phytoplankton group. Before running CHEMTAX, the samples were clustered according to the contribution of the pigments 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, alloxanthin, chlorophyll *b*, chlorophyll  $c_2$ , chlorophyll  $c_2$ -MGDG [14:0/ 14:0], divinyl-chlorophyll *a*, fucoxanthin, neoxanthin, peridinin, prasinoxanthin, violaxanthin, uriolide and zeaxanthin. The statistical similarity matrix among samples was calculated using the Manhattan distances, and the samples were clustered according to Ward's method using the Statistica v.5.5 software. Following the procedures of Latasa (2007) and Latasa et al. (2010), 29 random initial pigment ratio matrices were created considering 8 phytoplankton pigment groups: cryptophytes (Crypto), diatoms (Diat), dinoflagellates (Dino), haptophytes (Hapto), pelagophytes (Pelago), prasinophytes (Pras), Prochlorococcus (Prochl) and Synechococcus (Syn). The Hapto group combined haptophytes Types 6 + 7, which include Chrysochromulina and the cosmopolitan coccolithophore Emiliana huxleyi, and haptophytes Type 8, which comprise Phaeocystis (Zapata et al. 2004). Prasinophytes contain prasinoxanthin as major carotenoid and share chlorophyll b, neoxanthin, violaxanthin and zeaxanthin with other chlorophytes, which in turn present lutein instead of prasinoxanthin as their dominant carotenoid. As lutein was almost always below detection limits, we assumed that chlorophytes in Blanes Bay were basically represented by prasinophytes. Eight successive CHEM-TAX runs were performed with the 29 matrices. A single average matrix was obtained from the eighth run of the 29 matrices. This average matrix was run again to estimate the contribution of each phytoplankton pigment group to the T\_Chl a in the sample. This procedure was performed independently with each cluster of samples.

### Statistical analyses

The significance of the regressions between phytoplankton cell abundances and HPLC pigment concentrations was tested by means of the *t*-test. Linear correlations were calculated among the monthly anomalies of the variables (the difference between the variable value for a particular month in a given year and the mean of the values corresponding to that month in all the years of the series); chl *a* concentrations were previously subjected to a square root transformation to improve the normality and homoscedasticity of the data. Some analyses were also carried out separately for 'winter' and 'summer'; in this context, winter comprised the months of January, February and March, and summer July, August and September. Temporal trends were examined by means of linear regression of the monthly anomalies of the dependent variable with respect to sampling date. Bivariate linear regression equations with chl a concentrations as dependent variables and time and PI7 as predictor variables were also calculated. The significance of the slopes was determined by the tstatistic and the standardized slopes (equal in absolute value to the correlation coefficient), which indicate how much a dependent variable increases (in terms of standard deviations) when the independent valuable increases 1 standard deviation, were used to compare interannual trends among chl a variables. Figs. 6 & S1 were produced using the Ocean Data View software (Schlitzer 2016).

### RESULTS

### Hydrography

The highest surface water temperature, 26.2°C, was recorded on 12 September 2007 and the lowest (11.0°C) on 26 March 2003. Monthly-averaged values showed a clear seasonality (Figs. 2A & 3A), with summer maxima (mean  $\pm$  SE of the mean SE of 24.2  $\pm$  $0.3^{\circ}C$  in August) and winter minima ( $12.8 \pm 0.2^{\circ}C$  in February). Monthly averages of salinity (after 2007) ranged in general between 38 and 38.1 and were lowest  $(37.7 \pm 0.1)$  in June (Fig. 3A). In general, the water column presented a vertically homogeneous distribution of temperature in autumn and winter; stratification of the upper waters started around May and a 5 to 10 m mixed layer became established between June and September. Vertical homogenization took place again in October (see Fig. S1 in the Supplement). The record of 24 h precipitation (Fig. 2B) observed at Malgrat, taken here as a proxy for freshwater runoff, showed a typical Mediterranean pattern of high episodic events superimposed to a regime driven by dry summer periods and rains in spring and autumn. As expected (Stull 1988), mean daily wind speed was lower for the 2000-2005 data set, measured 2 m above the ground, than for the 2005–2014 one, measured at 10 m. Few daily means exceeded 5 m s<sup>-1</sup> (Fig. S2 in the Supplement); strong winds were generally from the north (data not shown). There was a weak but significant correlation between daily 24 h precipitation and mean wind



Fig. 2. Temporal variation of (A) surface water temperature, (B) daily precipitation at the Malgrat meteorological station, 5 km south of Blanes, and (C) temporal variation of fluorometric chlorophyll *a* (Fl\_Chl *a*) and total chlorophyll *a* (T\_Chl *a*) concentrations



Fig. 3. Monthly average climatology (September 2000–December 2014) of (A) water temperature (°C) and salinity, (B) nitrate and silicate concentrations, (C) ammonium and nitrite concentrations and (D) phosphate concentration and N:P ratio. Error bars = 1 SE. Salinity included only measurements taken after 2007



Fig. 4. Monthly average climatology (September 2000– December 2014) of fluorometric chlorophyll a (Cl\_Chl a) and total chlorophyll a (T\_Chl a) concentrations. Error bars = 1 SE

speed (2000–2005 data set, N = 1925, R<sup>2</sup> = 0.015, p < 0.0001; 2005–2014 data set, N = 3275, R<sup>2</sup> = 0.04, p < 0.0001). Nutrient concentrations were generally low and increased during winter (Fig. 3B,C,D). Average (±1 SE) monthly values peaked in March (Fig. 3B) for nitrate (2.52 ± 0.52  $\mu$ M) and silicate (2.26 ± 0.44  $\mu$ M), nitrite showed a summer minimum, and ammonium did not present a clear seasonal cycle (Fig. 3C). Phosphate (Fig. 3D) presented relatively low seasonal

variability, with a maximum monthly mean of 0.16  $\pm$  0.22  $\mu$ M in March (a value of 0.94  $\mu$ M recorded on 19 April 2001 was not included in the calculations) and a minimum of 0.083  $\pm$ 0.014  $\mu$ M in September. The N:P ratio was mostly affected by the nitrate variability and, except in March, was lower than the Redfield value of 16 (Fig. 3D).

# Chl *a* distribution and HPLC pigment composition

There was good agreement between fluorometric and HPLC chl *a* measurements (Fl\_Chl *a* = 0.84 × T\_Chl*a* + 0.152, N = 157, R<sup>2</sup> = 0.70, p < 0.0001). Fluorometric chl *a* (Fl\_Chl *a*) and total chl *a* (T\_Chl *a*) presented a parallel seasonal evolution, with a main maximum in March and a minimum between July and September (Figs. 2C & 4). Chl *a* concentrations were generally <1 µg l<sup>-1</sup> but there were several samples with T\_Chl *a* values exceeding 1.5 µg l<sup>-1</sup>, as noted, for example, on 7 February 2002, 4 March 2003, 16 December 2003, which registered the highest T\_Chl *a* concentration of the series (3.37  $\mu$ g l<sup>-1</sup>), 16 May 2006, 12 March 2008 and 29 November 2011 (Fig. 2C).

Among the 28 pigments detected by the HPLC analyses (Table S2, Fig. S3 in the Supplement), the most abundant ones were MV\_Chl *a*, fucoxanthin, 19'-hexanoyloxyfucoxanthin, chlorophyll *b*, chlorophyll *c2*, chlorophyll *c3*, diadinoxanthin and 19'-butanoyloxyfucoxanthin. Most pigments presented their highest monthly average concentrations between mid-autumn and mid-spring, and their lowest ones in summer. Among the exceptions were peridinin and chlorophyll  $c_2$  MGDG [14:114], which did not have a clear seasonal cycle, and zeaxanthin, which had maxima in April and August.

# Seasonal variability of the phytoplankton community

Among the phytoplankton groups characterized by CHEMTAX, the main global contributors to T\_Chl *a* (Fig. 5A) were haptophytes, diatoms, prasinophytes and cryptophytes, followed by *Synechococcus*, pelagophytes, dinoflagellates and *Prochlorococcus* (in



Fig. 5. Contribution to total chlorophyll *a* (T\_Chl *a*) of the CHEMTAXderived phytoplankton groups for (A) the whole data set (A) and (B) monthly averages

| Month  | Cryptophytes —   |   |  | Diatoms  |   |   | Dinoflagellates   |   |  | ——— Haptophytes ———  |   |   |
|--|--|---|--|--|---|---|---|---|--|--|---|---|
|  | Mean $\pm$ SD  | Median  | %  | Mean ± SD  | Median  | %   | Mean ± SD   | Median  | %  | Mean ± SD  | Median  | %   |
| T  | 07.1 . 01.4  | 0.01  |  | 107.00 . 045.0   | 4.1   | 00.0  | C 2 .   | 4.0   | 1  | 170.1 . 77.1   | 101.0   | 07.0  |
| January  | $37.1 \pm 21.4$  | 30.1  | 0  | $137.00 \pm 243.0$   | 41  | 22.3  | $0.3 \pm 3.2$   | 4.9   | 1  | $170.1 \pm 77.1$   | 101.0   | 27.0  |
| February   | $49.5 \pm 32.5$  | 45.8  | 0.3  | $180.6 \pm 228.5$  | 102.2   | 23  | $14.7 \pm 17.9$   | <i>t.</i> Z   | 1.9  | $256.9 \pm 232.0$  | 181.9   | 32.7  |
| March  | $60.8 \pm 67.2$  | 32.9  | 7.2  | 321.3 ± 292.9  | 230.6   | 38.3  | $14.4 \pm 20.0$   | 5.7   | 1.7  | $205.9 \pm 174.9$  | 128.2   | 24.5  |
| April  | $55.6 \pm 66.0$  | 29.8  | 10.2   | $131.1 \pm 206.5$  | 27  | 24  | $7.1 \pm 8.1$   | 4.3   | 1.3  | $185.2 \pm 118.3$  | 167.5   | 33.9  |
| May  | $23.2 \pm 21.5$  | 11.3  | 5.5  | $165.1 \pm 243.6$  | 73.5  | 38.4  | $11.1 \pm 8.6$  | 8.8   | 2.6  | $167.9 \pm 115.1$  | 121.5   | 39.1  |
| June   | $19.2 \pm 25.5$  | 9.1   | 6.1  | $78.5 \pm 105.2$   | 28.6  | 24.8  | $14.4 \pm 17.1$   | 9.9   | 4.6  | $135.8 \pm 78.9$   | 123.1   | 43  |
| July   | $16.4 \pm 18.8$  | 9.9   | 6  | $11.7 \pm 12.5$  | 6.1   | 4.3   | $32.0 \pm 54.1$   | 8.6   | 11.6   | $134.0 \pm 125.5$  | 101.7   | 49.1  |
| August   | $42.2 \pm 48.6$  | 17.6  | 13.5   | $15.5 \pm 19.4$  | 6.9   | 5   | $23.6 \pm 29.5$   | 15.3  | 7.5  | $109.0 \pm 51.5$   | 92.5  | 34.8  |
| September  | $11.4 \pm 11.5$  | 5.2   | 4.7  | $5.9 \pm 7.0$  | 4.1   | 2.4   | $28.5 \pm 34.3$   | 20.2  | 11.6   | $102.7 \pm 41.7$   | 88.1  | 41.9  |
| October  | $13.6 \pm 8.5$   | 16.5  | 4.8  | $5.7 \pm 3.3$  | 5.1   | 2   | $16.8 \pm 21.9$   | 9.2   | 5.9  | $152.8 \pm 52.5$   | 147.4   | 53.3  |
| November   | $27.6 \pm 17.9$  | 22.3  | 4.3  | $6.6 \pm 5.6$  | 6.3   | 1   | $211.4 \pm 365.1$   | 47.9  | 33.1   | $185.7 \pm 100.1$  | 148.9   | 29.1  |
| December   | $91.8 \pm 203.3$   | 29.7  | 12.3   | $35.7 \pm 96.8$  | 10.3  | 4.8   | $121.9 \pm 135.3$   | 66.3  | 16.4   | $229.6 \pm 159.2$  | 192.2   | 30.8  |
|  |  |   |  |  |   |   |   |   |  |  |   |   |
|  |  |   |  |  |   |   |   |   |  |  |   |   |
|  | ——— Pelago   | ophytes-  |  | —— Prasino   | phytes –  |   | Prochlo   | rococcus  | s ——   | ——————————————————————————————————————   | hococcus  |   |
|  | —— Pelago<br>Mean ± SD   | ophytes -<br>Median   | %  | —— Prasino<br>Mean ± SD  | phytes –<br>Median  | %   | <i>—— Prochlo</i><br>Mean ± SD  | <i>rococcus</i><br>Median   | s  | ——— Synec<br>Mean ± SD   | h <i>ococcus</i><br>Median  | %   |
| January  | —— Pelago<br>Mean ± SD<br>40.1 ± 21.6  | ophytes -<br>Median<br>37.9   | %  | Prasino<br>Mean ± SD<br>185.0 ± 88.2   | phytes –<br>Median<br>144.7   | %   | Prochlo<br>Mean ± SD<br>12.2 ± 13.1   | Median  | s<br>2   |  | hococcus<br>Median<br>2.6   | %<br>   |
| <br>January<br>February  | PelageMean ± SD40.1 ± 21.649.8 ± 35.5  | ophytes -<br>Median<br>37.9<br>36.5   | %<br>6.5<br>6.3  | PrasinoMean ± SD185.0 ± 88.2193.1 ± 63.1   | phytes –<br>Median<br>144.7<br>184.7  | %<br>30<br>24.5   | ProchloMean ± SD12.2 ± 13.15.0 ± 4.5  | 7<br>3.9  | 2<br>0.6   |  | hococcus<br>Median<br>2.6<br>0.5  | %<br>0.9<br>0.2   |
| January<br>February<br>March   | $\frac{1}{1} Pelage \\ Mean \pm SD \\ 40.1 \pm 21.6 \\ 49.8 \pm 35.5 \\ 27.1 \pm 21.2 \\ \end{array}$  | ophytes -<br>Median<br>37.9<br>36.5<br>27.9   | %<br>6.5<br>6.3<br>3.2   | PrasinoMean ± SD185.0 ± 88.2193.1 ± 63.1148.9 ± 94.6   | phytes –<br>Median<br>144.7<br>184.7<br>129.5   | %<br>30<br>24.5<br>17.7   | ProchloMean ± SD12.2 ± 13.15.0 ± 4.52.0 ± 2.5   | 7<br>3.9<br>1.3   | 2<br>0.6<br>0.2  | Synection Synectio | hococcus<br>Median<br>2.6<br>0.5<br>5.7   | %<br>0.9<br>0.2<br>1.6  |
| January<br>February<br>March<br>April  | $\begin{array}{c} \hline & \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$  | ophytes -<br>Median<br>37.9<br>36.5<br>27.9<br>17.6   | %<br>6.5<br>6.3<br>3.2<br>3.9  | $\begin{array}{c} \\ \text{Mean \pm SD} \\ \hline \\ 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ \end{array}$   | phytes –<br>Median<br>144.7<br>184.7<br>129.5<br>49.4   | %<br>30<br>24.5<br>17.7<br>12.5   | ProchloMean ± SD12.2 ± 13.15.0 ± 4.52.0 ± 2.51.4 ± 1.5  | 7<br>3.9<br>1.3<br>1  | 2<br>0.6<br>0.2<br>0.2   | $ SynectMean \pm SD5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.7$   | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4   | %<br>0.9<br>0.2<br>1.6<br>9.5   |
| January<br>February<br>March<br>April<br>May   | Pelagy<br>Mean ± SD<br>40.1 ± 21.6<br>49.8 ± 35.5<br>27.1 ± 21.2<br>21.4 ± 19.9<br>12.9 ± 14.5   | 27.9<br>37.9<br>36.5<br>27.9<br>17.6<br>11.6  | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4   | $\begin{array}{c} \\ \\ \text{Mean \pm SD} \end{array}$ $\begin{array}{c} 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ 33.3 \pm 31.4 \end{array}$  | phytes –<br>Median<br>144.7<br>184.7<br>129.5<br>49.4<br>28.2   | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7  | ProchloMean ± SD12.2 ± 13.15.0 ± 4.52.0 ± 2.51.4 ± 1.51.7 ± 2.3   | 7<br>3.9<br>1.3<br>1<br>0.9   | 2<br>0.6<br>0.2<br>0.2<br>0.3  | $ SynectMean \pm SD5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.8$  | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2   | %<br>0.9<br>0.2<br>1.6<br>9.5<br>2.7  |
| January<br>February<br>March<br>April<br>May<br>June   | $\begin{array}{c} \hline & \mbox{Pelag} \\ \hline & \mbox{Mean} \pm \mbox{SD} \\ \hline & \mbox{40.1} \pm 21.6 \\ \hline & \mbox{49.8} \pm 35.5 \\ \hline & \mbox{27.1} \pm 21.2 \\ \hline & \mbox{21.4} \pm 19.9 \\ \hline & \mbox{12.9} \pm 14.5 \\ \hline & \mbox{11.9} \pm 10.7 \\ \hline \end{array}$   | 27.9<br>37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9  | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7  | $\begin{array}{c} \hline & \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$  | phytes –<br>Median<br>144.7<br>184.7<br>129.5<br>49.4<br>28.2<br>22.4                                     | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3   | ProchloMean ± SD12.2 ± 13.15.0 ± 4.52.0 ± 2.51.4 ± 1.51.7 ± 2.31.3 ± 1.4  | 7<br>3.9<br>1.3<br>1<br>0.9<br>1.3  | 2<br>0.6<br>0.2<br>0.2<br>0.3<br>0.4   | $ SynectMean \pm SD5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.828.0 \pm 30.7$   | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8   | %<br>0.9<br>0.2<br>1.6<br>9.5<br>2.7<br>8.8   |
| January<br>February<br>March<br>April<br>May<br>June<br>July   | $\begin{array}{c} \hline & \mbox{Pelage}\\ \hline & \mbox{Mean} \pm \mbox{SD}\\ \hline & \mbox{40.1} \pm 21.6\\ \hline & \mbox{49.8} \pm 35.5\\ \hline & \mbox{27.1} \pm 21.2\\ \hline & \mbox{21.4} \pm 19.9\\ \hline & \mbox{12.9} \pm 14.5\\ \hline & \mbox{11.9} \pm 10.7\\ \hline & \mbox{12.8} \pm 11.0\\ \hline \end{array}$  | 37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9<br>12.5  | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7<br>4.7                                     | $\begin{array}{c} \hline & \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$  | phytes –<br>Median<br>144.7<br>184.7<br>129.5<br>49.4<br>28.2<br>22.4<br>16.3                             | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3<br>6.6                                    | $ ProchloMean \pm SD12.2 \pm 13.15.0 \pm 4.52.0 \pm 2.51.4 \pm 1.51.7 \pm 2.31.3 \pm 1.41.0 \pm 1.0$  | 7<br>3.9<br>1.3<br>1<br>0.9<br>1.3<br>0.9   | 2<br>0.6<br>0.2<br>0.3<br>0.4<br>0.3   | $ SynectMean \pm SD5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.828.0 \pm 30.736.6 \pm 38.1$  | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8<br>18.7                                       | 0.9<br>0.2<br>1.6<br>9.5<br>2.7<br>8.8<br>13.4  |
| January<br>February<br>March<br>April<br>May<br>June<br>July<br>August   | $\begin{array}{c} \hline & \mbox{Pelage}\\ \hline & \mbox{Mean $\pm$ SD} \\ \hline & \mbox{40.1 $\pm$ 21.6} \\ \hline & \mbox{49.8 $\pm$ 35.5} \\ \hline & \mbox{27.1 $\pm$ 21.2} \\ \hline & \mbox{21.4 $\pm$ 19.9} \\ \hline & \mbox{12.9 $\pm$ 14.5} \\ \hline & \mbox{11.9 $\pm$ 10.7} \\ \hline & \mbox{12.8 $\pm$ 11.0} \\ \hline & \mbox{9.0 $\pm$ 9.1} \\ \end{array}$   | 37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9<br>12.5<br>7.8                                   | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7<br>4.7<br>2.9                              | $\begin{array}{c} \hline & \label{eq:prasino} \\ \hline & \mbox{Mean \pm SD} \\ \hline \\ 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ 33.3 \pm 31.4 \\ 27.2 \pm 17.8 \\ 17.9 \pm 12.8 \\ 26.1 \pm 26.0 \\ \hline \end{array}$                               | phytes –<br>Median<br>144.7<br>129.5<br>49.4<br>28.2<br>22.4<br>16.3<br>17.4                              | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3<br>6.6<br>8.3                             | $ ProchloMean \pm SD12.2 \pm 13.15.0 \pm 4.52.0 \pm 2.51.4 \pm 1.51.7 \pm 2.31.3 \pm 1.41.0 \pm 1.03.3 \pm 4.7$   | 7<br>3.9<br>1.3<br>1<br>0.9<br>1.3<br>0.7<br>1.3                                      | 2<br>0.6<br>0.2<br>0.2<br>0.3<br>0.4<br>0.3<br>1.1                           | $ SynectMean \pm SD 5.7 \pm 6.7 1.3 \pm 1.7 13.1 \pm 22.5 52.2 \pm 52.7 14.4 \pm 12.8 28.0 \pm 30.7 36.6 \pm 38.1 73.6 \pm 49.6 \\$  | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8<br>18.7<br>85.2                               | %<br>0.9<br>0.2<br>1.6<br>9.5<br>2.7<br>8.8<br>13.4<br>23.5   |
| January<br>February<br>March<br>April<br>May<br>June<br>July<br>August<br>September                                    | $\begin{array}{c} \hline & \mbox{Pelage}\\ \hline & \mbox{Mean} \pm \mbox{SD}\\ \hline & \mbox{40.1} \pm 21.6\\ & \mbox{49.8} \pm 35.5\\ & \mbox{27.1} \pm 21.2\\ & \mbox{21.4} \pm 19.9\\ & \mbox{12.9} \pm 14.5\\ & \mbox{11.9} \pm 10.7\\ & \mbox{12.8} \pm 11.0\\ & \mbox{9.0} \pm 9.1\\ & \mbox{4.2} \pm 5.6\\ \hline \end{array}$  | 37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9<br>12.5<br>7.8<br>0                              | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7<br>4.7<br>2.9<br>1.7                       | $\begin{array}{c} \hline & \label{eq:prasino} \\ \hline & \mbox{Mean \pm SD} \\ \hline \\ 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ 33.3 \pm 31.4 \\ 27.2 \pm 17.8 \\ 17.9 \pm 12.8 \\ 26.1 \pm 26.0 \\ 12.5 \pm 9.5 \\ \end{array}$                      | phytes –<br>Median<br>144.7<br>184.7<br>129.5<br>49.4<br>28.2<br>22.4<br>16.3<br>17.4<br>8.3              | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3<br>6.6<br>8.3<br>5.1                      | $ ProchloMean \pm SD12.2 \pm 13.15.0 \pm 4.52.0 \pm 2.51.4 \pm 1.51.7 \pm 2.31.3 \pm 1.41.0 \pm 1.03.3 \pm 4.78.9 \pm 12.1$   | 7<br>Median<br>7<br>3.9<br>1.3<br>1<br>0.9<br>1.3<br>0.7<br>1.3<br>4.1                | 2<br>0.6<br>0.2<br>0.3<br>0.4<br>0.3<br>1.1<br>3.6                           | $ SynectMean \pm SD 5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.828.0 \pm 30.736.6 \pm 38.173.6 \pm 49.659.8 \pm 42.8$   | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8<br>18.7<br>85.2<br>50                         | 0.9<br>0.2<br>1.6<br>9.5<br>2.7<br>8.8<br>13.4<br>23.5<br>24.4  |
| January<br>February<br>March<br>April<br>May<br>June<br>July<br>August<br>September<br>October                         | $\begin{array}{c} \hline & \mbox{Pelage}\\ \hline & \mbox{Mean} \pm \mbox{SD}\\ \hline & \mbox{40.1} \pm \mbox{21.6}\\ \hline & \mbox{49.8} \pm \mbox{35.5}\\ \hline & \mbox{27.1} \pm \mbox{21.2}\\ \hline & \mbox{21.4} \pm \mbox{19.9}\\ \hline & \mbox{12.9} \pm \mbox{14.5}\\ \hline & \mbox{11.9} \pm \mbox{10.7}\\ \hline & \mbox{12.8} \pm \mbox{11.0}\\ \hline & \mbox{9.0} \pm \mbox{9.1}\\ \hline & \mbox{4.2} \pm \mbox{5.6}\\ \hline & \mbox{7} & \mbox{8} \pm \mbox{9} \mbox{0} \end{array}$ | 37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9<br>12.5<br>7.8<br>0<br>3                         | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7<br>4.7<br>2.9<br>1.7<br>2.7                | $\begin{array}{c} \hline & \label{eq:prasino} \\ \hline & \mbox{Mean \pm SD} \\ \hline \\ 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ 33.3 \pm 31.4 \\ 27.2 \pm 17.8 \\ 17.9 \pm 12.8 \\ 26.1 \pm 26.0 \\ 12.5 \pm 9.5 \\ 23.9 \pm 26.8 \\ \end{array}$     | phytes –<br>Median<br>144.7<br>129.5<br>49.4<br>28.2<br>22.4<br>16.3<br>17.4<br>8.3<br>19.8               | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3<br>6.6<br>8.3<br>5.1<br>8.3               | $ ProchloMean \pm SD12.2 \pm 13.15.0 \pm 4.52.0 \pm 2.51.4 \pm 1.51.7 \pm 2.31.3 \pm 1.41.0 \pm 1.03.3 \pm 4.78.9 \pm 12.113.9 \pm 10.8$  | 7<br>Median<br>7<br>3.9<br>1.3<br>1<br>0.9<br>1.3<br>0.7<br>1.3<br>4.1<br>10.5        | 2<br>0.6<br>0.2<br>0.2<br>0.3<br>0.4<br>0.3<br>1.1<br>3.6<br>4.9             | $ SynectMean \pm SD 5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.828.0 \pm 30.736.6 \pm 38.173.6 \pm 49.659.8 \pm 42.843.7 \pm 23.5$  | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8<br>18.7<br>85.2<br>50<br>43.4                 | %           0.9           0.2           1.6           9.5           2.7           8.8           13.4           23.5           24.4           15.2                             |
| January<br>February<br>March<br>April<br>May<br>June<br>July<br>August<br>September<br>October<br>November             | $\begin{array}{c} \hline & \mbox{Pelage}\\ \hline & \mbox{Mean $\pm$ SD} \\ \hline & \mbox{40.1 $\pm$ 21.6} \\ \hline & \mbox{49.8 $\pm$ 35.5} \\ \hline & \mbox{27.1 $\pm$ 21.2} \\ \hline & \mbox{21.4 $\pm$ 19.9} \\ \hline & \mbox{12.9 $\pm$ 14.5} \\ \hline & \mbox{11.9 $\pm$ 10.7} \\ \hline & \mbox{12.8 $\pm$ 11.0} \\ \hline & \mbox{9.0 $\pm$ 9.1} \\ \hline & \mbox{4.2 $\pm$ 5.6} \\ \hline & \mbox{7.8 $\pm$ 9.0} \\ \hline & \mbox{31.8 $\pm$ 29.0} \\ \hline \end{array}$                 | 27.9<br>37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9<br>12.5<br>7.8<br>0<br>3<br>26.8         | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7<br>4.7<br>2.9<br>1.7<br>2.7<br>5           | $\begin{array}{c} \hline & \mbox{Prasino} \\ \hline Mean \pm SD \\ \hline \\ 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ 33.3 \pm 31.4 \\ 27.2 \pm 17.8 \\ 17.9 \pm 12.8 \\ 26.1 \pm 26.0 \\ 12.5 \pm 9.5 \\ 23.9 \pm 26.8 \\ 69.2 \pm 55.5 \\ \end{array}$ | phytes –<br>Median<br>144.7<br>129.5<br>49.4<br>28.2<br>22.4<br>16.3<br>17.4<br>8.3<br>19.8<br>58 9       | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3<br>6.6<br>8.3<br>5.1<br>8.3<br>10.8       | $\begin{array}{c} Prochlo \\ Mean \pm SD \\ \hline 12.2 \pm 13.1 \\ 5.0 \pm 4.5 \\ 2.0 \pm 2.5 \\ 1.4 \pm 1.5 \\ 1.7 \pm 2.3 \\ 1.3 \pm 1.4 \\ 1.0 \pm 1.0 \\ 3.3 \pm 4.7 \\ 8.9 \pm 12.1 \\ 13.9 \pm 10.8 \\ 12.7 \pm 10.6 \\ \end{array}$                     | 7<br>Median<br>7<br>3.9<br>1.3<br>1<br>0.9<br>1.3<br>0.7<br>1.3<br>4.1<br>10.5<br>8 5 | 2<br>0.6<br>0.2<br>0.2<br>0.3<br>0.4<br>0.3<br>1.1<br>3.6<br>4.9<br>2        | $ SynectMean \pm SD 5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.828.0 \pm 30.736.6 \pm 38.173.6 \pm 49.659.8 \pm 42.843.7 \pm 23.522.7 \pm 25.6$   | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8<br>18.7<br>85.2<br>50<br>43.4<br>14 7         | %           0.9           0.2           1.6           9.5           2.7           8.8           13.4           23.5           24.4           15.2           3.6               |
| January<br>February<br>March<br>April<br>May<br>June<br>July<br>August<br>September<br>October<br>November<br>December | $\begin{array}{c} \hline \\ Pelage \\ Mean \pm SD \\ \hline \\ 40.1 \pm 21.6 \\ 49.8 \pm 35.5 \\ 27.1 \pm 21.2 \\ 21.4 \pm 19.9 \\ 12.9 \pm 14.5 \\ 11.9 \pm 10.7 \\ 12.8 \pm 11.0 \\ 9.0 \pm 9.1 \\ 4.2 \pm 5.6 \\ 7.8 \pm 9.0 \\ 31.8 \pm 29.0 \\ 38.2 \pm 35.2 \\ \end{array}$  | 27.9<br>37.9<br>36.5<br>27.9<br>17.6<br>11.6<br>10.9<br>12.5<br>7.8<br>0<br>3<br>26.8<br>29.2 | %<br>6.5<br>6.3<br>3.2<br>3.9<br>2.4<br>3.7<br>4.7<br>2.9<br>1.7<br>2.7<br>5<br>5<br>5 | $\begin{array}{c} \\ \text{Mean \pm SD} \\ \hline \\ 185.0 \pm 88.2 \\ 193.1 \pm 63.1 \\ 148.9 \pm 94.6 \\ 68.5 \pm 55.9 \\ 33.3 \pm 31.4 \\ 27.2 \pm 17.8 \\ 17.9 \pm 12.8 \\ 26.1 \pm 26.0 \\ 12.5 \pm 9.5 \\ 23.9 \pm 26.8 \\ 69.2 \pm 55.5 \\ 178.9 \pm 254.0 \\ \end{array}$      | phytes –<br>Median<br>144.7<br>129.5<br>49.4<br>28.2<br>22.4<br>16.3<br>17.4<br>8.3<br>19.8<br>58.9<br>94 | %<br>30<br>24.5<br>17.7<br>12.5<br>7.7<br>8.3<br>6.6<br>8.3<br>5.1<br>8.3<br>10.8<br>24 | $\begin{array}{c} Prochlo \\ Mean \pm SD \\ \hline \\ 12.2 \pm 13.1 \\ 5.0 \pm 4.5 \\ 2.0 \pm 2.5 \\ 1.4 \pm 1.5 \\ 1.7 \pm 2.3 \\ 1.3 \pm 1.4 \\ 1.0 \pm 1.0 \\ 3.3 \pm 4.7 \\ 8.9 \pm 12.1 \\ 13.9 \pm 10.8 \\ 12.7 \pm 10.6 \\ 13.4 \pm 11.6 \\ \end{array}$ | 7<br>3.9<br>1.3<br>1<br>0.9<br>1.3<br>0.7<br>1.3<br>4.1<br>10.5<br>8.5<br>9.9         | 2<br>0.6<br>0.2<br>0.2<br>0.3<br>0.4<br>0.3<br>1.1<br>3.6<br>4.9<br>2<br>1.8 | $ SynectMean \pm SD5.7 \pm 6.71.3 \pm 1.713.1 \pm 22.552.2 \pm 52.714.4 \pm 12.828.0 \pm 30.736.6 \pm 38.173.6 \pm 49.659.8 \pm 42.843.7 \pm 23.522.7 \pm 25.613.7 \pm 11.8$   | hococcus<br>Median<br>2.6<br>0.5<br>5.7<br>40.4<br>13.2<br>15.8<br>18.7<br>85.2<br>50<br>43.4<br>14.7<br>17 2 | %           0.9           0.2           1.6           9.5           2.7           8.8           13.4           23.5           24.4           15.2           3.6           1.8 |

Table 1. Global monthly means  $\pm$  standard deviation (SD) and medians (in terms of ng l<sup>-1</sup> of chl *a*), and percentage contribution to total chl *a* (T\_Chl *a*) (%) of the CHEMTAX-derived phytoplankton groups

the following, unless otherwise stated, the group name will be used to indicate the CHEMTAXderived contribution to T\_Chl a of the group). In general, there was a marked seasonality (Table 1, Figs. 5B, 6 & 7), but interannual variability of the timing of population maxima was high. On average, diatoms peaked in March (40.5%), May (39%), and November (37%) and were the prevailing group in the high T\_Chl a episodes, contributing between 44 and 83% of T\_Chl a in these cases. The exception was the 16 December 2003 event, in which the dominant groups were prasinophytes, cryptophytes and haptophytes. According to available phytoplankton counts, the most abundant diatom taxa in the proliferations, particularly those of autumn-winter, were Chaetoceros spp., Pseudo-nitzschia spp., Asterionellopsis glacialis (e.g. in December 2009), Lioloma pacificum (e.g. in November 2009) and Thalassionema nitzschioides (e.g. in December 2013 and May 2014). However, other taxa, such as Proboscia alata, were dominant on some occasions (e.g. May-June 2007). The contribution of prasinophytes, cryptophytes and pelagophytes tended to be more

important from December to March, although cryptophytes also presented a marked peak in August. The haptophytes followed a comparable pattern but varied relatively little throughout the year, while dinoflagellates were particularly important from winter to summer, with a minimum between September and November. It must be noted that for prasinophytes, cryptophytes and haptophytes, the December averages were enhanced by the exceptional population densities of December 2003, although all these groups presented a peak in December, even if that sample was eliminated from the calculations. Regarding the cyanobacteria, Prochlorococcus practically disappeared from the surface waters between February and August, and the highest contributions of Synechococcus were in April and between July and October.

Counts of selected phytoplankton taxa were compared with their CHEMTAX-derived contribution to chl *a*. The square of the correlation coefficients between flow cytometry counts of *Prochlorococcus* and *Synechococcus* and their contributions to chl *a* were 0.63 (N = 151, p < 0.0001) and 0.60 (N = 151, p <



Fig. 6. Seasonal and interannual variation of the contribution to total chlorophyll *a* by the CHEMTAX-derived phytoplankton groups (in ng l<sup>-1</sup>). Figure produced using the Ocean Data View software (Schlitzer 2016)

0.0001), respectively (see Fig. S4 in the Supplement). For diatom and dinoflagellate abundances, the squares of the correlation coefficients with their corresponding chl *a* contributions were 0.19 (N = 126, p < 0.0001) and 0.13 (N = 126, p < 0.0001), respectively (data not shown).

### Interannual variability

The time series of temperature (Fig. 8A), PI7 precipitation index (the total amount of precipitation during the 7 d ending 2 d before each sampling date), wind speed at 2 m (2000–2005) and 10 m (2005–2014)



Fig. 7. Monthly average climatologies (September 2000–December 2014) of the contribution to total chlorophyll *a* by (A) cryptophytes and pelagophytes, (B) dinoflagellates, (C) haptophytes and diatoms, (D) prasinophytes, (E) *Prochlorococcus* and (F) *Synechococcus*. Error bars = 1 SE

above the ground, and anomalies of salinity and ammonium did not present any statistically significant interannual trend (data not shown). In contrast, nitrate, nitrite (data not shown), phosphate, and silicate anomalies decreased with time (Fig. 8B,C,D, Table 2), as happened also for the anomalies (after square root transformation of the data) of TMV\_ Chl *a*, T\_Chl *a*, Fl\_Chl\_*a* and all CHEMTAX groups except pelagophytes, which increased with time, and haptophytes and prasinophytes, for which correlations were not significant (Figs. 6, 9 & 10, Table 2). Comparable results were obtained for the winter (January-March) and summer (July-September) data subsets (data not shown), although in this case, with reduced number of observations, many of the relationships became not significant. A correlation

analysis among potential factors influencing the variability of chl a concentration anomalies, such as temperature, the PI7 precipitation index, wind speed (only 2005-2014) and major nutrient (phosphate, nitrate and silicate) concentrations revealed some interesting patterns (Table 3). The temperature anomaly was only significantly negatively correlated with the PI7 index, whereas all nutrient anomalies were significantly positively correlated among themselves. In turn, the nitrate and silicate anomalies were positively correlated with that of the PI7 index and the phosphate anomaly was positively correlated with wind speed (2005-2014). When these same relationships were calculated separately for the winter (January-March) and summer (July-August) periods of 2005 to 2014, the results were qualitatively similar,



Fig. 8. Temporal variation of the monthly anomalies of (A) surface water temperature, (B) nitrate, (C) phosphate and (D) silicate. The significant linear regression lines are indicated in the graphs; the corresponding equations are shown in Table 2

but the only significant correlations were the winter ones between silicate and PI7 anomalies (N = 26, R<sup>2</sup> = 0.20, p < 0.01) and between phosphate and wind speed anomalies (N = 26, R<sup>2</sup> = 0.24, p < 0.05). Based on the findings for the whole data set, time and the PI7 index were used as predictor variables in bivariate linear regressions with the group chl *a* concentration anomalies as dependent variables. The standardized slopes, shown in Table 4, can be used as an indication of the relative importance of long-term (time) versus episodic (PI7) environmental forcing. As expected, the slopes with respect to time in the bivariate regression are close to the corresponding ones in Table 2.

# DISCUSSION

#### Seasonal patterns

In agreement with the findings of previous studies, Blanes Bay presented a strong seasonality with a marked yearly cycle of warm summers and cool winters (Margalef 1957, 1964, Cebrián et al. 1996, Duarte et al. 1999). Salinity values lower than 38 are typical of coastal surface waters in the region. The relatively high autumn and winter salinities can be associated with intrusions of oceanic waters (Cebrián et al. 1996); local freshwater discharges may induce sporadic low salinity values at the sampling station but are generally insufficient to affect the seasonal salinity patterns. The salinity minimum in June may be related to spring increases of freshwater inputs from upstream rivers (in particular the Rhône) influenced by snow melt (Masó & Tintoré 1991); in addition, seasonal stratification hinders the mixing of freshwaters entering at the surface, therefore contributing to maintaining relatively low salinity values. Monthly Fl\_Chl a and T\_Chl a averages in Blanes Bay present a winter maximum that starts to build up in October and a summer minimum, coinciding with a similar variation in the concentration of nitrate, silicate and, to a minor extent, phosphate. These seasonal patterns are typical of the Catalan Sea (Estrada 1999, Segura-Noguera et al. 2011, Arin et al. 2013) and other Mediterranean marine areas (D'Ortenzio & Ribera d'Alcalà 2009, Siokou-Frangou et al. 2010, Estrada & Vaqué 2014). The occurrence of a winter bloom in the Mediterranean, before the establishment of thermal stratification, has been related to the

Table 2. Slope (time units are yr <sup>1</sup>), standardized (Std.) slope, squared correlation coefficient and p-values corresponding to the regression lines, with respect to sampling date (in years), of the monthly anomalies of phosphate and silicate, and the anomalies (after square root transformation) of the CHEMTAX groups, total monovinyl-chl\_a (TMV Chl a), total chl a (T\_Chl a) and fluorometric chl a (Fl\_Chl a). All units are (chl a, ng l<sup>-1</sup>)<sup>-1/2</sup> except for phosphate, nitrate and silicate ( $\mu$ M). The number of observations ranged from 216 for temperature to 157–162 for the other variables. NS: not significant. The second row for cryptophytes and dinoflagellates gives the values after deletion of the December 2003 sample, which had unusually high abundances of these groups (see 'Results')

| Variable S      | Slope (chang<br>per year) | e Std.<br>slope | $r^2$ | р        |
|-----------------|---------------------------|-----------------|-------|----------|
| Phosphate       | -0.005                    | -0.318          | 0.101 | < 0.001  |
| Nitrate         | -0.045<br>-0.062          | -0.157          | 0.025 | < 0.05   |
| Cryptophytes    | -0.278                    | -0.374          | 0.140 | < 0.0001 |
|                 | -0.238                    | -0.384          | 0.148 | < 0.0001 |
| Diatoms         | -0.314                    | -0.189          | 0.036 | < 0.05   |
| Dinoflagellates | -0.128                    | -0.272          | 0.074 | < 0.0001 |
|                 | -0.098                    | -0.273          | 0.074 | < 0.0001 |
| Haptophytes     | -0.137                    | -0.145          | 0.021 | NS       |
| Pelagophytes    | 0.139                     | 0.245           | 0.06  | < 0.01   |
| Prasinophytes   | -0.108                    | -0.138          | 0.019 | NS       |
| Prochlorococcus | -0.06                     | -0.217          | 0.047 | < 0.01   |
| Synechococcus   | -0.130                    | -0.208          | 0.043 | < 0.01   |
| TMV_Chl a       | -0.448                    | -0.280          | 0.079 | < 0.0001 |
| T_Chl a         | -0.467                    | -0.293          | 0.086 | < 0.0001 |
| Fl_Chl a        | -0.284                    | -0.178          | 0.032 | < 0.05   |

T\_Chl a anomaly TMV\_Chl a anomaly (Chl a, ng l<sup>-1</sup>)<sup>-1/2</sup> 30 15 0 -15 (Chl a, ng l<sup>-1</sup>)<sup>-1/2</sup> 30 В 15 0 -15 30 Fl\_Chl *a* anomaly (Chl *a*, ng l<sup>-1/2</sup> 30 С 15 0 -15 -30 2000 2006 2002 2004 2008 2010 2012 2014 Year

recurrent periods of calm weather during this season (D'Ortenzio & Ribera d'Alcalà 2009). In addition, in shallow waters like those of the Blanes station, it may be facilitated by the fact that there is sufficient light available practically all year (Gasol et al. (2016).

Haptophytes were the globally most abundant group (Fig. 5A), while the most important components of the chl a maxima were the diatoms, which showed a strong global correlation (r = 0.79, p < 0.0001) with T\_Chl a and accounted for 44% of the March T\_Chl a peak (Fig. 5B). This dominance of diatoms in phytoplankton bloom situations is a general finding in coastal and open sea areas of the NW Mediterranean (Ribera d'Alcalà et al. 2004, Estrada & Vaqué 2014). The prevalence of Chaetoceros spp. and Asterionellopsis glacialis in the late winter peaks and the contribution of Lioloma pacificum and Thalassionema nitzschioides in autumn agree with findings from previous studies (Margalef 1964). Prasinophytes, haptophytes and pelagophytes (Fig. 7) also presented winter maxima and summer minima. Cryptophytes showed a comparable pattern but with an additional peak in August. The association of diatoms, prasinophytes and haptophytes in the autumn to late-winter bloom period was noted by Gutiérrez-Rodríguez et al. (2011), who linked the dominance of these groups to high nutrient and moderately mixed conditions. According to Unrein et al. (2014), haptophytes (in particular the 3 to 5 µm size class) are present in Blanes Bay year-round and account for a high proportion of the bacterivory at the site. In our study, the seasonal vari-

> Fig. 9. Temporal variation of the monthly anomalies (after square root transformation of the data, indicated by the -1/2 exponent of the units) of (A) total monovinyl-chlorophyll *a* (TMV\_Chl *a*), (B) total chlorophyll *a* (T\_Chl *a*) and (C) fluorometric chlorophyll *a* (Fl\_Chl *a*). The linear regression lines are indicated in the graphs; the corresponding coefficients are shown in Table 2



Fig. 10. Temporal variation of the monthly anomalies (an.) (after square root transformation of the data) of (A) cryptophytes, (B) diatoms, (C) dinoflagellates, (D) haptophytes, (E) pelagophytes, (F) prasinophytes, (G) Prochlorococcus and (H) Synechococcus. The significant regression lines are indicated in the graphs; the corresponding coefficients are shown in Table 2

Table 3. Correlation coefficients among phosphate, nitrate, silicate, precipitation index (PI7), temperature and wind speed (WS, 2005–2014) anomalies. N = 159-161 for all coefficients, except those involving WS (in *italics*), for which N = 110-111; in this case, anomalies of all the other variables were recalculated for the period 2005–2014. Significant values (p < 0.05) are in **bold** 

| Variable                         | Phosphate<br>anomaly | Nitrate<br>anomaly | Silicate<br>anomaly | PI7<br>anomaly | WS<br>anomaly |
|----------------------------------|----------------------|--------------------|---------------------|----------------|---------------|
| Nitrate anomaly                  | 0.345                |                    |                     |                |               |
| Silicate anomaly                 | 0.456                | 0.632              |                     |                |               |
| PI7 anomaly (1 m <sup>-2</sup> ) | 0.055                | 0.208              | 0.332               |                |               |
| Temperature anomaly (°C)         | 0.018                | 0.018              | 0.092               | -0.2           |               |
| WS anomaly (m s <sup>-1</sup> )  | 0.191                | 0.02               | -0.041              | 0.044          | 0.064         |

ability of haptophytes was relatively small and the monthly variability was high, as evidenced by large error bars (Fig. 7), a finding that may be related to the ecological heterogeneity of this chemotaxonomic group, which included forms such as Emiliana huxleyi and Chrysochromulina (haptophytes Types 6 and 7), as well as *Phaeocystis* (haptophyte Type 8). The pelagophytes were classified among the 'mesotrophic' groups by Latasa et al. (2010), and in fact Marty et al. (2002) found a spring increase of their pigment marker 19'-butanoyloxyfucoxanthin in the DYFA-MED site. The presence of cryptophytes in Blanes Bay during the coldest months had been noted by Unrein et al. (2014), but Cerino & Zingone (2006) reported maximum concentrations in the Gulf of Naples in spring-summer and autumn. This diversity of observations concerning HPLC-derived groups such as pelagophytes and cryptophytes highlights the need for gathering information to improve our insight into

their taxonomical composition and associated ecological traits. The remaining eukaryotic group, the dinoflagellates, was more abundant between May and August and, with the exception of the December average value, influenced by the high concentrations observed in December 2003; their calculated monthly average contribution to total chl *a* did not exceed 20 ng l<sup>-1</sup> and was always <5% (Table 1, Figs. 5B & 7). The presence of dinoflagellates in sum-

mer, under stratified, nutrient-poor conditions (Margalef 1978), can be related to their potential mixotrophy and to their ability to undergo vertical migrations. However, some dinoflagellate taxa may also proliferate in winter, in particular following prolonged periods of good weather, as has been shown for *Alexandrium minutum* in some Catalan harbors (Van Lenning et al. 2007, Estrada et al. 2008). Note also that the CHEM-TAX method only takes peridinin-containing dinoflagellates into account; many dinoflagellate taxa do not contain this pigment or are heterotrophic and would not be accounted for by CHEMTAX.

The photosynthetic cyanobacteria, *Synechococcus* and *Prochlorococcus*, showed quite distinct seasonal distributions in Blanes Bay (Table 1, Figs. 5B & 7). *Synechococcus* presented their highest concentrations during the warmer months and accounted for 39% of T\_Chl *a* in September, while *Prochlorococcus* was generally present in low concentrations and

Table 4. Slopes (units are  $yr^{-1}$ ), standardized (Std.) slopes, p-values for the *t*-statistic, squared correlation coefficients and p-values of the *F*-ratio corresponding to the bivariate linear regression lines, with respect to sampling date (in yr) and to the precipitation index (PI7) anomaly (units are  $1 m^{-2}$ ), of the monthly anomalies (after square root transformation) of the CHEMTAX groups. The number of observations was 162. NS: non-significant. The second row for cryptophytes and dinoflagellates gives the values after deletion of the December 2003 sample, which had unusually high abundances of these groups (see 'Results'). Significant positive slopes with respect to time and negative with respect to PI7 are in **bold** 

| Dependent variable<br>(Chl a, ng l <sup>-1</sup> ) <sup>-1/2</sup> | Slope            | Time (yr)<br>Std. slope p-value |                    | - | —— PI'<br>Slope | 7 anomaly (l 1<br>Std. slope | Multiple r <sup>2</sup> | p-value        |                    |
|--|------------------|---------------------------------|--------------------|---|-----------------|------------------------------|-------------------------|----------------|--------------------|
| Cryptophytes   | -0.273<br>-0.236 | -0.367<br>-0.378                | <0.0001<br><0.0001 |   | 0.027<br>0.016  | 0.185<br>0.132               | <0.05<br>NS             | 0.174<br>0.163 | <0.0001<br><0.0001 |
| Diatoms  | -0.303           | -0.183                          | < 0.05             |   | 0.055           | 0.172                        | < 0.05                  | 0.065          | < 0.01             |
| Dinoflagellates  | -0.121<br>-0.092 | -0.254<br>-0.257                | <0.001<br><0.001   |   | 0.021<br>0.009  | $0.224 \\ 0.131$             | <0.01<br>NS             | 0.119<br>0.085 | <0.0001<br><0.001  |
| Haptophytes  | -0.137           | -0.145                          | NS                 | _ | 0.002           | -0.012                       | NS                      | 0.021          | NS                 |
| Pelagophytes   | 0.142            | 0.250                           | < 0.001            |   | 0.014           | 0.125                        | NS                      | 0.076          | < 0.01             |
| Prasinophytes  | -0.099           | -0.126                          | NS                 |   | 0.037           | 0.242                        | < 0.001                 | 0.077          | < 0.01             |
| Prochlorococcus  | -0.062           | -0.225                          | < 0.01             | - | 0.010           | -0.194                       | < 0.05                  | 0.085          | < 0.001            |
| Synechococcus  | -0.131           | -0.211                          | < 0.01             | - | 0.009           | -0.073                       | NS                      | 0.048          | < 0.05             |

increased between autumn and early winter, with a maximum contribution to T\_Chl *a* of 5% in October. Both genera are favored by their small size under low nutrient conditions (Moutin et al. 2002, Lomas et al. 2014), although Prochlorococcus has been associated with more oligotrophic situations than Synechococcus (Partensky et al. 1999, Latasa et al. 2010). Based on a seasonal survey in the Bay of Banyuls-sur-mer (a coastal site in the Mediterranean Sea, at the northern foothills of the Pyrenees), Charles et al. (2005) observed that Prochlorococcus and Synechococcus peaked between August and November and suggested that the dominance of cyanobacteria over eukaryotes was associated with low nutrient concentrations and N:P ratios below 10. In Blanes Bay, average N:P ratios below 10 were recorded in May and between August and November (Fig. 3D). These periods coincide in part with times of high Synechococcus abundance, but Prochlorococcus concentrations are still significant in December and January, when N:P ratios are relatively high. It is likely that relative nutrient availability is only one of many factors affecting picophytoplankton abundance. Several studies have addressed the positive relationship between temperature and picocyanobacteria abundance (Flombaum et al. 2013, Hunter-Cevera et al. 2016). Agawin et al. (1998) illustrated the relationship between temperature and Synechococcus variability in Blanes Bay, and Šantić et al. (2011) reported that both Synechococcus and Prochlorococcus tended to increase in several stations of the Adriatic Sea during the warmer period. However, as noted by Vaulot et al. (1990) and Šantić et al. (2011) among others, both cyanobacterial taxa were able to grow across a wide temperature range. This feature is illustrated by the occurrence of the autumn to early winter maximum of Prochlorococcus in Blanes Bay. A similar timing for the highest presence of prochlorophytes (divinyl-chl a) was documented by Marty et al. (2002) in open waters of the NW Mediterranean. However, temperature, nutrient availability, irradiance in surface waters and a number of biotic factors, such as grazing intensity, vary along the seasonal cycle of the coastal Mediterranean, and it is difficult to disentangle which variables or combinations thereof are responsible for a seasonal change in another variable. In addition, the 2 genera present a diversity of ecotypes that show spatio-temporal variability and preferences for different levels of the water column (Garczarek et al. 2007, Latasa et al. 2010, Mella-Flores et al. 2011, van de Poll et al. 2015). This genetic diversity is likely to underlie the 2 Synechococcus maxima in April and August. On the other

hand, the modest abundance of *Prochlorococcus* in the summer samples could perhaps be related to their susceptibility to the high solar UV radiation in the surface waters (Sommaruga et al. 2005, Llabrés et al. 2010).

# Episodic nutrient enrichment and interannual variability

Significant linearly decreasing temporal trends were identified (Table 2) for the monthly anomalies of phosphate, nitrate, nitrite (data not shown) and silicate, pooled chl a concentrations (TMV\_Chl a, T\_Chl a, FL\_Chl a) and all CHEMTAX-derived groups except haptophytes, prasinophytes and pelagophytes. Haptophytes and prasinophytes, as well as ammonium concentration anomalies (data not shown) also presented negative, albeit non-significant trends, while pelagophyte anomalies increased with time. Superimposed to the general temporal trends, the monthly sampling recorded isolated events with T\_Chl a concentrations exceeding 1.5 µg 1<sup>-1</sup>. Comparison of Fig. 2B,C suggests that particularly intense rain storms may have fuelled some of these high chl a blooms, as appears to be the case for the samples taken on 4 March 2003 (T\_Chl a = 1.68 $\mu$ g l<sup>-1</sup>), 16 December 2003 (T\_Chl *a* = 3.37  $\mu$ g l<sup>-1</sup>) and 29 November 2011 (T\_Chl  $a = 2.15 \ \mu g \ l^{-1}$ ), which followed heavy rains on 25-26 February 2003, 1-7 December 2003 and 4-6 and 15 November 2011, respectively. The significant relationships between precipitation (as represented by the PI7 index, see 'Materials and methods') and the chl a concentration of the various CHEMTAX groups were positive, except for Prochlorococcus, as shown by the PI7 slopes in the bivariate linear regression of CHEM-TAX Chl a concentrations with time and PI7 anomaly as independent variables (Table 4). In this context, it must be noted that although the plume of the main river close to Blanes, the Tordera (Fig. 1), tends to go to the south without affecting Blanes Bay, heavy rains may originate localized freshwater discharges and runoff. Due to the shorter period covered by the records, the effect of wind events is more difficult to ascertain, although a relationship is suggested by the positive correlation between phosphate and wind speed anomalies for the winter data subset of 2005 to 2014. The influence of continental runoff and wind storms (which may appear in association) for the phyto- and bacterioplankton community of Blanes Bay was studied by Guadayol et al. (2009) for the period between December 1997 and September 2006

(with a 2 yr gap in 1999 and 2000); they found that in this particular location, river runoff was very important for phytoplankton dynamics, whereas resuspension events caused by waves were of secondary importance. Winter wind events of certain intensity and direction could also be important in introducing nutrient-rich oceanic waters, as shown by Arin et al. (2013) for the coastal area of Barcelona and by Goffart et al. (2015) for the Bay of Calvi (Corsica). However, our data for Blanes Bay do not have enough spatio-temporal resolution to document this point. The fertilization effect of freshwater runoff in Catalan coastal waters was also discussed by Estrada (1979), who documented a Skeletonema costatum proliferation off Arenys de Mar (~30 km to the south of Blanes) following a heavy rainfall event in the region. In addition, precipitation may contribute to wet nutrient input from the atmosphere and, together with dry deposition processes, may be important in Blanes Bay, as shown by Marín (2017). We do not have information on long-term trends in our study region; according to Goffart et al. (2015), atmospheric deposition values seem to present decadal fluctuations and have recently (2003-2007) shown high variability (Ternon et al. 2010).

The general decreasing trends in nutrient and phytoplankton group concentrations could be due to several causes, including changes in stratification (due to increased temperature or freshwater input) and variations in episodic forcing factors such as those discussed above (continental runoff, wind events and atmospheric deposition). However, there was no relationship between nutrient concentration and temperature anomalies. The water column in Blanes Bay is mixed during most of the year, and during our study period, temperature did not present any significant trend, a finding that can be explained by the relatively short period of observation; indeed, the study of a long-term (since 1974) series measured at l'Estartit marine station (43° 03' N, 03° 15' 15" E), located to the north of Blanes, has shown a temperature increase of 0.3°C per decade for the shallower 50 m of the water column (Martin-Vide 2016). Among nutrient concentration anomalies, phosphate showed the strongest negative correlation with time (Table 2) while, as discussed above, nitrate and silicate were positively correlated with the precipitation index anomaly, and phosphate (period 2005 to 2014) was correlated with the wind speed anomaly (Table 3). However, there were no significant interannual trends in PI7 and wind speed, an observation that in the case of PI7 (no comparable information is available for wind speed) is consistent with conclusions

from longer precipitation time series in the region (Servei Meteorològic de Catalunya 2017). Another possibility for explaining the decreasing nutrient and chl a concentrations in Blanes Bay waters is the implementation of policies of wastewater treatment and phosphate reduction during the last decades (Rygaard et al. 2009, Gasol et al. 2012). The first sewage treatment plant started to operate in Blanes in 1972 and was replaced by an improved facility in 1997; in 2003, it was upgraded to tertiary reclamation treatment that has been interrupted since 2012 (Rygaard et al. 2009, E. Sallarès, Agència Catalana de l'Aigua, unpubl. data). A reduction in anthropogenic nutrient inputs could be a plausible factor behind the general oligotrophication trend in Blanes Bay, but longer time series are needed to ascertain the relative importance of climatic and anthropogenic forcing. Our findings agree with those of Mozetič et al. (2012), who concluded that anthropogenic drivers (the ban on phosphorus in the late 1980s and improvement of sewage treatment) together with reduced precipitation in the surrounding continental area could be responsible for the nutrient and chl a reduction trends detected in the Northern Adriatic for the period 1970 to 2007.

Table 4 indicates that the relative importance of the temporal trends and the runoff events was different for the various CHEMTAX groups. Taking into account the classification of Latasa et al. (2010), who assigned a 'trophic preference index' to different phytoplankton groups (cryptophytes, dinoflagellates, prasinophytes and diatoms were considered 'eutrophic', Synechococcus, pelagophytes and haptophytes as 'mesotrophic' and Prochlorococcus as 'oligotrophic'), it appears that the response to shortterm fertilization events associated with freshwater runoff was strongest for the most 'eutrophic' taxa, prasinophytes, diatoms, dinoflagellates and cryptophytes, and lowest for Prochlorococcus, as shown by their standardized slopes for the PI7 precipitation index. In contrast, there was little relationship between the effects of nutrient reduction (as expressed by standardized slopes with respect to time) and the trophic preference index classification. In this context, the positive temporal trend of the 'mesotrophic' pelagophytes is difficult to explain; it could indicate a need to revise the grouping of Latasa et al. (2010) or could be a result of other ecological changes in the Blanes Bay ecosystem. As noted above, the lack of significant slopes in the haptophytes could be a result of the heterogeneity of this group, which is likely to include taxa with substantial ecological differences.

#### **Concluding remarks**

The nano- and microphytoplanton succession in the waters of Blanes Bay had been previously characterized, by means of optical microscopic observations, as typical of temperate marine ecosystems: dominated by diatoms in the winter-spring bloom, followed by haptophytes and dinoflagellates later in the season (Margalef 1964, Mura et al. 1996, Estrada 1999, Siokou-Frangou et al. 2010, Estrada & Vaqué 2014). The present study, using HPLC pigment chemotaxonomy and flow cytometry, provides a more comprehensive view of the composition of the phytoplankton community, including cells in the picoplankton size range. Our data confirmed the prevalence of diatoms in the winter-spring bloom and in most episodic highbiomass situations and indicated the occurrence of more or less marked winter-spring maxima and summer minima in cryptophytes, haptophytes, pelagophytes and prasinophytes, in agreement with the variability in the concentrations of major nutrients. Prochlorococcus showed a fall-winter maximum and a spring-summer minimum, while dinoflagellates did not decrease their contribution in summer, and Synechococcus presented peaks in April and August. In contrast, the abundance of diatoms, prasinophytes, cryptophytes and dinoflagellates responded positively to episodic coastal fertilization associated with precipitation events. Most phytoplankton groups presented a decreasing interannual trend that could be attributed to a reduction in nutrient availability, as suggested by the coincidence with decreasing concentrations of phosphate and the other major nutrients. A possible driver for this oligotrophication trend is the improvement in wastewater treatment, but longer time series are needed to ascertain the role of climatic factors. Our results highlight the need for sustained ecosystem observations at adequate temporal resolution. In the context of coastal management, our findings suggest, on the one hand, that in spite of increasing human pressure, coastal eutrophication can be controlled if appropriate management of continental nutrient inputs is performed, and on the other hand, highlight the importance of considering not only warming (Doney 2006) but also the potential effects of extremes of precipitation or other meteorological events (Lavaysse et al. 2012) in a context of climate change.

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