



## Decreased seasonality and high variability of coastal plankton dynamics in an urban location of the NW Mediterranean



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### ABSTRACT

Contrary to what happens in open waters, where chlorophyll values and plankton dynamics can be predicted with a reasonable accuracy on an annual basis, biological parameters analyzed for coastal waters often show slight seasonality, and are exposed to numerous and convergent forcing factors that make it difficult to draw clear patterns. On top of this large natural variability, coastal locations subjected to urban sprawl suffer further human impact that may increase the unpredictability of plankton dynamics. Here we present the results of a multi-year time series of monthly samplings carried out in a coastal location by the city of Barcelona (NW Mediterranean) that is highly exposed to anthropogenic disturbances. Our data confirm the existence of complex patterns throughout the year. Freshwater inputs proved to be an important source of nutrients, yet the response of the planktonic organisms was vague and not systematic, contrary to the results of a previous study at a nearby coastal site less affected by human activities. The severity of anthropogenic disruptions was partially masked by the co-occurrence of natural physical phenomena, e.g., waste spills often come with downpours and large river discharge. In the NW Mediterranean, there seems to be a gradient of decreasing predictability on plankton dynamics from offshore to coastal waters with little human influence, where seasonality can be largely modified by local processes but the biological response is systematic and fairly predictable, and finally to urban coastal locations, where the seasonal background is diluted by numerous perturbations and there exists a variable pattern of biological responses. Our study underlines the importance of specific coastal processes in determining the structure and dynamics of the planktonic community, and the need to characterize coastal areas setting aside some of the assumptions valid for open ocean regions (e.g., (1) in the open ocean seasonality dominates annual nutrient fluxes, which are tightly linked to mixing and turbulence, while nutrient inputs at the coast can occur anytime throughout the year and may not be coincident with increased water-column mixing (Cloern, 1996; Cloern and Jassby, 2008); and (2) in coastal regions the concentration of nutrients during nutrient pulses can be greatly imbalanced with regard to Redfield elemental ratios (Jickells, 1998; Justić et al., 1995 and references therein)).

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### 1. Introduction

In the Mediterranean Sea, as in most temperate and subtropical oceanic areas, the annual plankton dynamics are recurrent and strongly driven by the winter mixing that initiates the most prominent production and population succession events. Mixing transfers nutrients from deep waters to the surface, where the availability of light and the stratification of the water column allow for a large phytoplankton production peak in winter–spring. Highly stratified water column conditions and diminished nutrient concentrations in summer give prominence to smaller phytoplankton species and recycling heterotrophs (Longhurst, 1995, 1998; Margalef, 1978). Lower autumn phytoplankton peaks are not uncommon when mixing starts to break

the stratification. Despite the many physico-chemical factors known to influence or modulate this system dynamics, such as fronts (Mahadevan and Archer, 2000), internal waves (Kahru, 1983), mesoscale processes (Lévy, 2008), wind-driven turbulence (Gargett, 1989; Pesant et al., 2002) or atmospheric inputs (Guerzoni et al., 1999), a high degree of predictability remains in open ocean conditions.

A different picture emerges for coastal areas. Chlorophyll, as a proxy for system production, tends to have annual cycles more or less blurred by a multitude of local, site-specific factors such as river discharge, complex bottom bathymetries, local winds, tides, atmospheric deposition, among others. Thus, the strong nutrient constraint on the seasonal occurrence of phytoplankton production in the open ocean weakens in coastal waters that receive nutrient inputs from land through a range of sources and regimes (Cloern, 1996; Jickells, 1998). In addition, the growth stimulation that takes place in open waters as a result of increased light availability during the spring stratification is of less importance in many coastal areas, because the water column is shallow

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and the organisms are permanently within the photic layer. As a consequence, seasonality may be greatly modified and predictability at the system level dramatically decreased.

Mediterranean climate is characterized by a strong irregularity in precipitation (Cebrián et al., 1996; Font, 2000; Llasat and Puigcerver, 1997; Valero et al., 2009). Rainfall is most common in autumn and spring, but strong storms can occur anytime throughout the year and they often result in significant inputs of terrestrial runoff into the coastal zone (Liquete et al., 2009). Precipitation events affect the discharge regime of streams, including many rivulets that remain dry during long periods of time but are responsible for large short-lived discharge events (Estrela et al., 2001; Liquete et al., 2007). In the NW Mediterranean, previous studies have shown the importance of episodic forcing on the dynamics of the planktonic community and their potential to induce changes on the order of seasonal variability (Duarte et al., 1999; Guadayol et al., 2009). Guadayol et al. (2009) proposed the existence of a sequence of events that led to production peaks after episodic storm and river discharge events in Blanes Bay. This increased the understanding of plankton system dynamics in the area.

In addition to the variability of natural forcing factors, there are human-induced perturbations. Human pressure along the world's coasts has increased during the last decades, and the impacts on the coastal environment have consequently multiplied (IPCC, 2007). Continuous human pressure can induce progressive changes in the water properties that may alter the natural dynamics of plankton or the community composition, and drive gradual, long-term ecosystem shifts. Eutrophication of coastal waters, along with the artificial stabilization of the coastline through dikes and harbors, are but two consequences (Claussen et al., 2009; Justić et al., 1995; Tett et al., 2003, 2007). The combination of excess nutrients and limited water mass movements can foster the emergence of chronically polluted areas or favor the development of algal blooms (Vila et al., 2001). Beyond long-term changes, human-induced perturbations may interact with the natural forcing variability on shorter time scales to affect coastal plankton dynamics (Romero et al., 2013).

The city of Barcelona and its nearby littoral is a good example of a densely populated coastal area. The metropolitan region houses about 3 million inhabitants, but over 4.6 million people live within the catchment area of the two major rivers that flow into the city's nearshore waters, the Besòs River and the Llobregat River (ACA, 2005). Coastal management in the area may thus benefit from a better understanding of the combined effects of natural and anthropogenic forcing on coastal plankton dynamics. Apart from the intrinsic pressure related to urban sprawl in terms of air and water pollution, impacts exerted by human activities on the coast are highly variable, both in form and in time. In the summer, touristic and recreational activities (bathing, leisure crafts) involve a direct and continuous perturbation of coastal waters, but their effects are usually restricted to a narrow water strip close to the coastline. On the contrary, in autumn and spring, urban sewage spills that come with freshwater flushes and rainfall overflows affect extensive areas, though the impact is limited in time and fairly uneven along the coast (ACA, 2005; EEA, 1999; UNEP/MAP/MED POL, 2003).

Within this framework, we hypothesize that the seasonal signal characteristic of open Mediterranean waters is blurred in the littoral fringe, particularly near densely populated areas, since nutrient inputs can be expected to be large and occasionally uncoupled from meteorological forcing. In order to address this issue, the present study focuses on the dynamics of plankton in a highly urbanized coastal location by the city of Barcelona, using time series data. We compare this study site to the results described for Blanes Bay (70 km north from Barcelona, Guadayol et al., 2009), and to a 13-year time series from Banyuls-sur-mer (200 km north from Barcelona), two stations where the natural forcing conditions are fairly similar to those found in Barcelona, but where the anthropogenic influence is lower.

## 2. Material and methods

### 2.1. Sampling area and sampling scheme

The study was conducted ca. 0.5 km offshore of Barceloneta beach, in Barcelona's coastal waters (NW Mediterranean, 41°22'55" N, 2°11'58" E, Fig. 1). The sampling station has a depth of about 10 m with a sandy bottom. The predominant current acting near the coast is the southwest littoral drift, a by-product of the Liguro-Provençal Current, which is the main oceanographic current in the Catalan margin (Flexas et al., 2002). An accurate description of the Barcelona continental shelf and its main geological, hydrographic and oceanographical features are given in Liquete et al. (2007). The area receives the discharge of two rivers, the Besòs River to the north and the Llobregat River to the south, and is influenced by numerous human activities (tourism, recreational sailing, dredging for beach maintenance, works on coastal infrastructures). In addition, the sampling site is located between two storm overflows (Fig. 1).

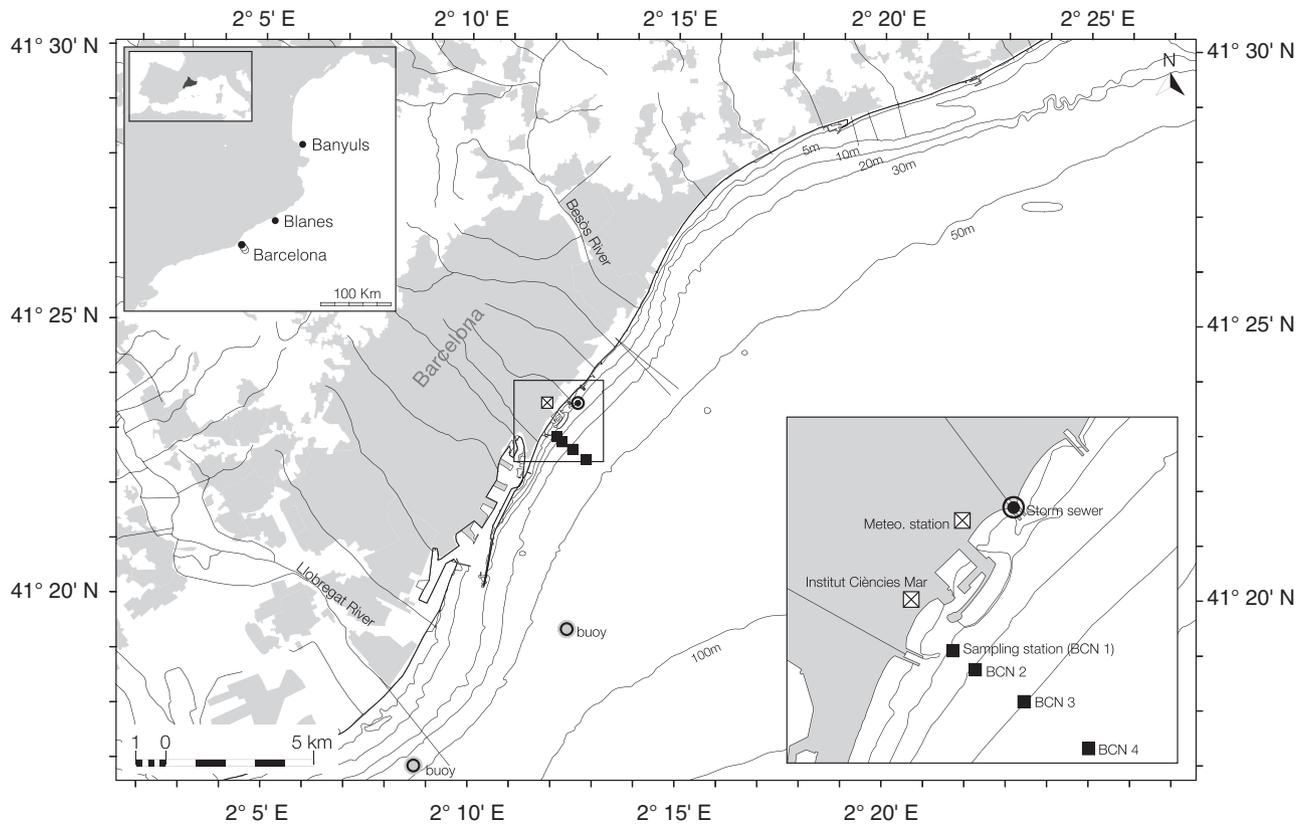
We carried out monthly samplings from October 2005 to October 2008, framed within a larger regular sampling program (the Coastal Oceanographic Observatory, COO) running since 2002 that has provided basic variables also included in the analyses (namely inorganic nutrients, chlorophyll *a*). Water was taken from a depth of 3 m by means of a Niskin bottle, and immediately transferred to 2.5 L polycarbonate carboys that had previously been washed with a dilute solution of hydrochloric acid, milli-Q water, and sample water. Carboys were carefully protected from sunlight and taken to the laboratory. CTD vertical profiles of salinity and temperature were also obtained (Sea-Bird Electronics SBE 25 CTD SN97 probe).

### 2.2. Hydrological and meteorological time series

Meteorological data was provided by the Spanish Meteorological Agency. Data was acquired from a meteorological station located at the seafront, close to our sampling site (41°23'27" N, 2°12'05" E, 21 m height, Fig. 1 inset). They supplied hourly-accumulated rainfall and hourly-averaged data for air temperature, wind speed and wind direction. Wind parameters were measured at 10 m above the ground and consisted of vectorial averages. Time series for the Besòs River discharge was provided by the Catalan Water Agency. Wave height and water temperature were obtained from a directional wave buoy deployed southwards of our sampling station (41°19'18" N, 2°12'24" E, Fig. 1) over a depth of 68.5 m (REDCOS network, Puertos del Estado). When necessary, data from a second buoy placed near the Llobregat River mouth (41°16'42" N, 2°08'29" E, 45 m depth, XIOM network, Generalitat de Catalunya) were used to fill gaps in the time series, applying a simple linear model that explained >96% of the variance. Both buoys provided hourly-averaged data. Storm sewer outflow data was obtained from Clavegueram de Barcelona S.A. (CLABSA). Barcelona's sewer system has underground deposits to collect rainfall and terrestrial runoff for routine sewage disposal, but when large downpours occur, part of these waters are jointly released into the sea through storm overflows. Outlet flow rates are not measured directly but are determined from measurements of the water level. Only values above a minimum threshold (0.1 m) are considered, and this hinders the detection of low-volume discharges. We analyzed the data from their station L20, north of our sampling station (41°23'31" N, 2°12'10" E, Fig. 1).

### 2.3. Analytical procedures

Inorganic nutrients (nitrate, nitrite, ammonium, phosphate and silicate) were determined with an Alliance Evolution II autoanalyzer following the methods in Hansen and Koroleff (1999) with minor modifications. Samples were kept frozen at –20 °C until analysis.



**Fig. 1.** Map of the study area, close to the city of Barcelona (NW Mediterranean). Gray shaded regions indicate urban areas and long straight lines near the coastline represent marine outfalls. The Besòs River flows into the sea up north of the city, while the mouth of the Llobregat River is located southwards. Meteorological stations are represented by white squares with crossed lines, and gray circles stand for wave buoys. The black squares mark our sampling station (BCN 1) and three more stations (BCNs 2, 3, 4) used for comparison of seasonal patterns. Several storm sewer overflows are located north and south of the sampling area; we used data from the storm overflow marked with a black circle. The inset map on the left shows a wider area with the stations of Blanes and Banyuls-sur-mer, also used for comparison in the study.

Particulate carbon and nitrogen (PC and PN) were collected in duplicate on pre-combusted Whatman GF/F filters (up to 1000 mL per filter). Samples were kept frozen at  $-80^{\circ}\text{C}$  until analysis. Measurements were carried out with a PerkinElmer 2400 CHN analyzer. An acetanilide standard was used daily. The precision of the method is  $\pm 0.3 \mu\text{mol C L}^{-1}$  and  $\pm 0.1 \mu\text{mol N L}^{-1}$ . To discern whether this particulate material corresponded to organisms or mostly consisted of inorganic particles, we acidified one replicate filter prior to analysis. When exposed to acidic steams, inorganic carbon compounds (mainly calcified structures) react to form volatile carbon dioxide, so that by comparing carbon concentration in acidified and non-acidified filters we can estimate the organic and inorganic fractions. No significant differences were found between both treatments, implying that particulate carbon concentrations were mostly organic.

The concentration of total phosphorus (TP) was determined by wet oxidation and colorimetry (Hansen and Koroleff, 1999). Briefly, 25 mL aliquots were mixed with 2 mL of the oxidant reagent in glass vials, and autoclaved at  $121^{\circ}\text{C}$  for 30 min. Once the vials were cooled down to room temperature, 600  $\mu\text{L}$  of ascorbic acid were added and left to act for 2 min. We finally added 600  $\mu\text{L}$  of the combined reagent and kept the samples in the dark for 15 min. Readings were done at 880 nm with a CaryWin UV Spectrophotometer.

Total and fractionated ( $>10 \mu\text{m}$ ,  $>20 \mu\text{m}$ ) chlorophyll *a* concentrations were determined by fluorometry (Yentsch and Menzel, 1963). For total chlorophyll, 20–40 mL samples were filtered through Whatman GF/F glass fiber filters. For the  $>10 \mu\text{m}$  fraction, we filtered 30 to 50 mL samples through 10- $\mu\text{m}$  pore size Whatman Nuclepore polycarbonate filters, and for the larger fraction ( $>20 \mu\text{m}$ ) we filtered 200 mL samples through 20- $\mu\text{m}$  nylon-mesh filters. All samples were done in triplicate. Filters were extracted in 90% acetone and left in the dark at  $4^{\circ}\text{C}$  for

24 h. The fluorescence of the extract was measured with a Turner Designs fluorometer.

Heterotrophic bacteria, autotrophic pico- and nanoplankton were quantified by flow cytometry (Gasol and del Giorgio, 2000). Samples (1.8 mL) were fixed with 0.18 mL of a 10% paraformaldehyde and 0.5% glutaraldehyde mixture. For heterotrophic bacteria, subsamples of 200  $\mu\text{L}$  were stained with SYTO13 (Molecular Probes) at  $2.5 \mu\text{mol L}^{-1}$  (diluted in DMS), left to stain for 15 min in the dark and then ran at low speed (ca.  $12 \mu\text{L min}^{-1}$ ) through a Becton Dickinson FACSCalibur flow cytometer with a laser emitting at 488 nm. As an internal standard, we added 10  $\mu\text{L}$  per sample of a  $10^6 \text{ mL}^{-1}$  solution of yellow-green 0.92  $\mu\text{m}$  latex beads (Polysciences). For phytoplankton, subsamples of 600  $\mu\text{L}$  were left unstained and were run at high speed (ca.  $60 \mu\text{L min}^{-1}$ ). Again, a volume of 10  $\mu\text{L}$  of a  $10^6 \text{ mL}^{-1}$  solution of beads was used as an internal standard. As a cross-control to determine cell abundances and to ensure stability of the flow, the flow speed was calibrated every 10 samples by measuring sample volume before and after a 10 min run.

Autotrophic and heterotrophic nanoflagellates (ANF and HNF, respectively) were estimated by epifluorescence microscopy according to the procedure described in Porter and Feig (1980). Samples for nanoflagellates were fixed with glutaraldehyde (1% final concentration), stained with DAPI ( $5 \mu\text{g mL}^{-1}$ ), and filtered on 0.8  $\mu\text{m}$  black polycarbonate membranes. The filters were then mounted on microscope slides and kept frozen at  $-20^{\circ}\text{C}$ . Counts were done on a Nikon Labophot epifluorescence microscope at  $\times 1250$  magnification. Between 180 and 200 nanoflagellates were counted per filter, and they were sized using a calibrated ocular micrometer in 4 classes ( $<5 \mu\text{m}$ , 5–10  $\mu\text{m}$ , 10–20  $\mu\text{m}$ ,  $>20 \mu\text{m}$ ). Autotrophic and heterotrophic organisms were distinguished by the red fluorescence of chlorophyll under blue light excitation.

## 2.4. Biomass calculations

Chlorophyll *a* values were converted to carbon using a factor of 50  $\mu\text{g C per } \mu\text{g chl}$ , a value within the range reported by Delgado et al. (1992) for surface waters in the NW Mediterranean. Bacterial biomass was estimated by flow cytometry following the methodology described in Gasol and del Giorgio (2000), using a carbon conversion factor of 0.35  $\text{pg C } \mu\text{m}^{-3}$  (Bjørnsen, 1986), which resulted in an average value of 21  $\text{fg C cell}^{-1}$ . Cell volume of nanoflagellates was established from the mean value of each size class, assuming a prolate spheroid shape. In this latter case, conversion to carbon was calculated with the equation  $\text{pg C cell}^{-1} = 0.433 \cdot (\mu\text{m}^3)^{0.863}$  (Verity et al., 1992). Carbon biomass of the organisms was used to determine the percentage of autotrophic and heterotrophic carbon.

## 2.5. Statistics

Water-column data was base-10 logarithmically transformed, linearly and seasonally detrended prior to statistical analyses. Log-transformed but non-detrended data were also used in correlation analyses for comparison. To filter out seasonal trends, the dynamics of the different parameters were analyzed using a cubic spline adjustment by minimizing the GCV (generalized cross-validation) score on the annually assembled data. The percentage of variability explained by the statistical cubic spline adjustment can be largely ascribed to seasonal patterns, and we therefore consider it a measure of the importance of seasonality in each parameter. References to seasonality, or to the percentage of seasonal variability throughout the text are referred to this adjustment value (e.g.,  $R^2$  seasonality values presented in Table 2 for Barcelona, Blanes and Banyuls-sur-mer). A 7-day moving average was applied to the meteorological and hydrological time series to remove daily variability and high-frequency noise. Meteorological and hydrological data were then linearly and seasonally detrended as explained above.

Pearson's product-moment correlation coefficient was used to estimate statistic relationships between variables. When performing multiple tests, *p-values* were adjusted applying the Bonferroni correction to control for the familywise error rate (Dunn, 1961). To detect delays in the response of water column variables with regard to the physical forcing, or between physical forcing variables, we performed cross correlations. Briefly, a cross correlation is a statistical method that computes the correlation between two time series shifted in time relative to one another (Jenkins and Watts, 1968). The resulting coefficients can be plotted versus the time lag to determine the offset between the two variables, i.e., the time scale of response. This type of analysis usually requires data sets with the same sampling frequency, although the method has been successfully used to examine data collected at different intervals (Guadayol et al., 2009). Statistical analyses were performed with Statistica (StatSoft Inc.), JMP statistical software (SAS) and R (R Foundation for Statistical Computing).

## 3. Results

### 3.1. Seasonal dynamics and interannual variability

#### 3.1.1. Hydrological and meteorological parameters

The interannual and seasonal dynamics of several physical parameters are shown in Fig. 2. Water temperature showed a strong seasonal signal, while precipitation and river flow presented high variability associated to the seasonal pattern and numerous peaks during the year. Maximum precipitation events in the study area occurred mostly in spring and early autumn. Extreme rainfall episodes decreased over the study period, and precipitation in 2008 was more evenly distributed throughout the year. In contrast, 2006 was particularly dry, with only minor rainfall episodes from the beginning of March until mid August. River discharge was closely related to precipitation and flow peaks

followed most rainfall events (Fig. 2). We considered the discharge of the Besòs River, north of our site, since the southwest littoral drift brings the plume past our sampling station. The mean Besòs flow was of about  $4 \text{ m}^3 \text{ s}^{-1}$ , but large peaks occurred during extreme rainfall episodes (daily values of  $86 \text{ m}^3 \text{ s}^{-1}$  in February 2003 or  $82 \text{ m}^3 \text{ s}^{-1}$  in September 2006).

Storm sewer data presented very high variability and several flow peaks, not always coincident with large episodes of rainfall. On average, the sewer outflow was higher in winter and late spring, but these values were somewhat biased by a few extreme events. An increase was also observed in autumn, at the time when precipitation is maximal in the area.

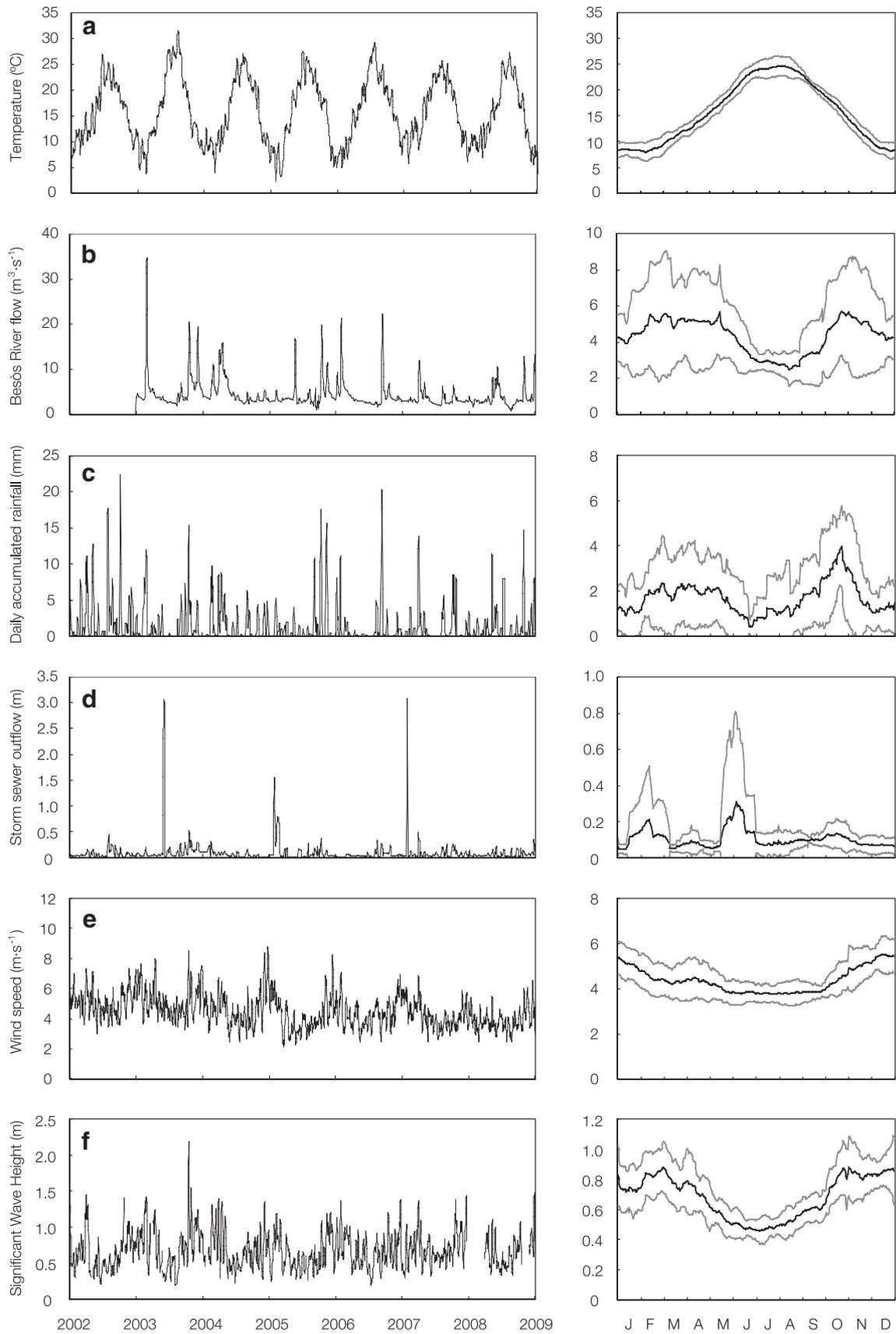
Significant wave height (hereafter,  $H_s$ ) ranged from 0.14 to 3.20 m, with an average daily value of  $0.68 \pm 0.39$  m. There was a trend in this parameter to slightly increase from late autumn to early spring (Fig. 2). Tides are very small in the Catalan coast (25 cm at maximum height).  $H_s$  can thus be considered a rough proxy for turbulence and mixing in the water. Wind velocities in the area averaged  $4.38 \pm 1.71 \text{ m s}^{-1}$  (Fig. 2), with higher values normally found in autumn and winter. Similar patterns were described by Guadayol and Peters (2006).

#### 3.1.2. Water column parameters

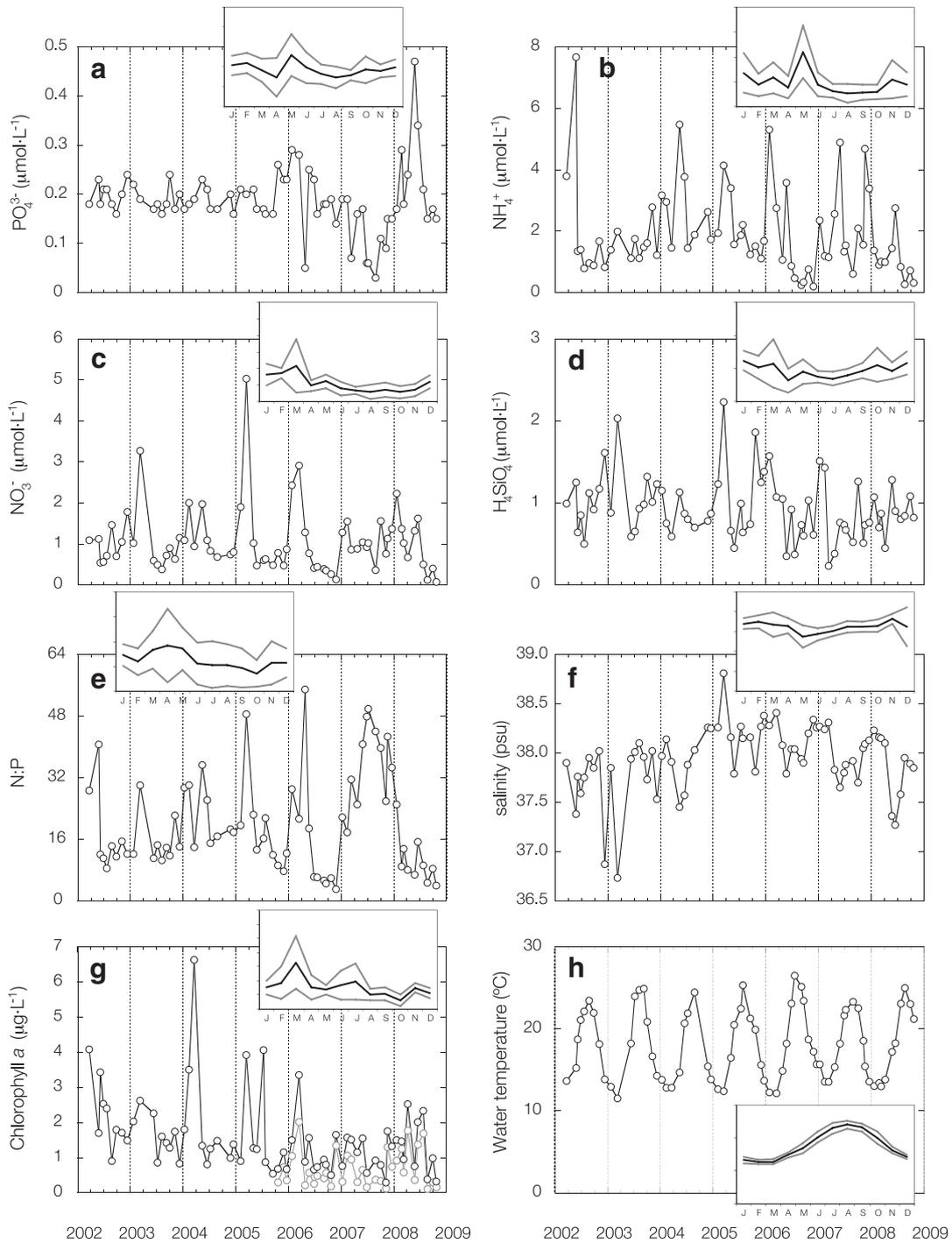
Salinity ranged from 36.87 to 38.54, and showed local minima following rainfall episodes (Fig. 3). Despite the absence of a clear seasonal pattern, slightly lower values were registered in spring, while in winter salinity was seldom below 38. Water temperature varied between 11.54 and 26.50 °C.

Broadly, nutrient concentrations were higher in winter and lower in summer (particularly silicate and nitrate, Fig. 3), but these background dynamics were obscured by numerous peaks during the year. Seasonality accounted for 30% of the annual variability for nitrate, but it represented <20% for silicate and between 9 and 14% for phosphate and ammonium, respectively. We calculated the dissolved N:P ratio throughout the whole study period to examine the potential co-limitation between N and P. There were remarkable differences between years; for the most part, nitrogen exceeded phosphorus during the first months of the year (with regard to the Redfield ratio), but the ratio dropped below Redfield values in the summer, indicating a nitrogen limitation. In 2007, phosphorus inputs were low and the ratio remained always above 16.

Higher winter chlorophyll maxima were found during the first years of the study period, and summer minima were also higher during the first years (2002–2005). Although the time series is not long enough to predict generalized long-term changes, chlorophyll showed a significant linear decrease throughout the 7 years. Chlorophyll *a* values increased from early winter to spring time, and they decreased in the summer, but there were many secondary peaks throughout the year, sometimes as high as the winter maximum (Fig. 3g). Size-fractionated chlorophyll analyses showed a visible coincidence between the >10  $\mu\text{m}$  and >20  $\mu\text{m}$  fractions (data not shown), meaning that most organisms retained in the 10- $\mu\text{m}$  filter were also collected in the >20- $\mu\text{m}$  mesh and corresponded to microphytoplankton. In the winter, high values of chlorophyll matched high proportions of large pigmented organisms (chl > 10  $\mu\text{m}$  represented ~50–80% of total chlorophyll *a*) whereas chlorophyll peaks occurring in spring or early summer did not always display such correspondence. Seasonal patterns accounted for <20% of annual chlorophyll variability, and they represented ~30% for the fraction of chl > 10  $\mu\text{m}$ . Pico- and nanophytoplankton were most abundant when total chlorophyll concentrations were low (Fig. 4). *Synechococcus* sp. and small eukaryotes peaked in the summer (from May to September), while maximum concentrations of *Prochlorococcus* sp. occurred in autumn. High numbers of autotrophic nanoflagellates were also found during summer periods. Note that pigmented nanoflagellates were not too abundant during the phytoplankton winter bloom, but instead they



**Fig. 2.** Interannual and seasonal dynamics of (a) air temperature; (b) river flow; (c) daily-accumulated rainfall; (d) storm overflow; (e) wind speed, and (f) significant wave height. The time series show 7-day moving average values. Plots on the right panel show the average annual cycle of each variable, computed as the mean (black line) ± standard deviation (gray lines) for each day of the year after smoothing with a 30 d moving average.



**Fig. 3.** Time series of (a–d) phosphate, ammonium, nitrate and silicate; (e) dissolved N:P ratio; (f) salinity; (g) total and fractionated chlorophyll *a* ( $\text{chl} > 10 \mu\text{m}$ , gray line), and (h) water temperature. Inset plots show the average annual cycle of each variable, i.e., each month displays the average value (black line)  $\pm$  standard deviation (gray line) of that particular month, calculated for the whole time series. The scale of the y-axis in the inset plots is the same scale of the time series.

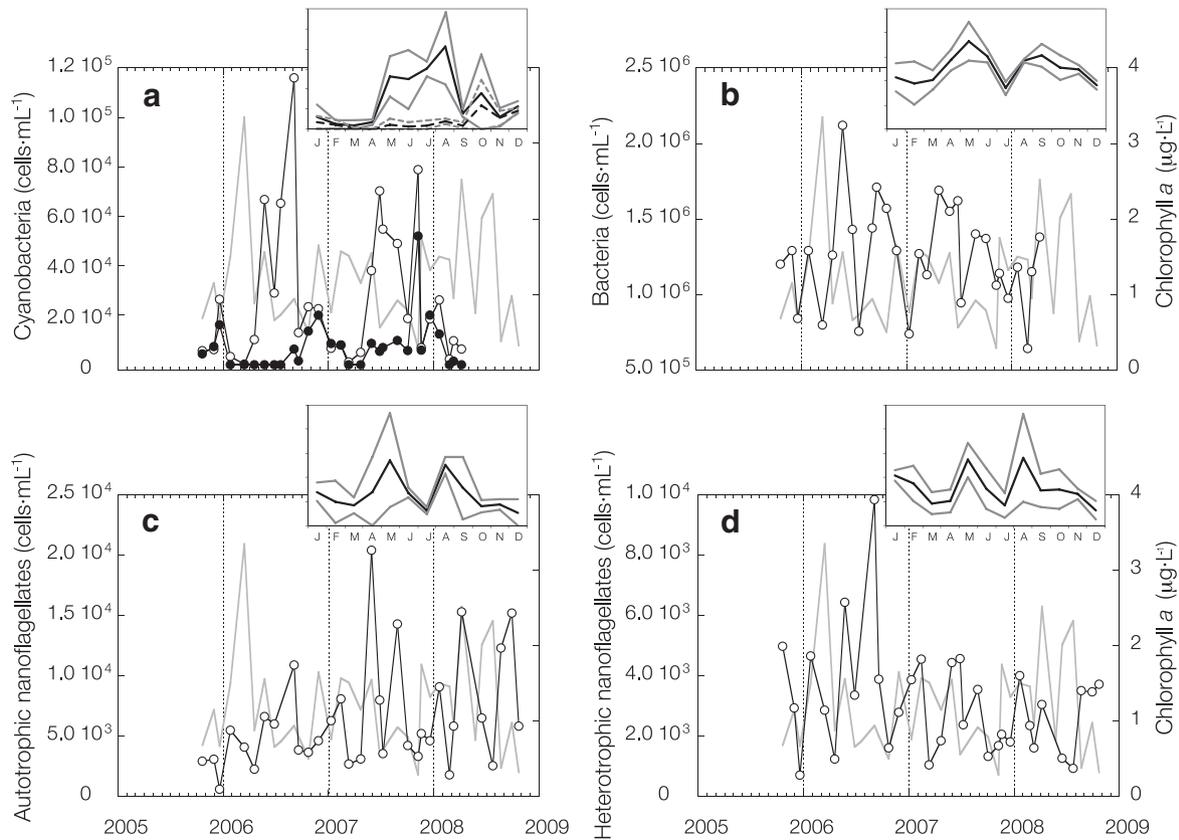
notably contributed to spring chlorophyll peaks (spring maxima for chlorophyll and ANF overlapped, Fig. 4c).

Bacterial abundance ranged from  $6.4 \cdot 10^5$  to  $2.1 \cdot 10^6$  cells  $\cdot \text{mL}^{-1}$ , with an average concentration of  $1.2 \pm 0.3 \cdot 10^6$  cells  $\cdot \text{mL}^{-1}$  (Fig. 4b). Two annual recurrent peaks were found in 2006 and 2007; the first one appeared in mid-spring (April–May) whereas the second one took place at the end of the summer (August–September). Lower concentrations were observed in the winter samples. The dynamics of heterotrophic nanoflagellates was fairly well coupled to that of bacteria, and increases in the abundance of heterotrophic nanoflagellates matched peaks in bacterial concentration throughout the year (Fig. 4d).

Despite the proximity to the city and the shallowness of the sampling station, PC and PN concentrations in the study area were generally low (Table 1). Average values for both variables were higher in winter and spring – seasonal variability was responsible for 31–38% of annual variations. The C:N ratio for the particulate matter was on average 7.7 and never fell below 5.9 (Redfield C:N ratio  $\approx$  6.6).

### 3.1.3. Comparison of chlorophyll and nutrients among different systems

Cloern and Jassby (2008) emphasized the large intra- and interannual variability found in many coastal areas around the world. To put the results in a broader context, we compared our data with those



**Fig. 4.** Abundance of (a) *Synechococcus* sp. (open circles) and *Prochlorococcus* sp. (black circles, dotted lines); (b) heterotrophic bacteria; (c) autotrophic nanoflagellates, and (d) heterotrophic nanoflagellates. Gray lines stand for reference and represent the concentration of chlorophyll a. Inset plots show the average annual cycle of each variable, i.e., each month displays the average value (black line)  $\pm$  standard deviation (gray line) of that particular month, calculated for the whole time series. The scale of the y-axis in the inset plots is the same scale of the time series.

presented in Cloern and Jassby (2008) by means of the two indices they propose: (a) the difference between the largest and smallest chlorophyll annual values in the series and (b) the timing of the annual chlorophyll maximum. Additionally, we gathered data for nutrients and chlorophyll concentration from three nearby stations located further

**Table 1**

Basic statistics for several biological and chemical water-column parameters from the sampling station in Barcelona (BCN 1). Sample size (N), mean  $\pm$  standard deviation, and range of values (min–max). Abbreviations: Chl = chlorophyll; Syne = *Synechococcus* sp.; Picoeuk. = picoeukaryotes; ANF = autotrophic nanoflagellates; HNF = heterotrophic nanoflagellates; TP = total phosphorus; PC = particulate carbon; PN = particulate nitrogen; and C:N refers to the C to N ratio of the particulate matter.

Variable	N	Time period	Mean $\pm$ sd	Min–max
Salinity	69	03/2002–10/2008	38.12 $\pm$ 0.25	36.87–38.54
Water temp. ( $^{\circ}$ C)	69	03/2002–10/2008	17.77 $\pm$ 4.32	11.54–26.50
$\text{PO}_4^{3-}$ ( $\mu\text{mol L}^{-1}$ )	69	03/2002–10/2008	0.186 $\pm$ 0.065	0.025–0.466
$\text{NH}_4^+$ ( $\mu\text{mol L}^{-1}$ )	69	03/2002–10/2008	1.920 $\pm$ 1.413	0.200–7.662
$\text{NO}_3^-$ ( $\mu\text{mol L}^{-1}$ )	69	03/2002–10/2008	1.043 $\pm$ 0.789	0.067–5.026
$\text{H}_4\text{SiO}_4$ ( $\mu\text{mol L}^{-1}$ )	69	03/2002–10/2008	0.932 $\pm$ 0.387	0.230–2.231
N:P	69	03/2002–10/2008	19.68 $\pm$ 12.84	2.96–54.96
$\text{NH}_4^+:\text{NO}_3^-$	69	03/2002–10/2008	2.14 $\pm$ 1.24	0.47–6.82
Chl $\alpha$ ( $\mu\text{g L}^{-1}$ )	68	03/2002–10/2008	1.58 $\pm$ 1.09	0.29–6.63
Chl $> 10 \mu\text{m}$ ( $\mu\text{g L}^{-1}$ )	34	10/2005–10/2008	0.60 $\pm$ 0.53	0.02–1.95
Bacteria ( $10^6$ cells $\text{mL}^{-1}$ )	29	10/2005–10/2008	1.25 $\pm$ 0.33	0.64–2.12
Syne. ( $10^4$ cells $\text{mL}^{-1}$ )	29	10/2005–10/2008	2.72 $\pm$ 2.89	0.03–11.6
Picoeuk. ( $10^4$ cells $\text{mL}^{-1}$ )	29	10/2005–10/2008	0.62 $\pm$ 0.63	0.09–2.67
ANF ( $10^4$ cells $\text{mL}^{-1}$ )	33	10/2005–10/2008	0.65 $\pm$ 0.46	0.06–2.04
HNF ( $10^4$ cells $\text{mL}^{-1}$ )	33	10/2005–10/2008	0.31 $\pm$ 0.18	0.07–0.98
TP ( $\mu\text{mol L}^{-1}$ )	30	10/2005–10/2008	0.25 $\pm$ 0.09	0.11–0.52
PC ( $\mu\text{mol L}^{-1}$ )	26	10/2005–10/2008	19.9 $\pm$ 9.0	7.3–41.4
PN ( $\mu\text{mol L}^{-1}$ )	26	10/2005–10/2008	2.6 $\pm$ 1.1	1.0–5.7
C:N	26	10/2005–10/2008	7.7 $\pm$ 1.1	5.9–10.6

offshore, and from two locations in the NW Mediterranean that are much less affected by human activities, Blanes Bay and Banyuls-sur-mer (Fig. 1, Table 2).

Chlorophyll at our Barcelona station ranged from 0.29 to 6.63  $\mu\text{g L}^{-1}$ , with an annual mean of 1.58  $\mu\text{g L}^{-1}$ . These values correspond to some of the lowest values reported in the study by Cloern and Jassby (2008), indicating a rather oligotrophic situation despite the proximity of the city. Nonetheless, chlorophyll values decrease rapidly as we move away from the coast, and notably as we go further north, away from the urban influence. Chlorophyll averages  $\sim 1 \mu\text{g L}^{-1}$  (50% decrease) just 1 km offshore from our station, and ca. 0.5–0.7  $\mu\text{g L}^{-1}$  in Blanes and Banyuls. The normalized range for Barcelona's coastal waters, i.e., the annual range divided by the annual mean, resulted in  $\sim 4.0$ , and was even higher in offshore stations, Blanes and Banyuls. These values are greater than the median (2.2) obtained for the whole data set in Cloern and Jassby (2008), and suggest large intra-annual variability in the area. Regarding the timing of the annual maximum, we found the largest peaks in February, March, and April. The year-to-year shift in timing is a common feature in many other locations, and provides further evidence for the complexity associated with coastal waters.

To compare the regularity of patterns across coastal stations, we fitted a statistical cubic spline to the time series of chlorophyll and nutrients in Blanes Bay and Banyuls, following the procedure applied in Barcelona and detailed in the methods (Table 2). Broadly, the adjustment of the spline indicates the importance of seasonal trends with regard to the annual dynamics of each parameter. Chlorophyll showed regular cycles in Banyuls and Blanes, where  $>40\%$  of the annual variability was explained by seasonal patterns (i.e., a large winter peak and low values in the summer). Seasonality blurred as we approached the area of Barcelona, where it accounted for  $<20\%$  of the overall variability. Seasonal patterns increased as we moved offshore, and reached ca.

**Table 2**

Chlorophyll and nutrient concentration in several coastal stations of the NW Mediterranean. North to south (see map in Fig. 1): Banyuls-sur-mer (SOLA station), Blanes (BBMO station), and Barcelona (BCNs 1 to 4, a series of stations located on an onshore–offshore transect perpendicular to the coast). BCN 1 corresponds to our study site. Range = (max–min); N range is the normalized range,  $N\ range = (max - min) / mean$ .  $R^2_{Season}$  refers to the percentage of the annual variability that is explained by seasonal patterns (see Materials and methods section for details). Chlorophyll (Chl) is in  $\mu g\ L^{-1}$  and nutrients in  $\mu mol\ L^{-1}$ .

		World average <sup>a</sup>	Banyuls	Blanes	BCN 1	BCN 2	BCN 3	BCN 4
Lat N, long E			42°29'22, 3°08'37"	41°40'13, 2°48'01"	41°22'55, 2°11'58"	41°22'50, 2°12'11"	41°22'40, 2°12'31"	41°22'30, 2°12'59"
Depth (m)			27	24	10	20	30	40
Distance to coast (km)			1.0	0.7	0.5	0.7	1.3	1.9
Chl	Range	0.5–10.2	0.03–4.21	0.02–3.45	0.29–6.63	0.11–4.75	0.09–4.89	0.08–4.39
	N range	0.8–7.0	7.54	5.10	4.03	3.90	4.40	4.55
	Mean	6	0.55	0.67	1.58	1.19	1.09	0.95
	Median	–	0.37	0.51	1.36	0.90	0.80	0.75
	$R^2_{Season}$	–	0.416	0.436	0.186	0.276	0.325	0.354
NO <sub>3</sub> <sup>-</sup>	Mean	–	0.83	1.67	1.04	1.03	1.02	0.95
	Median	–	0.31	0.63	0.87	0.63	0.60	0.58
	$R^2_{Season}$	–	0.562	0.319	0.299	0.300	0.361	0.370
	Mean	–	0.28	0.82	1.92	2.06	2.19	1.98
	Median	–	0.22	0.64	1.48	0.98	1.32	1.15
$R^2_{Season}$	–	0.060	0.033	0.141	0.070	0.141	0.140	

<sup>a</sup> Values from Cloern and Jassby (2008).

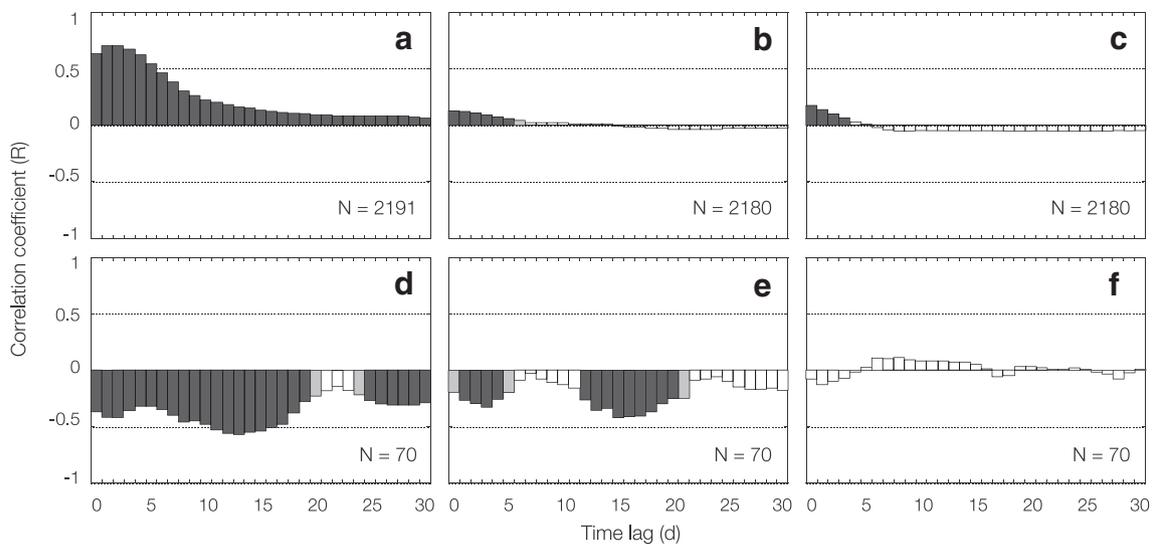
35% of the total variability at the furthestmost station. Coastal proximity therefore set a variability axis to the data, probably because of local bottom morphology, river and rivulet discharges and many other local forcing phenomena.

**3.2. Episodic forcing: relationships between physical drivers and water-column parameters**

Beyond the description of annual patterns, data from monthly samplings have been successfully used to determine the effects of large forcing episodes on the planktonic community, such as those derived from river discharge, rainfall, or gales and rough seas (Duarte et al., 1999; Guadayol et al., 2009). Physical factors present, however, multiple interactions. Most sudden river flushes are directly related to heavy rainfall, and extreme precipitation events are often driven by strong easterly winds, also responsible for rough seas. Aiming to clarify the extent of these interactions, we performed cross-correlations between the meteorological and hydrological time series. Data were linearly and seasonally detrended prior to the analyses, so that the observed relationships between variables would correspond to their association during specific episodes, and not just to seasonal adjustments.

First, we checked if episodes of rainfall and river discharge were simultaneous or there was a consistent delay between the start of precipitation and the peak in river discharge. Both variables proved to be significantly correlated from day 0, and high positive correlation coefficients lasted for weeks (Fig. 5a). Second, we examined the correlation between the Besòs River discharge and the salinity values registered in our sampling point to get an idea of how long it takes the river plume to reach the sampling station. According to the results (Fig. 5d), there is a significant drop in salinity immediately after the river discharge that would match an expected lag of 6 to 8 h from rough estimations using current velocities. However, the largest correlation starts about 1 week later and significant correlations extend to over 2 weeks. A significant decrease in salinity was also observed after rainfall, notably during the first week, but again the freshwater signal lasted for over 2 weeks (Fig. 5e).

One of the effects of urban sprawl is sewage water spill (Crain et al., 2009; EEA, 1999). Our sampling station is indeed located between two storm sewer overflows. Storm overflow data showed significant positive correlations with both rainfall and river discharge (Fig. 5b–c) during the first days, but negative links to salinity were non-significant (Fig. 5f). This hints at water releases at least partly



**Fig. 5.** Cross-correlations between hydrological and meteorological data. (a) Rainfall vs. lagged river discharge; (b) rainfall vs. lagged storm sewer outflow; (c) river discharge vs. lagged storm sewer outflow; (d) river discharge vs. lagged salinity; (e) rainfall vs. lagged salinity; (f) storm sewer outflow vs. lagged salinity. On the y-axis we have plotted the value of the correlation coefficient (R), while the x-axis represents the time lag in days. Dark gray bars represent correlation coefficients at a significant level of  $p < 0.01$  and light gray bars are significant at  $p < 0.05$ .



When non-detrended data were considered, silicate, nitrate and phosphate grouped together, while ammonium did not correlate with phosphate or silicate. Nitrate was significantly related to chlorophyll, notably with the fraction of chl > 10  $\mu\text{m}$ , and the quotient  $\text{NH}_4^+:\text{NO}_3^-$  was positively related to the bacterial abundance. Bacteria also matched with heterotrophic nanoflagellates. Negative links appeared between *Synechococcus* sp., silicate and nitrate. Total phosphorus peaked along with freshwater, ammonium and chlorophyll concentration, while phosphate correlated to particulate carbon. Water temperature was negatively related to nutrients and chlorophyll, and positively linked to cyanobacteria. Many of these significant correlations changed when tests were performed with seasonally detrended data. Nitrate and silicate were still significantly related to each other, but the link between nitrate and phosphate disappeared. Further, the positive match between chlorophyll, chl > 10  $\mu\text{m}$  and nitrate vanished, and instead a positive relationship with TP emerged. The link between bacteria and heterotrophic flagellates turned non-significant, but a positive connection with pigmented nanoflagellates appeared.

Correlation matrices give a good overall idea on groupings between variables and how these relationships change when filtering out seasonal patterns, but they do not allow the attribution of the observed shifts to specific forcing events. Moreover, despite the fact that many physical processes act as stimuli for biological changes, correlations between biological and physical parameters measured simultaneously do not always capture the connection between both factors. Because biological response may be delayed with regard to the onset of forcing factors, cross-correlations are useful to estimate the effects of episodic events on the community dynamics.

Cross-correlations with river discharge, significant wave, and storm sewer outflow height are plotted in Fig. 6. We used seasonally detrended data for both the water-column parameters and the time series of physical drivers. River flushes entailed rapid increases in silicate, nitrate, phosphate, and TP, while ammonium showed no significant response. A delayed positive signal appeared for silicate and TP (1–2 weeks). The addition of nutrients due to freshwater inputs was however not followed by a measurable increase in plankton abundance, and only the fraction of chlorophyll > 10  $\mu\text{m}$  and bacteria showed positive trends, although statistically non-significant. The effects of rainfall were very similar but with lower significance levels (not shown). Cross-correlations performed with significant wave height showed longer time lags for nutrient peaks (Fig. 6b). Silicate and nitrate correlated significantly to  $H_s$ , yet with 2-week lags, and TP increased somewhat later. Again, these increases in nutrients did not seem to be directly transferred to the organisms; positive correlations appeared during the first week with all groups except cyanobacteria, but none of these relationships were statistically significant. The results for storm sewer outflow showed the opposite pattern (Fig. 6c): nutrient concentrations did not change significantly following sewer outflows except for ammonium, and significant changes were found for the organisms. Chlorophyll > 10  $\mu\text{m}$  and *Synechococcus* sp. decreased shortly after sewer discharge, and they were followed by an increase of total chlorophyll, autotrophic nanoflagellates and bacteria (time lag of ~1–2 weeks).

#### 4. Discussion

The results show high inter- and intra-annual variability of both nutrients and planktonic organisms in our study area. Chlorophyll showed a significant linear decrease throughout the 7-year period; the decrease in chlorophyll may be related to changes in nutrient dynamics,

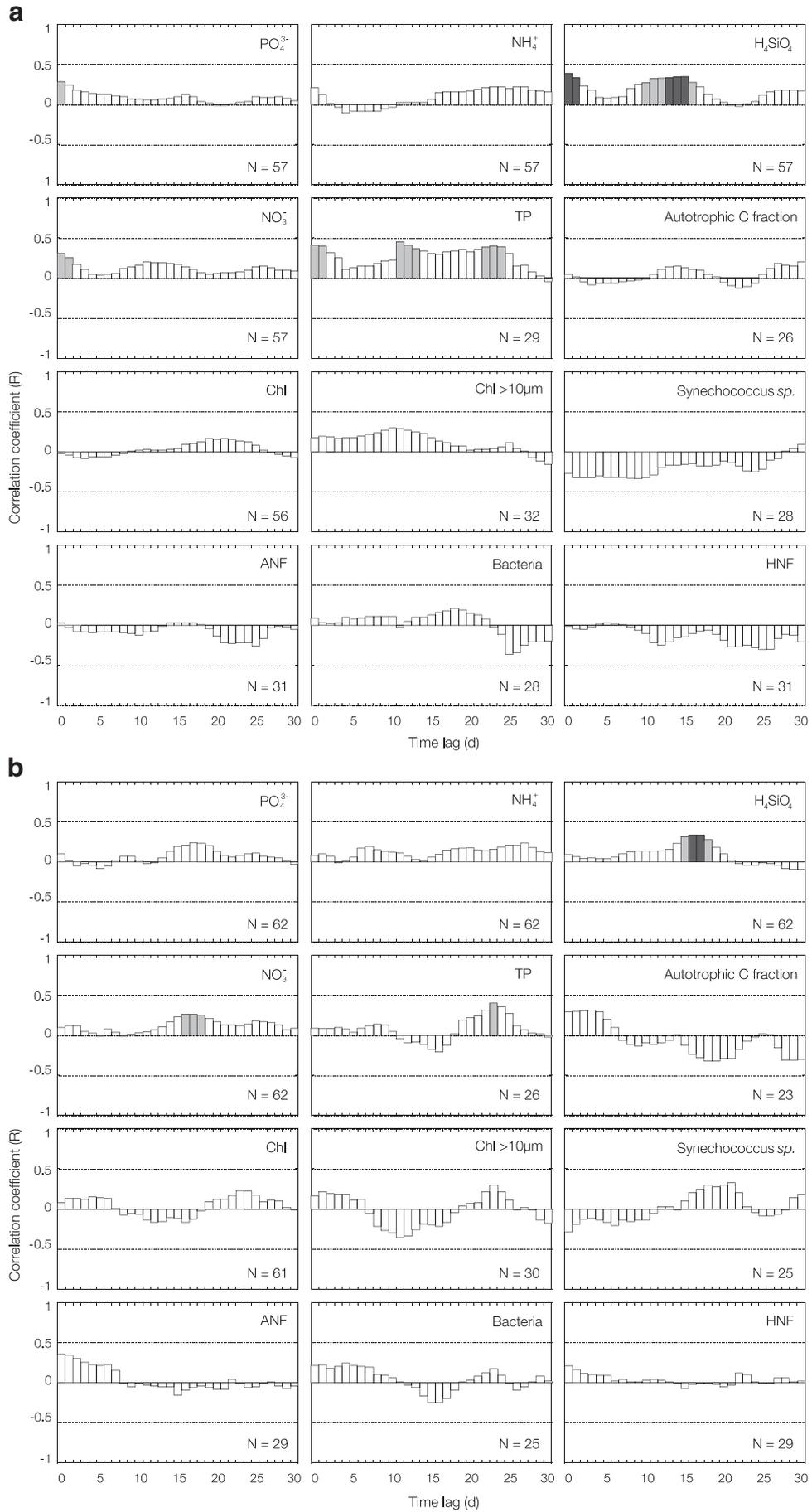
although none of the inorganic nutrients showed significant decreasing trends, or to changes in the food web structure. Interannual differences in the magnitude of the winter peak can be related to a number of physical factors, such as stronger vertical mixing, larger river discharge or the intrusion of offshore waters. Unfortunately, except for the river flow, we did not have sufficient data to adequately assess the relative contribution of these hydrographic factors. A recent study by Arin et al. (2013) addresses the importance of large-scale oceanographic forcing in the Barcelona region.

Nitrate and chlorophyll were the two variables that showed the highest seasonality, and yet seasonal patterns accounted for less than 30% of their total annual variability. Planktonic dynamics was however consistent with the typical annual cycles described for temperate regions (e.g., Marty et al., 2002). On average, higher abundances of microphytoplankton were found in the winter, while small autotrophs peaked in spring (ANF) and during the summer period (cyanobacteria). Major seasonal traits were outlined in the correlation matrix performed with non-detrended data. Thus, the coupling between cold waters, nitrate and silicate, along with the positive relationship between nitrate and chlorophyll, pointed to the annual winter phytoplankton bloom (nitrate-replete, well-mixed waters that foster diatom blooms in the winter), while cyanobacteria were most abundant at high water temperatures.

We compared the results with data collected from other coastal stations and found that seasonal patterns were of less importance with regard to the annual variability closer to Barcelona. This suggests that episodic forcing accounts for a large part of the observed intra-annual fluctuations in our site. Cross-correlation analyses and correlation matrices were aimed precisely at disclosing changes in the community after such episodic forcing, but the results showed that the response of the community after events was also complex and poorly predictable.

The irregular precipitation regime in the area is a strong physical feature, and it drives increased river discharge as seen from cross-correlations between both variables. Maximum correlation coefficients were obtained for lags of a few days, which are consistent with the fact that river discharge does not only depend on local rainfall, but also on the distribution of rainfall over the entire drainage basin. Freshwater flushes induced increases in most nutrients, highlighting the diverse contribution of natural factors and human activities to the river flow. The Besòs watershed comprises urban, industrial and agricultural lands, and the river has many tributaries flowing into the main stream close to its mouth. Agricultural runoff and industrial wastes contain high concentrations of nitrate and silicate (EEA, 1999, 2001; Garcia-Esteves et al., 2007; Torrecilla et al., 2005), while urban runoff is usually enriched in phosphate, ammonium, and organic matter (EEA, 1999, 2001; Ludwig et al., 2009; Suárez and Puertas, 2005). Because urban effluents are characterized by high ammonium concentrations, the absence of correlation between ammonium and freshwater discharge was puzzling. Nearshore waters in our study area are relatively enriched in ammonium. The ratio  $\text{NH}_4^+:\text{NO}_3^-$  was on average 2.14 throughout the 7-year period, and the background level of ammonium was ca. 2  $\mu\text{mol L}^{-1}$  (Table 1). High background concentrations may hinder the detection of statistical significant patterns if inputs are frequent but moderate, which may be the case in our study area. The low significant signal of nutrients after storm sewer outflows was also quite confusing, and we attribute it to the fact that sewer outflows are normally short-lived and moderate in volume (Romero et al., 2013), so that on the timescales here examined, nutrients could be already diluted or depleted by the organisms and the only visible response might be precisely the change in planktonic groups.

**Fig. 6.** Cross-correlations between water-column variables and (a) river discharge, (b) significant wave height, and (c) storm sewer outflow. On the y-axis we have plotted the value of the correlation coefficient (R), while the x-axis represents the time lag in days. Significant correlations are indicated by either dark gray bars ( $p < 0.01$ ) or light gray bars ( $p < 0.05$ ). Autotrophic C fraction refers to the relative proportion of autotrophic biomass to total biomass in terms of carbon. C biomass was estimated by applying the conversion factors specified in the text; total biomass is calculated by the addition of algae derived from chlorophyll *a*, bacteria and heterotrophic flagellates, while autotrophic biomass corresponds to the conversion of chlorophyll *a*. The rest of abbreviations as in Table 1.



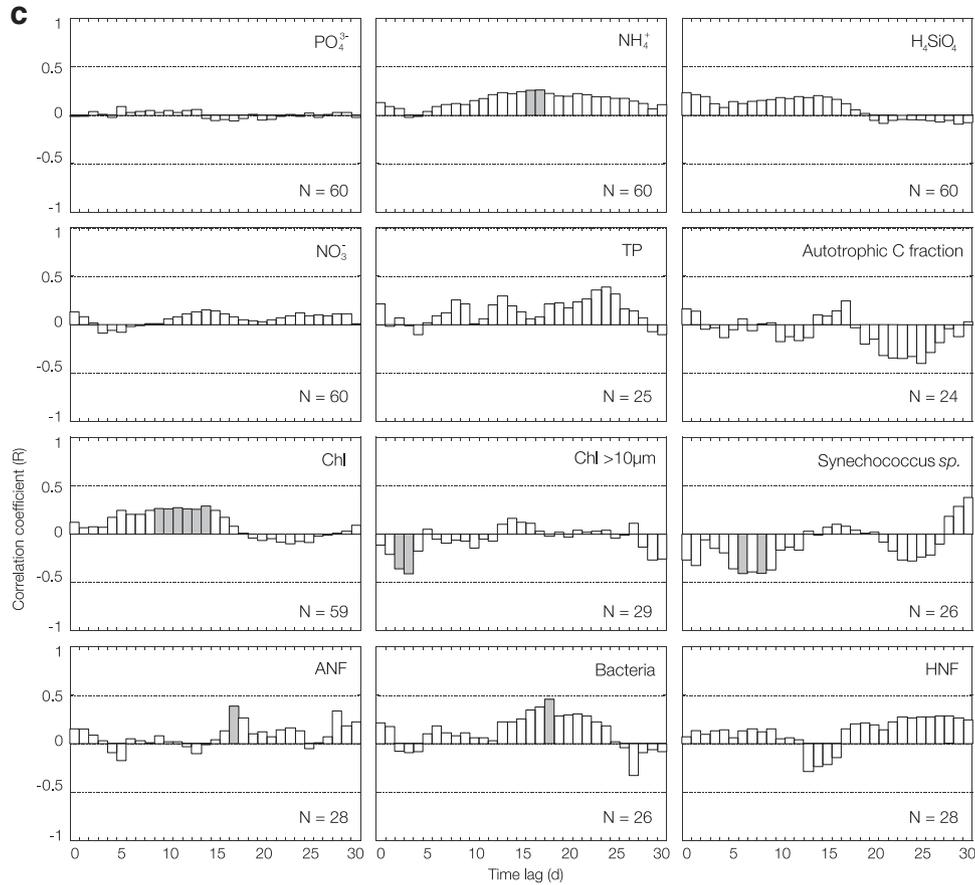


Fig. 6 (continued).

Wave height induced low and delayed nutrient increases. Rough seas in the area are mostly sustained by easterly storms, which often entail enhanced exchanges with offshore waters. The inflow of open waters during high wave episodes was suggested by the cross-correlogram between  $H_s$  and salinity, with a positive trend during the first week (not shown), and would match the observed increases in nitrate and silicate. Further, the sampling station is located close to the city harbor, in an area protected from storms by a series of submerged breakwaters. These barriers can contribute to a delay in the inflow of open waters and the nutrient signal, and possibly favor the nutrient patch lasting over time once it is within the sheltered region.

The existence of various mechanisms of nutrient enrichment with different inputs of inorganic nutrients was supported by the correlation matrices when seasonality was removed. Nitrate and silicate were tightly coupled even when seasonal patterns were filtered out, which hints at occasional open water inputs such as those described during rough seas. Ammonium and phosphate were partially mismatched from nitrate and silicate, and correlated to total phosphorus. An association between ammonium and total phosphorus can be expected in coastal locations exposed to the influx of urban sewage outfalls (EEA, 1999, 2001).

Theoretically, a greater load of dissolved organic matter and high ratios of ammonium to nitrate – both typical of urban spills – may overall favor the growth of small phytoplankton and bacteria, while nitrate and silicate inputs promote the growth of diatoms (Capriulo et al., 2002; Heil et al., 2007; Romero et al., 2012 and references therein). The advantage of particular groups was however not observed after episodes of river discharge or increased wave height, despite their different nutrient contribution. River discharge led to increases in most nutrients, yet only a positive, non-significant relationship

appeared for chlorophyll > 10  $\mu\text{m}$  and bacteria. Similar analyses performed by Guadayol et al. (2009) in Blanes Bay showed a clear increase of chlorophyll a few days after freshwater inputs, and a later increase of bacteria. In our study, significance may have been partly hindered by the lower number of samples, but there are differences between Blanes Bay and Barcelona's coastal waters that support the blurry biological response found in the latter. First, the river in Blanes is an ephemeral Mediterranean rivulet that runs dry most of the year but has large flow peaks during rainfall events, and therefore the magnitude of the forcing with regard to the normal conditions is larger than in the area near Barcelona. Second, owing to its population density, Barcelona nearshore waters are subject to a variety of human impacts that largely surpass those affecting the town of Blanes. Concurrent with river discharge there are numerous storm overflows and sewage pipes that release waters with a potential high load of organic substances (e.g., Llorens et al., 2008; Marchand et al., 1989), greater tourism and shipping, ongoing construction works and dredging for beach maintenance. These factors may all have an impact on the chemical composition and turbidity of the water column, and can eventually obscure the effects of the hydrological forcing.

On the other hand, we expected wave height to be of particular importance to large phytoplankton, because the combination of higher turbulent mixing and nutrient load favors autotrophs, and especially large phytoplankton (Karp-Boss et al., 1996; Peters et al., 1998, 2006). Moreover, mixing may induce sediment resuspension, favoring the entrainment of large phytoplankton cells from the bottom. High chlorophyll near-bottom layers (HCNBL) have been found in Barcelona's coastal waters during warm periods, mainly composed of big diatoms (Guallar and Flos, 2009). The theoretical advantage

of microphytoplankton, however, was not apparent in our results; positive effects of wave height appeared also for bacteria and nanoplankton, suggesting strong competition for nutrients.

Turbidity and decreased light availability could be affecting the capacity of phytoplankton to thrive during episodes of increased mixing. A number of studies have stressed the importance of the coupling between dissolved organic matter and water turbidity in determining which planktonic group may benefit from episodic nutrient enrichments. Turbidity and light attenuation were crucial in controlling phytoplankton growth in San Francisco Bay (Cloern, 1987, 1999). In Banyuls-sur-mer, bacterial biomass and production were enhanced following a winter storm, while phytoplankton cells were light-limited due to sediment resuspension and increased turbidity (Grémare et al., 2003). The situation is somewhat tricky in Barcelona's coastal waters. A moderate amount of suspended matter, as derived from the shallow water column, is to be expected in Barcelona, but the potential disadvantage for autotrophic cells might be easily overcome because (a) light is available down to the bottom and (b) during resuspension events, local sandy particles settle down fast. Further, wave action resuspends phytoplankton cells, adding a competing interaction. When turbidity is driven by terrestrial runoff and river discharge, however, finer sediment fractions may be transported (partly on account of intrinsic lithological features of the Besòs River basin, Liquete et al., 2007, 2009) and transient light attenuation can bring about bacterial advantage.

High concentrations of dissolved organic matter rather than turbidity could be the factor underlying the apparent lack of response to episodic forcing in our sampling site. Organic nutrients have been shown to be important in shaping the dynamics of coastal communities, and may stimulate a number of planktonic organisms, including heterotrophic bacteria and cyanobacteria, but also small flagellates and dinoflagellates (Glibert et al., 2004; Heil et al., 2007). In our system, an indication of the importance of organic nutrients could be the fact that total phosphorus, but not phosphate, was correlated to chlorophyll. We believe that the regular enrichment in organic matter and ammonium found in our study area as a result of urban spills sustains a very active background community of bacteria and small phytoplankton that is largely responsible for the high variability observed in the system beyond seasonal patterns. This dynamic microbial community could be responding to episodic nutrient inputs and mediating the response of other groups. Cross-correlations with sewer outflow were consistent with this hypothesis; sewer outflows were positively linked to ammonium, and induced significant increases of chlorophyll, notably of small autotrophic nanoflagellates, and bacteria. An enhancement of the microbial loop and mixotrophic organisms as a consequence of anthropogenic inputs has been previously observed in coastal areas (Burkholder et al., 2008; Capriulo et al., 2002). Mixotrophs and heterotrophic microbes can benefit from increased organic nutrients and favor the growth of other phytoplankton groups through nutrient regeneration.

## 5. Conclusions

The large difference in the anthropogenic footprint may be the main cause underlying both the limited seasonality and the lack of systematic responses to specific events in the coastal zone near Barcelona. In Blanes Bay, storms characterized by episodes of high wave height or river discharge had a high predictive power with regard to plankton dynamics, showing a consistent chain of effects (Guadayol et al., 2009). In Barcelona's coastal waters the outcome of episodic physical forcing had a lower predictability and was characterized by a blurry sequence of events. The meteorological, oceanographic, and geological framework (including key factors such as the wind and precipitation regime, light conditions or prevailing currents) is basically the same for both sites, and one would expect a somewhat similar biological response to common environmental drivers. Close to the city, however, the anthropogenic pressure sets a system of proportionally higher ammonium and

phosphate levels and lower silicate levels, all with respect to nitrate. Additionally, organic matter inputs may be also larger (Gray et al., 2002; Howarth et al., 1991; Marchand et al., 1989; Tolosa et al., 1996). Such general conditions favor the abundance of small phytoplankton and bacteria (Glibert et al., 2004; Heil et al., 2007; Romero et al., 2012), reducing their dependency on fresh phytoplankton-based organic matter and partly decoupling their dynamics from the large phytoplankton peaks. Further, nutrient pulses in the Barcelona site are poorly predictable. This buffering effect of the urban area seems to strongly constrain the timing and intensity of nutrient inputs after forcing events. A reduction in the forcing variability or its intensity theoretically favors small picoplankton (auto and heterotrophic) over large phytoplankton, such as diatoms that respond strongly to large inorganic nutrient inputs. Thus, paradoxically, a decrease in the variability of forcing conditions reduces the predictability in the response of plankton dynamics that now depend more heavily on complex internal trophic interactions and delicate balances.

We believe that the anthropogenic pressure in the area drives frequent, but moderate nutrient inputs that favor higher variability on shorter time scales (hours to days, Romero et al., 2013), and decreases the seasonal signal on the monthly to annual time scales studied here. This aspect is important from a theoretical as well as a practical point of view, because administrators of large urban regions are increasingly pressed by society to foresee and prevent anthropogenic impacts in their coastal areas. Our results show that close to urban areas, monitoring approaches based on monthly data are not enough to identify the causes underlying biological changes within the coastal community. Higher frequency measurements are required to improve our understanding of ecological processes, and although traditional sampling methods are costly, new genomic tools are a valuable resource that can assist in providing rapid information about ecosystem functioning for coastal monitoring programs (Bourlat et al., 2013).

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## References

- ACA, 2005. Catalan Water Agency. Caracterització de masses d'aigua i anàlisi del risc d'incompliment dels objectius de la directiva marc de l'aigua (2000/60/CE) a Catalunya. Departament de Medi Ambient i Habitatge, Generalitat de Catalunya.
- Arin, L., Guillén, J., Segura-Noguera, M., Estrada, M., 2013. Open sea hydrographic forcing of nutrient and phytoplankton dynamics in a Mediterranean coastal ecosystem. *Estuar. Coast. Shelf Sci.* 133, 116–128.
- Bjørnsen, P.K., 1986. Automatic-determination of bacterioplankton biomass by image-analysis. *Appl. Environ. Microbiol.* 51, 1199–1204.
- Bourlat, S.J., Borja, A., Gilbert, J., Taylor, M.L., Davies, N., Weisberg, S.B., Griffith, J.F., Lettieri, T., Field, D., Benzie, J., Glöckner, F.O., Rodríguez-Ezpeleta, N., Faith, D.P., Bean, T.P., Obst, M., 2013. Genomics in marine monitoring: new opportunities for assessing marine health status. *Mar. Pollut. Bull.* 74, 19–31.
- Burkholder, J.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8, 77–93.
- Capriulo, G.M., Smith, G., Troy, R., Wikfors, G.H., Pellet, J., Yarish, C., 2002. The planktonic food web structure of a temperate zone estuary, and its alteration due to eutrophication. *Hydrobiologia* 475 (476), 263–333.
- Cebrián, J., Duarte, C.M., Pascual, J., 1996. Marine climate on the Costa Brava (northwest Mediterranean) littoral. *Publicaciones Especiales del Instituto Español de Oceanografía* 22, 9–21.

- Claussen, U., Zevenboom, W., Brockmann, U., Topcu, D., Bot, P., 2009. Assessment of the eutrophication status of transitional, coastal and marine waters within OSPAR. *Hydrobiologia* 49–58.
- Cloern, J.E., 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Cont. Shelf Res.* 7, 1367–1381.
- Cloern, J.E., 1996. Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. *Rev. Geophys.* 34, 127–168.
- Cloern, J.E., 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquat. Ecol.* 33, 3–16.
- Cloern, J.E., Jassby, A.D., 2008. Complex seasonal patterns of primary producers at the land–sea interface. *Ecol. Lett.* 11, 1294–1303.
- Crain, C.M., Halpern, B.S., Beck, M.W., Kappel, C.V., 2009. Understanding and managing human threats to the coastal marine environment. *Year in Ecology and Conservation Biology* 1162, 39–62.
- Delgado, M., Latasa, M., Estrada, M., 1992. Variability in the size-fractionated distribution of the phytoplankton across the Catalan front of the north-west Mediterranean. *J. Plankton Res.* 14, 753–771.
- Duarte, C.M., Agustí, S., Kennedy, H., Vaqué, D., 1999. The Mediterranean climate as a template for Mediterranean marine ecosystems: the example of the northeast Spanish littoral. *Prog. Oceanogr.* 44, 245–270.
- Dunn, O.J., 1961. Multiple comparisons among means. *J. Am. Stat. Assoc.* 56 (293), 52–64. <http://dx.doi.org/10.1080/01621459.1961.10482090>.
- EEA, 1999. State and pressures of the marine and coastal Mediterranean environment. *Environmental Assessment Series No 5* European Environment Agency, Copenhagen, Denmark 92-9167-187-8 (137 pp.).
- EEA, 2001. Eutrophication in Europe's coastal waters. *Topic Report 7/2001*. European Environment Agency, Copenhagen, Denmark.
- Estrela, T., Menéndez, M., Dimas, M., Marcuello, C., Rees, G., Cole, G., Weber, K., Grath, J., Leonard, J., Ovesen, N.B., Fehér, J., Consult, V., 2001. Sustainable water use in Europe. Part 3. Extreme hydrological events: floods and droughts. *Environmental Issue Report No. 21*. EEA, Copenhagen, Denmark (84 pp.).
- Flexas, M.M., de Madroñ, X.D., García, M.A., Canals, M., Arnau, P., 2002. Flow variability in the Gulf of Lions during the MATER HFF experiment (March–May 1997). *J. Mar. Syst.* 33, 197–214.
- Font, I., 2000. *Climatología de España y Portugal*. Ediciones Universidad de Salamanca, 2nd ed. Salamanca, Spain.
- García-Esteves, J., Ludwig, W., Kerhervé, P., Probst, J.L., Lespinas, F., 2007. Predicting the impact of land use on the major element and nutrient fluxes in coastal Mediterranean rivers: the case of the Têt River (Southern France). *Appl. Geochem.* 22, 230–248.
- Gargett, A.E., 1989. Ocean turbulence. *Annu. Rev. Fluid Mech.* 21, 419–451.
- Gasol, J.M., Del Giorgio, P.A., 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci. Mar.* 64, 197–224.
- Glibert, P.M., Heil, C.A., Hollander, D., Revilla, M., Hoare, A., Alexander, J., Murasko, S., 2004. Evidence for dissolved organic nitrogen and phosphorus uptake during a cyanobacterial bloom in Florida Bay. *Mar. Ecol. Prog. Ser.* 280, 73–83.
- Gray, J.S., Wu, R.S.S., Or, Y.Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar. Ecol. Prog. Ser.* 238, 249–279.
- Grémare, A., Amouroux, J.M., Cauwet, G., Charles, F., Courties, C., De Bovee, F., Dinet, A., Devenon, J.L., De Madroñ, X.D., Ferre, B., Fraunie, P., Joux, F., Lantoiné, F., Lebaron, P., Naudin, J.J., Palanques, A., Pujo-Pay, M., Zudaire, L., 2003. The effects of a strong winter storm on physical and biological variables at a shelf site in the Mediterranean. *Oceanol. Acta* 26, 407–419.
- Guadayol, Ò., Peters, F., 2006. Analysis of wind events in a coastal area: a tool for assessing turbulence variability for studies on plankton. *Sci. Mar.* 70, 9–20.
- Guadayol, Ò., Peters, F., Marrasé, C., Gasol, J.M., Roldán, C., Berdalet, E., Massana, R., Sabata, A., 2009. Episodic meteorological and nutrient-load events as drivers of coastal planktonic ecosystem dynamics: a time-series analysis. *Mar. Ecol. Prog. Ser.* 381, 139–155.
- Guallar, C., Flos, J., 2009. High Chlorophyll Near-bottom Layer in the Barcelona Coastal Waters. *ASLO Aquatic Sciences Meeting*, Nice, France.
- Guerzoni, S., Chester, R., Dulac, F., Herut, B., Loje-Pilot, M.D., Measures, C., Migon, C., Molinaroli, E., Moulin, C., Rossini, P., Saydam, C., Soudine, A., Ziveri, P., 1999. The role of atmospheric deposition in the biogeochemistry of the Mediterranean Sea. *Prog. Oceanogr.* 44, 147–190.
- Hansen, H.P., Koroleff, F., 1999. Determination of nutrients. In: Grasshoff, K., Kremling, K., Ehrhardt, M. (Eds.), *Methods of Seawater Analysis*. Wiley-Verlag Chemie, Weinheim.
- Heil, C.A., Revilla, M., Glibert, P.M., Murasko, S., 2007. Nutrient quality drives differential phytoplankton community composition on the southwest Florida shelf. *Limnol. Oceanogr.* 52, 1067–1078.
- Howarth, R.W., Fruci, J.R., Sherman, D., 1991. Inputs of sediment and carbon to an estuarine ecosystem – influence of land-use. *Ecol. Appl.* 1, 27–39.
- IPCC, 2007. Intergovernmental panel on climate change. In: *Writing Team, Core, Pachauri, R.K., Reisinger, A.* (Eds.), *Climate Change 2007: Synthesis Report*. IPCC, Geneva, Switzerland ([http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4\\_syr.pdf](http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf)).
- Jenkins, G.M., Watts, D.G., 1968. *Spectral Analysis and Its Applications*. Holden-Day, San Francisco.
- Jickells, T.D., 1998. Nutrient biogeochemistry of the coastal zone. *Science* 281, 217–222.
- Justiç, D., Rabalais, N.N., Turner, R.E., Dortch, Q., 1995. Changes in nutrient structure of river-dominated coastal waters – stoichiometric nutrient balance and its consequences. *Estuar. Coast. Shelf Sci.* 40, 339–356.
- Kahru, M., 1983. Phytoplankton patchiness generated by long internal waves – a model. *Mar. Ecol. Prog. Ser.* 10, 111–117.
- Karp-Boss, L., Boss, E., Jumars, P.A., 1996. Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanogr. Mar. Biol.* 34, 71–107.
- Lévy, M., 2008. The modulation of biological production by oceanic mesoscale turbulence. *Lect. Notes Phys.* 744, 219–261. [http://dx.doi.org/10.1007/978-3-540-75215-8\\_9](http://dx.doi.org/10.1007/978-3-540-75215-8_9).
- Liquete, C., Canals, M., Lastras, G., Ambias, D., Urgeles, R., De Mol, B., De Batist, M., Hughes-Clarke, J.E., 2007. Long-term development and current status of the Barcelona continental shelf: a source-to-sink approach. *Cont. Shelf Res.* 27, 1779–1800.
- Liquete, C., Canals, M., Ludwig, W., Arnau, P., 2009. Sediment discharge of the rivers of Catalonia, NE Spain, and the influence of human impacts. *J. Hydrol.* 366, 76–88.
- Llasat, M.C., Puigcerver, M., 1997. Total rainfall and convective rainfall in Catalonia, Spain. *Int. J. Climatol.* 17, 1683–1695.
- Llorens, E., Thiery, F., Grieu, S., Polit, M., 2008. Evaluation of WWTP discharges into a Mediterranean river using KSOM neural networks and mass balance modelling. *Chem. Eng. J.* 142, 135–146.
- Longhurst, A., 1995. Seasonal cycles of pelagic production and consumption. *Prog. Oceanogr.* 36, 77–167.
- Longhurst, A., 1998. *Ecological Geography of the Sea*. Academic Press, San Diego, CA.
- Ludwig, W., Dumont, E., Meybeck, M., Heussner, S., 2009. River discharges of water and nutrients to the Mediterranean and Black Sea: major drivers for ecosystem changes during past and future decades? *Prog. Oceanogr.* 80, 199–217.
- Mahadevan, A., Archer, D., 2000. Modeling the impact of fronts and mesoscale circulation on the nutrient supply and biogeochemistry of the upper ocean. *J. Geophys. Res.* Oceans 105, 1209–1225.
- Marchand, M., Caprais, J.C., Pignet, P., Porot, V., 1989. Organic pollutants in urban sewage and pollutant inputs to the marine-environment – application to the French shoreline. *Water Res.* 23, 461–470.
- Margalef, R., 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanol. Acta* 1, 493–509.
- Marty, J.C., Chiavérini, J., Pizay, M.D., Avril, B., 2002. Seasonal and interannual dynamics of nutrients and phytoplankton pigments in the western Mediterranean Sea at the DYFAMED time-series station (1991–1999). *Deep Sea Res. Part II* 49, 1965–1985.
- Pesant, S., Legendre, L., Gosselin, M., Bauerfeind, E., Budeus, G., 2002. Wind-triggered events of phytoplankton downward flux in the Northeast Water Polynya. *J. Mar. Syst.* 31, 261–278.
- Peters, F., Arin, L., Marrasé, C., Berdalet, E., Sala, M.M., 2006. Effects of small-scale turbulence on the growth of two diatoms of different size in a phosphorus-limited medium. *J. Mar. Syst.* 61, 134–148.
- Peters, F., Marrasé, C., Gasol, J.M., Sala, M.M., Arin, L., 1998. Effects of turbulence on bacterial growth mediated through food web interactions. *Mar. Ecol. Prog. Ser.* 172, 293–303.
- Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25, 943–948.
- Romero, E., Peters, F., Guadayol, Ò., 2013. The interplay between mild physico-chemical forcing and plankton dynamics in a coastal area. *Limnol. Oceanogr.* 58, 903–920.
- Romero, E., Peters, F., Marrasé, C., 2012. Dynamic forcing of coastal plankton by nutrient imbalances and match–mismatch between nutrients and turbulence. *Mar. Ecol. Prog. Ser.* 464, 69–87.
- Suárez, J., Puertas, J., 2005. Determination of COD, BOD, and suspended solids loads during combined sewer overflow (CSO) events in some combined catchments in Spain. *Ecol. Eng.* 24, 201–219.
- Tett, P., Gilpin, L., Svendsen, H., Erlandsson, C.P., Larsson, U., Kratzer, S., Fouilland, E., Janzen, C., Lee, J.-Y., Grenz, C., Newton, A., Gomes Ferreira, J., Fernandes, T., Scory, S., 2003. Eutrophication and some European waters of restricted exchange. *Cont. Shelf Res.* 23, 1635–1671.
- Tett, P., Gowen, R., Mills, D., Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M., Malcolm, S., 2007. Defining and detecting undesirable disturbance in the context of marine eutrophication. *Mar. Pollut. Bull.* 55, 282–297.
- Tolosa, I., Bayona, J.M., Albaigés, J., 1996. Aliphatic and polycyclic aromatic hydrocarbons and sulfur/oxygen derivatives in northwestern Mediterranean sediments: spatial and temporal variability, fluxes, and budgets. *Environ. Sci. Technol.* 30, 2495–2503.
- Torrecilla, N.J., Galve, J.P., Zaera, L.G., Retamar, J.F., Álvarez, A.N.A., 2005. Nutrient sources and dynamics in a mediterranean fluvial regime (Ebro River, NE Spain) and their implications for water management. *J. Hydrol.* 304, 166–182.
- UNEP/MAP/MED POL, 2003. *Riverine transport of water, sediments and pollutants to the Mediterranean Sea*. MAP Technical Reports Series No. 141. United Nations Environment Programme/Mediterranean Action Plan, Athens, Greece. ISBN: 92-807-2381-2.
- Valero, F., Martín, M.L., Sotillo, M.G., Morata, A., Luna, M.Y., 2009. Characterization of the autumn Iberian precipitation from long-term datasets: comparison between observed and hindcasted data. *Int. J. Climatol.* 29, 527–541.
- Verity, P.G., Robertson, C.Y., Tronzo, C.R., Andrews, M.G., Nelson, J.R., Sieracki, M.E., 1992. Relationships between cell-volume and the carbon and nitrogen-content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* 37, 1434–1446.
- Vila, M., Camp, J., Garcés, E., Masó, M., Delgado, M., 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. *J. Plankton Res.* 23, 497–514.
- Yentsch, C.S., Menzel, D.W., 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Res.* 10, 221–231.