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Temporal distribution of cyanobacteria in the coast of a shallow temperate estuary (Río de la Plata): some implications for its monitoring

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Abstract The aim of this study was to analyze the temporal distribution of phytoplanktonic cyanobacteria in a site located in the freshwater tidal zone near the extraction point for the drinking water supply. Samples were taken considering three timescales as follows: hours, days, and weeks, during the period of highest development of cyanobacteria. The phytoplankton density, microcystin concentration (LR, RR, YR), and chlorophyll-a were related to meteorological variables (wind and temperature), tidal high, and physical-chemical variables (nutrients, pH, conductivity, light penetration). The results obtained in this study showed that the variables that primarily modulate the temporal distribution of cyanobacteria were temperature, pH, light penetration, conductivity, and nutrients (particularly NO_3^- and NH_4^+), while the winds and tide had a secondary effect, only evidenced at an hourly scale. Therefore, this timescale would be the most suitable for monitoring cyanobacterial populations, when the amount of cyanobacterial cells exceeds the alert I level proposed by the World Health Organization. This recommendation is particularly important for the water intake zones in Río de la Plata, which are vulnerable to the damage generated by cyanobacteria on the water quality.

Keywords Cyanobacteria · Sampling timescales · Monitoring · Estuary · Freshwater tidal zone

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Introduction

At least half the world's population resides in estuarine watersheds, and this proportion continues to grow (Adams 2005). Rapidly growing and diversifying anthropogenic inputs associated with agriculture, aquaculture, urbanization, coastal development, and industrial expansion are a primary cause of the decline in the quality of natural habitats in these sensitive waters (Nixon 1995; Paerl 1997). The Río de la Plata, an estuary located between Argentina and Uruguay, with 3.1×10^6 km^2, is an ecosystem exposed to human and climatic pressures: nutrient enrichment, changes in the land use and soil erosion, the inflow of urban runoff (EcoPlata 1998; FREPLATA 2005), increases in the atmospheric temperature (Bidegain and Renom 1998; Nagy et al. 2002c), in rainfall, in fluvial flow, and in the variability of the ENSO (El Niño-Southern Oscillation) phenomenon (García and Vargas 1998; Menéndez 2002; Nagy et al. 2002a, b). Also, a southward shift of the high pressure belt of the South Atlantic-SAHP has

taken place (Escobar 2002). All these global changes have been more evident in the past few decades, manifesting themselves with increasing trophic changes and the development of more frequent cyanobacterial blooms (CARP 1990; de León and Yunes 2001; FREPLATA 2005; Andrinolo et al. 2007). These blooms severely affect the biotic integrity and disrupt the functioning of the estuary and the potential usage of its waters (Gómez and Bauer 2000); furthermore, many cyanobacterial species are able to produce a variety of toxic metabolites, which can be harmful to both human and animal health (Giannuzzi et al. 2012). One of the main services of this ecosystem is the provision of water; on the Argentinean coast (South Coastal Fringe), this resource provides drinking water to about 9 millions of inhabitants (INDEC 2010). It is therefore relevant to know the factors that influence the distribution of the cyanobacterial populations, particularly near the shore, where multiple activities are conducted related to recreation and economic activities of one of the most densely populated areas of Argentina.

According to Huszar and Reynolds (1997), phytoplanktonic cyanobacteria have fast responses to environmental changes; therefore, the aim of this study was to explore the distribution of cyanobacteria on different timescales; this could help design a monitoring program considering the dynamics of the cyanobacterial community.

For this aim, samples were taken considering three timescales (hours, days, and weeks), during summer, which is the most favorable period for the development of cyanobacteria on the coast of the Río de la Plata (De Leon and Yunes 2001; Andrinolo et al. 2007). The biological variables measured in the samples included total cyanobacterial density and species composition, microcystin concentration (LR, RR, YR), chlorophyll-a, and total phytoplanktonic density. The biological variables were related to meteorological variables (wind and temperature), tidal high, and physical-chemical variables (nutrients, pH, conductivity, light penetration).

We hypothesized that, in the coastal freshwater zone of a plain estuarine system such as Río de la Plata, the principal forcing variables that control the development of cyanobacteria were the following: temperature, nutrients, pH, conductivity, and light penetration; while the tidal high and winds act subordinated to those variables, determining the degree of incidence in cyanobacterial density and cyanotoxin amount near the shore.

Study area

The Río de la Plata is a large coastal plain estuarine system, microtidal (amplitude <2.0 m), naturally rich in nutrients, and trophically dominated by plankton (López Laborde and Nagy 1999; Nagy 2000), with 37 % of its surface covered with freshwater. The tidal waves from the ocean, the tributary discharge, and the wind acting on the entire surface of the estuary are the main forcing variables of the dynamics of the Río de la Plata (Menéndez and Re 2006).

A sampling site on the southern coastline of the Rio de la Plata was selected, located at 34° 49′ 19.47″ S, 57° 57′ 48.47″ W (Fig. 1), where cyanobacterial blooms are frequently reported (INA-FREPLATA 2012). This site, located downstream of the Buenos Aires city, is an area used for recreational purposes and is located near the extraction point for the drinking water supply of the city of La Plata. Moreover, the anthropogenic activity upstream provides nutrients, organic matter, and other pollutants to the study area (AA-AGOSBA-ILPLA-SHN 1997; FREPLATA 2005).

Material and methods

Sampling and preparations of samples

Phytoplankton samples were collected by triplicate, using a sampling device that consisted of a PVC pipe 6.5 cm in diameter, which allowed the integration of the first 50 cm of the water column.

Three time scales were established to collect the samples: hourly, daily, and weekly. The weekly and daily samples were collected with low tide, avoiding rainy days, covering a total of 16 weeks (between 13/11/2012 and 14/03/2013) and 5 days, respectively (04/02/2013 to 08/02/2013). For the hourly sampling, a day in February was selected (08/02/2013), and ten samples were taken with an interval of 1 h between 9 AM and 6 PM, covering periods of low tide and high tide.

Phytoplankton samples (125 mL) were fixed with formalin, final concentration 2 %, and dyed with Lugol's solution.

Microcystin determination was performed on 2-L samples, which were stored at −20 °C. For nutrient analysis, 200 mL was collected and promptly filtered through glass fiber filters (Whatman GF/C) and transported refrigerated to the laboratory for further

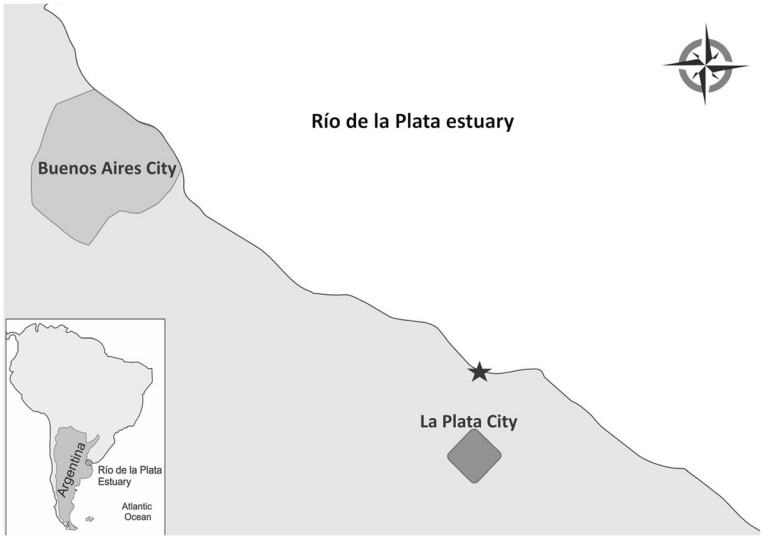

Fig. 1 Map of the study area, showing location of the sampling site (★)

analysis. Also, 1-L samples were immediately filtered through glass fiber filters (Whatman GF/C, 1.2-μm pore size) to be analyzed for chlorophyll-a content.

Measured parameters in the field included the following: temperature, pH, conductivity, turbidity, and dissolved oxygen (DO), all carried out with a multiparametric sensor Horiba U-50.

Photosynthetic active radiation (PAR) was measured at 40-cm depth (Li-cor Li-250A), and meteorological (wind direction and intensity) and tidal data were provided by the Naval Hydrographic Service and the Argentinean National Meteorological Service.

Phytoplankton analysis

Phytoplankton counts were carried out using an inverted microscope Olympus IX51 at ×400 and ×600, using 5- or 10-mL sedimentation chambers according to the amount of algae and suspended solids, which were left to settle at least 12 h. Each algae cell was the counting unit. Enough algae were counted to obtain an accuracy of 20 % in the density estimates of the more abundant species (Lund et al. 1958). The entire chamber was examined to find the scarcer species. Specific keys were used for taxonomic identification of phytoplankton.

Analytical methods

Soluble reactive phosphorus, nitrite, and ammoniacal nitrogen were determined colorimetrically, nitrate was reduced to nitrite before colorimetric measurement. All these determinations were made according to Mackereth et al. (1978).

To detect microcystins, each water sample (2 L) was subjected to three freezing-thawing cycles to disrupt the cells and release the toxins, filtered through Whatman G/FC filters, and the filtrate was run through a preactivated C-18 solid-phase extraction cartridge Sep-Pak C18 ODS (2 g, Waters). The toxins were eluted with 80 % methanol. Chromatographic analysis of microcystins was performed by HPLC with a photodiode array detector (Shimadzu 2010) and a C18 column (Hyperprep HS, 5-μm pore, 250×10 mm). The column was equilibrated with a mixture composed of 65 % A solution [water with 0.05 % (*v*/*v*) trifluoroacetic acid (TFA)] and 35 % B solution [acetonitrile with 0.05 % (*v*/*v*) TFA]. The mobile phase consisted of a discontinuous gradient of A and B solutions. The flow rate was 1.0 mL min^{-1}. Microcystins were identified on the basis of their UV spectra and retention time. Standard of microcystin-LR was purchased from Sigma (St Louis, MO, USA).

Chlorophyll-a was determined spectrophotometrically. Water samples (1 L) were filtered through glass fiber filters (Whatman G/FC), and the pigments were extracted from the filter using 90 % acetone as solvent (Clesceri et al. 1998). Chlorophyll-a concentration was then determined using a Shimadzu spectrophotometer and calculated according to Lorenzen (1967).

Statistical analysis

A redundance analysis (RDA) with all the cases analyzed (n=31) was conducted to explore the relationship between the average abundance of those species that had a frequency above 10 %, using a mean of the triplicates of the measured environmental variables. A preliminary detrended correspondence analysis (DCA) was conducted in order to know the length of the gradient of the distribution model of the species, which did not exceed four standard deviation units; as a result of this analysis, an RDA was performed, suitable for species that respond to a linear distribution model (terBraak and Smilauer 1998).

Species abundances were ln (x+1) transformed, and environmental data was standardized (terBraak 1986). Conductivity, pH, temperature, PO_4^{3-}, NH_4^+, NO_3^-, NO_2^{2-}, turbidity, and tidal amplitude were retained in the analysis since they had a variance inflation factor <10 (terBraak and Smilauer 1998). The overall significance of the ordination and the significance of the first two axes were tested with a Monte Carlo permutation test (p<0.01) using unrestricted permutations.

The relationship between the biological variables and the physical-chemical parameters were analyzed using Spearman's correlation.

Results

Physical-chemical parameters

During the sampling period, water temperature varied between 19 and 31 °C, conductivity between 113 and 631 μSm^{-1}, turbidity between 94 and 406 NTU, and PAR values from 5 to 313 $\mu M\ m^2\ s^{-1}$. DO in all cases exceeded 7 mg L^{-1} and reaching saturation, while pH was slightly alkaline peaking at 9.8. As for nutrients, PO_4^{3-} concentration ranged between 0.1 and 0.3 mg L^{-1}, NO_3^- ranged from 0.03 to 2.46 mg L^{-1}, NO_2^{2-} varied from 0.001 to 0.27 mg L^{-1}, and the NH_4^+ concentration ranged from 0.0007 to 0.3 mg L^{-1} (Table 1).

Meteorological and tidal data

Prevailing winds during the study period were from the northeast in 50 % of cases, reaching a maximum intensity of 32 km h^{-1}. In the remaining cases recorded, winds were from east, southeast, northwest, southwest and south, not exceeding 31 km h^{-1} in any case (Table 1).

The tidal regime of Río de la Plata is predominantly semidiurnal (two high tides and two low tides per day); during the study period, the recorded high tide values ranged from 0.25 to 1.2 m, this last value was recorded during the daily sampling.

Cyanobacterial composition

A total of 32 taxa of cyanobacteria were identified (Table 2); in 85 % of the total phytoplankton composition samples, the cyanobacteria had more than 50 % relative abundance. Among all the species analyzed, only three had more than 1 % relative abundances and were present in more than 80 % of the samples, *Merismopedia tenuissima*, *Microcystis aeruginosa*, and *Merismopedia punctata* (Table 2).

Chlorophyll-a and microcystins

The chlorophyll-a values, which varied from 7 to 90.3 $\mu g\ L^{-1}$, were significantly correlated to the total cyanobacterial density (Table 3). The presence of LR-microcystin was detected in 23 % of the cases, with concentrations always lower than 1.4 $\mu g\ L^{-1}$, and its concentration was positively correlated with the density of *M. aeruginosa* (p<0.05), while no significant correlations were observed between the concentration of LR-microcystin and the environmental variables measured. YR-microcystin and RR-microcystin were not detected in the analyzed samples.

Relationship between biological data and environmental factors

Cyanobacteria were present throughout the entire study, with minimum densities of 50 cells mL^{-1} and maximum densities of 35,679 cells mL^{-1}. Total cyanobacterial density was positively correlated with temperature, pH, ammonium concentration, and PAR, while being negatively correlated to turbidity and nitrate concentration (Table 3).

The *M. tenuissima* and *M. aeruginosa* populations reached maximum density values of 33,800 and 8,100 cell mL^{-1}, respectively, while *M. punctata* reached maximum densities of 9,900 cell mL^{-1}.

Table 1 Physical-chemical parameters (mean value and standard deviation), chlorophyll-a, microcystins, and meteorological and tidal data

Timescale	Date	Temp. (°C)	pH	Conductivity (μSm^{-1})	Dissolved oxygen (mg L^{-1})	Turbidity (NTU)	PAR (uM m^2 s^{-1})	P-PO4 (mg L^{-1})	N-NO3 (mg L^{-1})	N-NO2 (mg L^{-1})	N-NH4 (mg L^{-1})	Chlorophyll-a (µg L^{-1})	Microcystin-LR (µg L^{-1})	Wind intensity (km h^{-1})	Wind direction
Weekly	11/13/12	21.3±0.2	7.7±0	335.0±0	10.0±0.1	150.3±1.5	184.0	0.30	1.18	0.006	0.030	14.1	N/D	25.3	SE
	12/05/12	29.1±0.1	8.0±0.1	313.7±0.6	10.3±0.1	167.7±1.5	182.0	0.26	1.00	0.012	0.127	29.5	1.4	28	NE
	12/11/12	25.6±0	7.9±0.1	321.3±0.6	10.2±0	102.0±1	86.0	0.32	1.34	0.013	0.005	24.4	0.4	29.4	NE
	12/18/12	24.5±0	8.4±0.1	430.0±0	11.1±0	93.9±0.2	162.0	0.29	1.35	0.047	0.312	28.8	N/D	15.5	NE
	12/27/12	23.5±0.1	8.6±0.1	454.7±1.2	8.9±0	127.7±0.6	48.0	0.25	1.32	0.035	0.046	33.2	N/D	19.6	NE
	01/04/13	27.4±0	8.9±0	365.3±0.6	9.7±0	158.7±3.2	126.3	0.21	1.02	0.004	0.069	49.8	0.7	21	NE
	01/11/13	27.1±0	8.6±0	504.3±0.6	9.2±0	172.3±3.2	206.6	0.23	1.08	0.008	0.047	39.9	0.8	22.2	NE
	01/17/13	27.5±0	8.2±0	364.3±0.6	9.4±0.1	148.3±1.5	222.8	0.23	1.18	0.003	0.029	30.5	0.3	21	E
	01/25/13	24.5±0.1	7.9±0	406.7±1.2	10.4±0.1	173.7±1.5	60.8	0.19	0.82	0.002	0.032	37.7	0.7	32	NE
	02/01/13	31.3±0	8.9±0	113.0±0	9.1±0	125.7±0.6	313.3	0.33	0.80	0.272	0.004	90.3	0.7	16.8	NE
	02/08/13	24.8±0	8.5±0.1	417.0±0	9.2±0.2	163.7±3.2	149.3	0.22	0.96	0.007	0.048	14.5	N/D	23.6	NE
	02/14/13	30.3±0	9.9±0	631.3±0.6	11.8±0.2	134.0±2	88.0	0.14	0.03	0.003	0.001	68.7	N/D	13.8	NW
	02/22/13	21.7±0	8.1±0.1	245.0±0	9.0±0.4	248.0±1.7	52.6	0.18	1.35	0.005	0.002	10.3	N/D	16.8	E
	03/01/13	23.6±0	8.6±0	280.0±0	8.5±0.2	406.3±12.7	37.4	0.19	2.09	0.004	0.006	7.3	N/D	30.8	E
	03/08/13	21.7±0	7.7±0.1	285.3±0.6	10.9±0.7	357.0±4	5.3	0.25	2.40	0.005	0.001	10.3	N/D	22.2	NE
	03/14/13	19.7±0	8.4±0.1	417.7±0.6	10.7±1.2	308.7±3.2	32.4	0.26	2.46	0.002	0.012	12.0	N/D	21	SW
Daily	02/04/13	20.9±0	8.1±0	411.0±0	11.4±0.1	125.0±1	110.9	0.23	1.12	0.029	0.002	28.7	N/D	15.5	S
	02/05/13	23.0±0	8.3±0.1	349.0±1	10.8±0.4	109.0±1	110.8	0.23	1.17	0.034	0.006	42.4	N/D	13.8	NE
	02/06/13	24.5±0	8.9±0.1	379.0±0	10.0±0.3	122.7±2.1	212.1	0.25	0.34	0.094	0.018	69.0	N/D	21	NE
	02/07/13	25.9±0	9.0±0	326.0±0	9.1±0	148.7±1.2	124.8	0.22	0.47	0.009	0.020	47.0	N/D	23.6	NE
	02/08/13	27.6±0	9.1±0	407.7±0.6	8.4±0	181.0±2.6	115.6	0.23	0.97	0.007	0.044	30.8	N/D	23.6	NE
Hourly	09:40:00	24.8±0	8.5±0.1	417.0±0	9.2±0.2	163.7±3.2	149.3	0.25	2.40	0.005	0.001	14.5	N/D	7	E
	10:40:00	26.2±0.1	8.9±0	436.7±0.6	8.6±0	164.3±2.5	214.2	0.22	1.00	0.007	0.034	26.9	N/D	9	NE
	11:40:00	26.5±0	8.9±0	424.0±0	7.3±0.3	167.0±0	158.2	0.22	0.94	0.007	0.063	19.7	N/D	11	E
	12:40:00	27.6±0	9.1±0	407.7±0.6	8.4±0	181.0±2.6	115.6	0.23	0.97	0.007	0.044	30.8	N/D	13	SE
	13:40:00	30.2±0	9.2±0	360.0±0	7.5±0	185.3±0.6	103.0	0.23	0.94	0.005	0.041	29.9	N/D	15	E
	14:40:00	28.8±0	9.3±0	363.0±0	8.1±0	186.0±2.6	97.7	0.26	0.92	0.007	0.078	44.0	N/D	19	E
	15:40:00	29.6±0	9.4±0	368.0±0	7.9±0	185.0±1	130.5	0.24	0.91	0.007	0.053	33.3	N/D	22	SE
	16:40:00	29.6±0	9.3±0.1	340.0±0	7.6±0	178.7±6.7	68.2	0.26	0.91	0.008	0.067	33.3	N/D	22	SE
	17:40:00	29.6±0	9.3±0	341.0±0	8.2±0.1	163.3±1.5	38.0	0.27	0.90	0.008	0.050	32.9	N/D	24	SE
	18:40:00	28.8±0	9.2±0	325.0±1.7	8.3±0.1	167.7±2.9	30.1	0.27	0.96	0.008	0.057	34.6	N/D	28	SE

Table 2 List of the cyanobacteria species analyzed in this study, their frequency (Freq.), and relative abundance (R.A.) in relation to the total cyanobacterial density

	Freq.	R.A.
Anabaena sphaerica Born. et Flah.	1.9	*
Anabaenopsis circularis (G. S. West) Wolosz. et Miller	1.9	*
Aphanocapsa conferta (W. et GS We.) Leg.et Cronb.	11.5	*
Aphanocapsa delicatissima W. et G. S. West	17.3	*
Aphanocapsa grevillei (Hass.) Rabh.	15.4	*
Aphanocapsa holsatica (Lemm.) Cronb. Kom.	5.8	*
Aphanocapsa incerta (Lemm.) Cronb. Kom.	5.8	*
Aphanocapsa planctónica (GM Smith) Kom. et Anagn.	7.9	*
Aphanothece smithii Kom. Leg et Cronb.	1.9	*
Chroococcus prescottii Dro. et Dai.	1.9	*
Dolichospermum circinalis Rabenh. Ex Bornet et Flah.	5.8	*
Dolichospermum spiroides (Kleb) Wacklin et al.	3.8	*
Jaaginema subtilissimum (Kütz. ex de Toni) Anagn. et Kom.	3.8	*
Komvophoron constrictum (Szafer) Anagn. et Kom.	15.4	*
Komvophoron minutum (Skuja) Anagn. et Kom.	1.9	*
Merismopedia convoluta Breb. in Kütz.	7.7	*
Merismopedia glauca (Ehrenb.) Kütz.	7.7	*
Merismopedia punctata Meyen	80.8	**
Merismopedia tenuissima Lemm.	98.1	***
Merismopedia warmingiana Lag.	3.8	*
Microcystis aeruginosa (Kütz.) Kütz	90.4	**
Microcystis wesembergii (Kom.) Kom. in Kondrateva	1.9	*
Phormidium chalybeum (Mertens) Anag. et Kom.	1.9	*
Phormidium formosum (Bory ex Gom) Anagn. et Kom.	7.7	*
Planktothrix agardhii (Gom) Anagn. et Kom.	57.7	*
Pseudanabaena catenata Lauterborn	1.9	*
Pseudanabaena limnetica (Lemm.) Kom.	7.7	*
Pseudanabaena mucicola (Hüb.-Pest. and Naum.) Bourr.	34.6	*
Raphidiopsis mediterránea Skuja	11.5	*
Romeria elegans (Wolosz.) Koczw.	3.8	*
Romeria gracilis (Wolosz.) Koczw.	1.9	*
Romeria okensis (Meyer) Hindák	3.8	*

*<1 % relative abundance, **1–50 % relative abundance, ***>50 % relative abundance

According to the RDA performed with the densities of the cyanobacterial species and environmental variables, the first axis, which explained 61.1 % of the variance in the species data, showed that the increase of temperature, pH, ammonium, and phosphate concentration and conductivity favored the development of species analyzed. On the other hand, the increase in nitrate concentration and turbidity negatively influenced the populations of the taxa involved in the analysis. The second axis, which explained 15.7 % of the variance, revealed the secondary effects of high tide and nitrites' concentration on the species analyzed (Fig. 2).

Table 3 Spearman's correlation values between total cyanobacterial density (TC) and the environmental variables at weekly, daily, and hourly scales and at all cases analyzed

	Weekly (N=16)	Daily (N=5)	Hourly (N=10)	All cases (N=31)
Temperature	0.56 *	0.5	0.79 **	0.47 **
PAR	0.64 **	0.5	−0.55	0.37 *
Turbidity	−0.62 *	−0.1	0.16	−0.43 *
pH	0.53 *	0.5	0.79 **	0.38 *
P-PO4	−0.28	0.7	0.53	0.02
N-NO3	−0.45	−0.5	−0.77 **	−0.47 *
N-NO2	0.19	0.3	0.57	0.27
N-NH4	0.74 **	0.5	0.38	0.41 *
Tide	−0.13	−0.1	0.71 *	−0.00
Chlorophyll-a	0.91 **	0.6	0.61	0.81 **
Southeast wind intensity	−0.31	–	0.67 *	−0.01

Significant values are indicated with (*=0.05>p>=0.01) or (**=p<0.01)

Minus symbols (—) indicate absence of data

Temporal scales

The temporal distribution of the most abundant and frequent cyanobacterial populations, *M. tenuissima*, *M. punctata*, *M. aeruginosa*, and total densities of cyanobacteria are shown at a weekly, daily, and hourly scale in Figs. 3, 4, and 5. The results showed that at shorter scales (daily and hourly), the densities of these species and the total cyanobacterial density were less fluctuant than at longer scales (weekly).

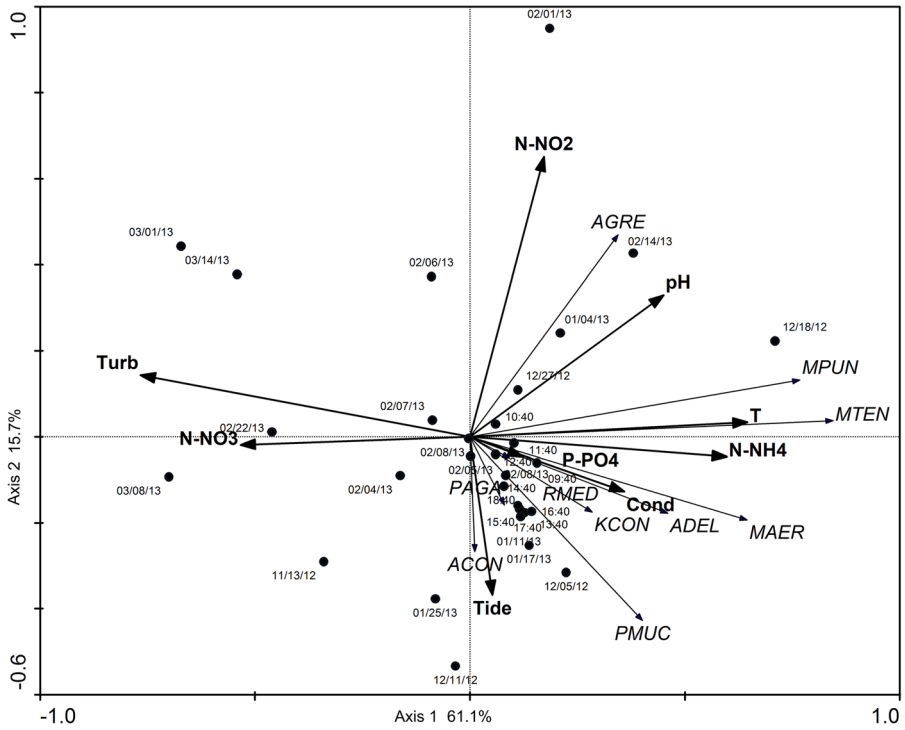

Fig. 2 RDA that related the environmental variables and the most frequent cyanobacterial species (n=31). Acronyms: ACON (*Aphanocapsa conferta*), ADEL (*Aphanocapsa delicatissima*), AGRE (*Aphanocapsa grevillei*), KCON (*Komvophoron constrictum*), MAER (*Microcystis aeruginosa*), MPUN (*Merismopedia punctata*), MTEN (*Merismopedia tenuissima*), PAGA (*Planktothrix agardhii*), PMUC (*Pseudanabaena mucicola*), RMED (*Raphidiopsis mediterranea*)

The correlation between the cyanobacterial density and the environmental data revealed different degrees of significance depending on the timescale employed. While the temperature and pH were significantly related to the increase in cyanobacterial density at weekly and hourly scale, the light penetration (PAR and turbidity), ammonium concentration, and conductivity were only significant at a weekly scale. The influence of the high tide and wind from the southeast was observed only during the hourly scale, and both parameters were correlated positively to the total cyanobacterial density; this temporal scale was the only one that covered periods of low tide and high tide. The daily scale did not show significant correlations with any of the parameters measured (Table 3).

Discussion and conclusion

Coastal systems are naturally vulnerable to eutrophication, and most of the world's estuaries show at least

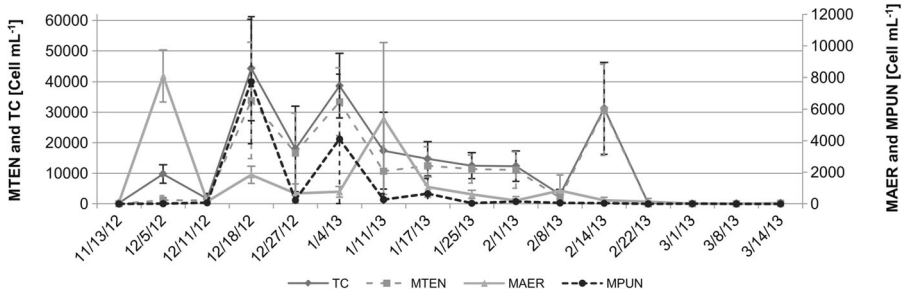

Fig. 3 Total cyanobacterial densities (*TC*) and densities of *Merismopedia tenuissima* (MTEN), *Microcystis aeruginosa* (MAER), and *Merismopedia punctata* (MPUN) in the weekly samples

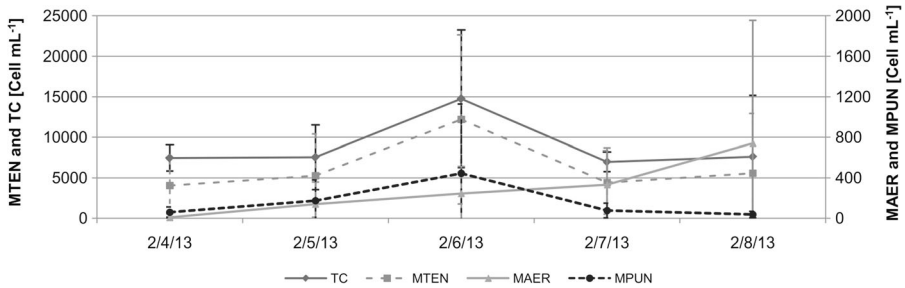

Fig. 4 Total cyanobacterial densities (*TC*) and densities of *Merismopedia tenuissima* (MTEN), *Microcystis aeruginosa* (MAER), and *Merismopedia punctata* (MPUN) in the daily samples

some symptoms, although their amount of nutrients is variable. The main symptoms include changes in the composition of the species in the phytoplankton, changes in the rate of nutrients (particularly N:P), increased biomass and primary production, and the development of harmful algal blooms, especially cyanobacteria (Pinckney et al. 1998; Paerl et al. 2011).

The statistical analysis performed in this study showed that the variables that primarily modulate the temporal distribution of cyanobacteria were temperature, pH, light penetration, conductivity, and nutrients (particularly NO_3^- and NH_4^+). These factors have been widely recognized in the literature as the main forcing variables that may influence the development of cyanobacteria (Levine 1983; Jacoby et al. 2000; Reynolds 2006; Havens 2008; Jiang et al. 2008). Nutrients and chlorophyll-a concentrations, observed during this study, exceeded several times the values of 0.025 mg L^{-1} for phosphorous and 50 μg L^{-1} for chlorophyll-a cited by Dodds et al. (1998) for eutrophicated flowing waters. Also, phosphorus concentrations exceeded the 0.1 mg L^{-1} cited by Havens (2008) as a condition conducive to the development and dominance of cyanobacteria. In relation to dissolved inorganic nitrogen (DIN) concentrations, the Río de la Plata is characterized by an excess in nitrogen forms, especially in ammonium, being the city of Buenos Aires one of the main input sources (Pizarro and Orlando 1985; CARP 1990; Bazán et al. 1996), which is evident in our study where the mean value of DIN was 1.2 mg L^{-1}. These results manifested the favorable conditions in the Southern Coastal Fringe of the Río de la Plata for the development of cyanobacteria.

The RDA analysis showed that the most abundant populations, *M. aeruginosa*, *M. tenuissima y M. punctata* were particularly related to changes in the concentrations of ammonium (positively) and nitrate (negatively). These observations are consistent with those reported by Kappers (1984) for *M. aeruginosa*, who found that this specie prefers ammonium over nitrate as an N source. According to Luque et al. (1994), the assimilation of nitrogen by cyanobacteria is subject to a strict regulation so that when ammonium is available, these organisms become unable to assimilate alternative inorganic nitrogen sources such as nitrate.

Subordinated to the physical and chemical forcing mentioned above, the tidal high was seen to influence the temporal distribution of cyanobacteria on the coast. In estuaries, the tide level modulates the physical, chemical, and biological properties (McLusky and Elliot

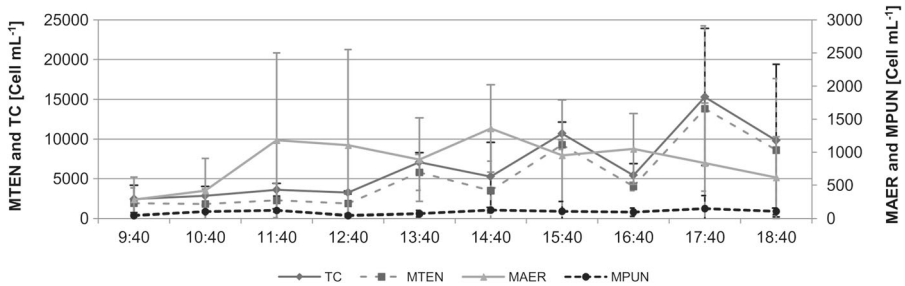

Fig. 5 Total cyanobacterial densities (*TC*) and densities of *Merismopedia tenuissima* (MTEN), *Microcystis aeruginosa* (MAER), and *Merismopedia punctata* (MPUN) in the hourly samples

2004). In Río de la Plata estuary, the tidal level is directly influenced by wind action; winds from the north produce the most significant declines of the water level, while those from the south and southeast correspond to the most significant increases. When the wind blows perpendicularly to the coast, it generates a surface current that causes the water mass to be displaced along with it (CARP 1990; López and Marcomini 2009) and the high tide acts delaying the fluvial currents towards the Atlantic Ocean.

In the sampling site selected in this study, when the winds were predominant from the southeast sector and in high tide, the highest densities of cyanobacteria were reported. Considering that the morphology of the coast changes along the coastline, it is to be expected that in different areas, the wind influence would act in a different way in the distribution of cyanobacteria.

Regarding to cyanotoxin production, in the Río de la Plata, there is evidence reporting toxicity episodes caused by cyanobacteria in the shore (De Leon and Yunes 2001; Andrinolo et al. 2007; Gianuzzi et al. 2012; INA-FREPLATA 2012). Some of the genera recognized as toxicity promoters in this ecosystem were identified in our study, as *Aphanocapsa*, *Dolichospermum*, *Merismopedia*, *Microcystis*, *Phormidium*, *Planktothrix*, *Pseudanabaena*, and *Raphidiopsis*. The concentrations of microcystin-LR obtained in this study were significantly correlated with the density of *M. aeruginosa*, reaching values that would compromise water consumption, as proposed by the World Health Organization (WHO). Also, the total cyanobacteria density exceeded the amount of cell per milliliter reported as dangerous for humans and animals (Chorus and Bartram 1999). According to Gianuzzi et al. (2012), the concentration of microcystins found in the coastal zone of Rio de la Plata represent a potential risk for the consumption of drinking water since the water intake is near the shore and the methods used by the water treatment plants are not always the indicated to remove those toxins. In our study, the concentration of microcystins did not correlate significantly with any of the environmental variables measured, but there are different studies that suggest a relationship between the concentrations of toxin and variables such as total phosphorous, total nitrogen, and irradiance (Rapala et al. 1997; Jacoby et al. 2000; Oh et al. 2001), while other authors consider that it is not possible to assess whether these relationships are causal or coincidental and additional research is required (Havens 2008).

Another factor recognized to promote the development and expansion of cyanobacteria in the aquatic ecosystems is the influence of El Niño Southern Oscillation (ENSO). According to Nagy (2005), in the Río de la Plata estuary, the alternation between severe positive or negative rainfall anomalies, along with the increase of nutrient concentrations, the changes in their relative rates, and the increase in mean temperatures, produces favorable conditions for the dominance of cyanobacteria. This author recognizes that the dry periods (La Niña) may enhance the further development of cyanobacteria in the estuary. Therefore, the responses observed in our study, coincident with a Niña-neutral period, might differ if one considers other climate scenarios.

The results obtained suggest the need to adjust the timescales in the monitoring of cyanobacteria, particularly during the most favorable periods for development in a temperate shallow estuary, with a moderate water residence time (46 days) and rich in nutrients such as the Río de la Plata (FREPLATA 2005). The temporal scale of the observations would help understand the dynamics of the cyanobacterial blooms in the coast in order to generate more precise mitigation plans for their harmful effects, considering that the duration of their massive developments can be highly variable and that the persistence of cyanobacterial toxins in the water can last up to 30 days (Lahti et al. 1997; Paerl 2008).

Considering the variability of environmental conditions typical of this estuarine ecosystem, and the significant correlations between the tide and winds obtained in this study, an hourly timescale would be the most suitable for monitoring cyanobacterial populations when the amount of cyanobacterial cells exceeds the level I of alert proposed by the World Health Organization (Chorus and Bartram 1999). This recommendation is particularly important for the water intake zones in Río de la Plata, which are vulnerable to the damage generated by cyanobacteria on water quality (Gianuzzi et al. 2012).

The distribution of cyanobacteria is central to hazard assessment; the design of monitoring program should be specifically tailored for each water body to optimize the relation of information output to work input. Local knowledge of bloom history and a good understanding of the local growth conditions for cyanobacteria will greatly enhance the capacity to anticipate bloom

formation. Also, a wider contribution to the knowledge of cyanobacterial ecology, hydrobiology, and the state of the environment are necessary for suitable monitoring. For these reasons, involving limnological expertise is particularly important in the planning of monitoring programs, in the evaluation of the data, and in periodic reassessment of the adequacy of ongoing programs (Chorus and Bartram 1999).

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