

Phytoplankton and Physicochemical Analysis on the Water System of the Temperate Estuary in South America: Bahía Blanca Estuary, Argentina

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ABSTRACT: Under the contemporary scenario of global climate change and increasing coastal eutrophication, baseline studies of phytoplankton diversity and distribution in relation to habitat conditions constitute the first approach to evaluate potential shifts at the base of the food webs. Here we present data of phytoplankton species composition and environmental variables in winter 2006 in a temperate estuary in South America, Argentina. The results showed that the Bahía Blanca Estuary is markedly turbid (particulate suspended matter up to 60 mg L⁻¹) with high nutrient concentrations. Apparently, besides high light attenuation coefficients (k up to 2.7 m⁻¹), the combination of a shallow (~7 m depth) and well mix water column in the innermost zone of the estuary allows light penetration (mean light intensity in the mixed zone I_m 57.8 $\mu\text{Em}^{-2}\text{s}^{-1}$ and euphotic zone to mixed zone ratio $Z_{eu};Z_m$ over 0.2), intensive nutrient regeneration and eventually phytoplankton massive growth (up to 1.8 x 10⁶ cells L⁻¹). Moreover, the relatively low residence time and the low river runoff seem to promote the accumulation of nutrients and phytoplankton biomass in the head of the estuary. Anthropogenic activities and autochthonous production are responsible of high particulate organic matter loads (8.3 ± 1.5 mg L⁻¹). Diatoms appeared as the dominant group in winter with the emergence of *Cyclotella* aff. *choctawatcheena* (5-15 μm) as the dominant species (up to 82 % of the total phytoplankton abundance). The distribution and habitat preferences of this diatom are briefly discussed in relation to the pelagic environment in the Bahía Blanca Estuary.

Key words: Phytoplankton diversity, Diatoms, Environmental variables, Mesotidal estuary, Southern Atlantic

INTRODUCTION

Monitoring coastal phytoplankton and environmental variables is of major importance for water quality assessment and ecosystem management (de Jonge, 2000). Surveys of phytoplankton species composition in relation to habitat conditions are the baselines for the understanding of ecosystem functioning (Cloern, 2001; Adolf *et al.*, 2006; Nixon *et al.*, 2009). Even when these studies are based on descriptive and qualitative features of phytoplankton (species composition, size structure, and functional groups), compilation of taxa distribution and habitat preferences are useful to determine the potential use of bioindicators of the ecosystem state (Beaugrand 2005).

In coastal shallow systems, the close interaction between the land, the atmosphere and the bottom sediments causes complex hydrological patterns and induces high variability in the pelagic environment. In

the particular case of estuaries, the continuous river-sea transition and the tidal influence are considered the main determining features of the phytoplankton biomass distribution (Calliari *et al.*, 2005; Domingues *et al.*, 2005; Hagy III *et al.*, 2005). On the other hand, local physicochemical and biological factors such as water column depth, light availability, nutrient turnover, grazing pressure and species-specific interactions could eventually mask the effect of the longitudinal hydrological gradient typical of estuaries (e.g. salinity and turbidity gradients) and highly affect the phytoplankton development (e.g. Lucas *et al.*, 1999; Kocum *et al.*, 2002).

The Bahía Blanca Estuary, Argentina, is characterized by low freshwater discharge and eventually a smooth gradient of water density from the head towards the mouth (Freije *et al.*, 2008). Long-term monitoring program of phytoplankton and

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environmental variables has been carried out biweekly since 1978 in a fixed station, Puerto Cuatrerros, located in the inner zone of the estuary (Popovich *et al.*, 2008b and reference therein). The analysis of this long-term data series revealed significant changes in the phenology of the phytoplankton linked to a shift in the hydro-climatic conditions around the mid 90's (Guinder *et al.*, 2010; Winder *et al.*, 2010; Guinder, 2011). On the other hand, spatial monitoring of the phytoplankton distribution along the mouth-head axis of the estuary are rather poor, indeed there is only one work (Popovich and Marcovecchio, 2008) carried out during the period August 1992 - August 1993.

Here, we present data of phytoplankton and physico-chemical variables measured simultaneously along a longitudinal transect of the main channel of the Bahía Blanca Estuary during winter 2006. This baseline monitoring program is of local relevance for further ecological studies after the hydro-climatic shift registered in the region (Guinder *et al.*, 2010). Moreover, this descriptive and up to date study constitutes an important contribution to the knowledge of the phytoplankton distribution in relation to the environmental variables in the Southwestern Atlantic coast.

The Bahía Blanca Estuary (38°42' - 39°25'S, 61°50' - 62°22'W) is located on the southwestern Atlantic

coast, Argentina, in a temperate climate region. The estuary is mesotidal with a semidiurnal cycle, characteristically shallow, turbid (annual mean of particulate suspended matter of 77.6 mg/l; Guinder *et al.*, 2009a) and eutrophic (Freije *et al.*, 2008). The water column is well mixed all year-round mainly affected by tides and wind stress (Guinder *et al.*, 2009a; 2009b). Yearly water temperature ranges from 4 °C to 26 °C and salinity range from about 17.3 and 41.9 with maximum values in summer. The estuary extends over 2,300 km² and comprises several NW-SE tidal channels, extensive tidal flats (1,150 km²) with patches of low salt marshes, and islands (410 km²). An extensive area is covered by halophytes, mainly *Sarcocornia perennis* and *Spartina alterniflora* (Negrin *et al.*, 2011). The main channel (Canal Principal) of the estuary has a total length of 60 km (Perillo *et al.*, 2001), varying in width from about 3-4 km at the mouth (22 m mean depth) to 200 m at the head (3 m mean depth). (Fig. 1). Two freshwater tributaries enter the estuary from the northern shore, the Sauce Chico River and the Napostá Grande Stream, with a mean annual runoff of 1.9 and 0.8 m³ s⁻¹, respectively. During autumn, the rainy season in the area, the Sauce Chico River may reach high peaks of up to 106 m³s⁻¹ (Melo and Limbozzi, 2008). The Ingeniero White port, one of the most important ports in South America, the Bahía Blanca City and the towns of General Cerri and Ingeniero White (350,000 inhab.) are located in the northern boundary of the estuary.

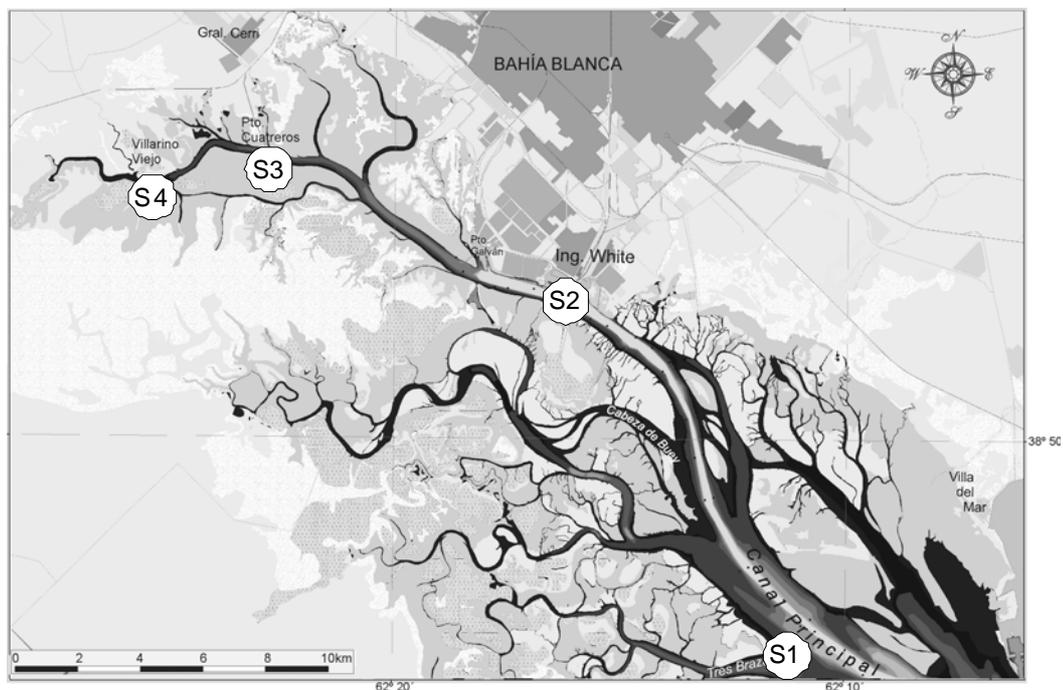


Fig. 1. Map of the inner zone of the Bahía Blanca Estuary showing the 4 sampling stations (S1-S4). Depths from S1 (external) to S4 (internal) in meters: 11.5, 15.5, 10.0 and 6.3, respectively. S1: Canal Tres Brazas; S2: Ingeniero White; S3: Puerto Cuatrerros and S4: Villarino Viejo

MATERIALS & METHODS

The sampling was carried out during winter 2006 (from July 3 to September 19) on a fortnightly frequency combining samples from the coast and from a boat in four stations (S1-S4) along the main channel of the Bahía Blanca Estuary (Fig. 1), during high tide. In each sampling station, *in situ* profiles (1 m intervals) of pH, temperature (in °C), dissolved oxygen (DO, in mg/L) and salinity (in PSU) were measured using a digital multi-sensor Horiba U-10. Water samples were collected from the surface and from the bottom (1 m from the sea floor) of the water column using a van Dorn bottle (2.5 L) to assess phytoplankton abundance and particulate suspended matter (PSM), particulate organic matter (POM), dissolved nutrients and chlorophyll *a* concentrations. The samples for phytoplankton enumeration were fixed with Lugol solution. For species identification, samples were taken with a Nansen net (30 µm mesh) and fixed with a formaldehyde solution (final concentration: 0.4%). Using a submersible radiometer Li-Cor LI-192SB, vertical profiles of PAR (photosynthetically active radiation, in µE m² s⁻¹) were measured in the water column at 10 cm intervals.

Chlorophyll *a* (in µg/L) was measured spectrophotometrically using the methods described in APHA (1998). Phytoplankton species identification was done using a Zeiss Standard R microscope and a Nikon Eclipse microscope with x1000 magnification and phase contrast. Phytoplankton >3 mm was counted with a Sedgwick–Rafter chamber (1 mL) which was a suitable volume according to the amount of suspended solids. The entire chamber was examined at x200 and each algal cell was counted as a unit according to McAlice (1971). Thereafter, the Shannon diversity index *H'* was estimated (Shannon and Weaver 1963) using the equation: $H' = -\sum [(ni/n) \log_2 (ni/n)]$, where n_i is the number of individuals of species *i* in the sample and *n* is the total number of individuals in the sample. The cell volumes were calculated assigning simple geometric shapes to the species (Hillebrand *et al.*, 1999) and converted into carbon content (µg C/L) according to Menden-Deuer and Lessard (2000).

SPM and POM concentrations (both in mg/L) were determined gravimetrically filtering 300–500 ml of water on pre-combusted and weighed GF/F filters. Thereafter the filters were dried at 60 °C for 24 h and weighed for SPM estimation. Afterwards, they were combusted at 500 °C for 30 min and weighed again for POM determination as the difference between both weight values (Calliari *et al.*, 2005).

From vertical PAR profiles, light extinction coefficient (*k*, m⁻¹) in the water column was calculated

considering that light is exponentially attenuated with depth. In addition, the mean light intensity in the mixed layer I_m was calculated with the equation (Riley, 1957): $I_m = I_0 (1 - e^{-kZ_m}) (kZ_m)^{-1}$, where I_0 (in µE m² s⁻¹) is the light intensity received at the water surface and Z_m is the mixed zone (in m). The depth of the euphotic zone (Z_{eu} , m) was estimated as the depth at which irradiance is 1% of the surface value, (i.e. $Z_{eu} = 4.6 k^{-1}$). Thereafter, the Z_{eu} to Z_m ratio ($Z_{eu}:Z_m$) was calculated.

For dissolved nutrient determinations, water samples were filtered through Whatman GF/C filters and frozen in plastic bottles until analysis. Dissolved nitrate NO₃⁻, nitrite NO₂⁻, ammonium NH₄⁺, phosphate PO₄³⁻ and silicate SiO₂ concentrations were measured according to APHA (1998) using a Technicon AA-II Autoanalyzer expanded to five channels. Total dissolved inorganic nitrogen concentration (DIN) was calculated as the sum of nitrite, nitrate and ammonium.

RESULTS & DISCUSSION

Vertical profiles of the physico-chemical variables measured *in situ* (Fig. 2) showed that the water column was vertically homogeneous in all sampling sites. The salinity showed a smooth diminution from the external (S1) to the internal (S4) station (mean salinity in the water column: 31.6 - 29.7, respectively) consistent with the expected result of the advection of the lateral salinity gradients typical of estuaries with major freshwater input at the head. Nevertheless, the salinity gradient of the Bahía Blanca Estuary was considerably milder than other temperate estuaries (Kocum *et al.*, 2002; Calliari *et al.*, 2005; Adolf *et al.*, 2006) due to the reduced river runoff (Melo and Limbozzi, 2008). Both, the water temperature (8.9 to 8.2 °C) and the pH (8.4 to 8.7) were constant along the main channel. Dissolved oxygen concentrations were higher (~1 mg/L) in the innermost sampling stations (S3 and S4), probably in relation to the shallowness of the water column and the intense turbulence processes induced by the effect of wind and tides (Guinder *et al.*, 2009a) which allow continuous gas diffusion through the sea-atmosphere interphase. Conversely, the rise in dissolved oxygen could have been the result of phytoplankton production, as cellular densities were considerably higher in S3 and S4.

The particulate suspended matter PSM concentration was relatively high (> 25 mg/L) with maximal values (up to 60 mg/L) in the external station S1 (Fig. 3a). In consequence, the light extinction coefficients *k* were elevated (up to 1.69 m⁻¹, Fig. 3b) and the mean light intensity in the mixed zone I_m was relatively low (mean value: 57.8 µE m⁻²s⁻¹) and showed

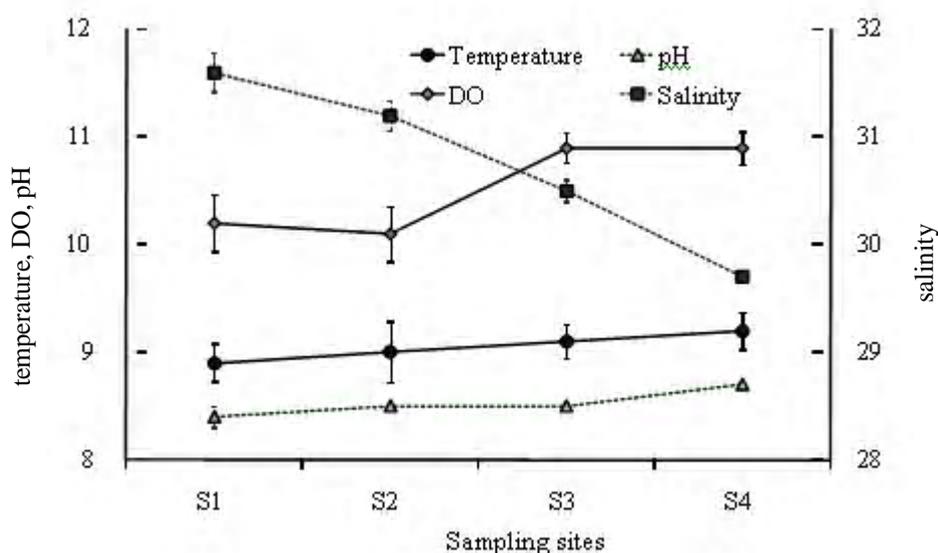


Fig. 2. Variability of the physicochemical variables measured *in situ* in the sampling sites (S1-S4) along the main channel of the Bahía Blanca Estuary. The vertical bars represent the standard deviations (SD) of the means of the vertical profiles in the water column, based on measurements done each 1 m depth

an increasing trend towards the head of the estuary (fig. 3c). Maximal values of k were registered on the coastal stations, especially in Puerto Cuatros (S3) on late-winter (up to 2.7 m^{-1}). On the other hand, the light intensity in the surface layer I_0 was between 554.5 and $945.5 \mu\text{E m}^{-2}\text{s}^{-1}$, which indicates that in the Bahía Blanca Estuary, the light penetration in the water column is highly attenuated by the suspended matter, as it has been previously reported (Guinder *et al.*, 2009b). In addition, the ratio $Z_{\text{eu}}:Z_{\text{m}}$ incremented towards the innermost station (Fig.3b) in concordance with the I_{m} (Fig. 3c), both as a result of the diminution of the water column depth (i.e. smaller Z_{m} , see Fig. 1 for the water column depths of the sampling stations) and the enlargement of the euphotic zone Z_{eu} . The $Z_{\text{eu}}:Z_{\text{m}}$ ratio was always over the critical mixing depth of 0.16 proposed by Alpine and Cloern (1988), thus phytoplankton growth was non-limited by light availability in the water column as it has been observed in other turbid estuaries (e.g. Kocum *et al.*, 2002).

The particulate organic matter concentration POM was high compared to other similar coastal ecosystems (Hemminga *et al.*, 1993; Goosen *et al.*, 1999; Calliari *et al.*, 2005) and remained relatively constant along the main channel (Fig. 3a). The Bahía Blanca Estuary ecosystem is particularly productive *per se* due to the extensive tidal flats and island covered by halophytes (e.g. *Spartina alterniflora* and *Sarcocornia perennis*) that are responsible of considerable contributions of

organic matter to the system (e.g. Negrin *et al.*, 2011). Moreover, this estuary is highly eutrophic due to external inputs of nutrients and organic matter from diffusive and punctual anthropogenic sources (industrial and urban wastewaters, fertilizers from agricultural activities in the surrounding lands) and continental runoff (Botté *et al.*, 2007; Melo and Limbozzi, 2008; also see figure 8 in Guinder *et al.* (2009b) for a general diagrammatic representation of the main factors affecting the SPM and POM loads in the inner zone of the estuary). In addition, in this shallow estuary characterized by a high tidal energy regime, the close benthic-pelagic interaction may play a major role in nutrients regeneration and carbon cycle (Parodi and Barría de Cao, 2003), as has been observed in other shallow coastal ecosystems with high microphytobentos production (de Jonge, 1992; Kromkamp *et al.*, 1995).

Dissolved inorganic nutrients exceeded limiting levels for phytoplankton development throughout the sampling stations (Fig. 4). Silicate and phosphate were in higher levels in the innermost stations whereas nitrogen concentrations (DIN = nitrite + nitrate + ammonium) showed a decreasing trend towards the inner zone (nitrite: $0.6 \pm 1.2 \mu\text{M}$, nitrate: $5.6 \pm 2.5 \mu\text{M}$ and ammonium: $19.3 \pm 7.0 \mu\text{M}$). In the Bahía Blanca Estuary, as in several shallow coastal ecosystem, the main sources of dissolved silicates in the internal zone are freshwater discharges and land erosion, whereas phosphate is released into the pelagic habitat from the

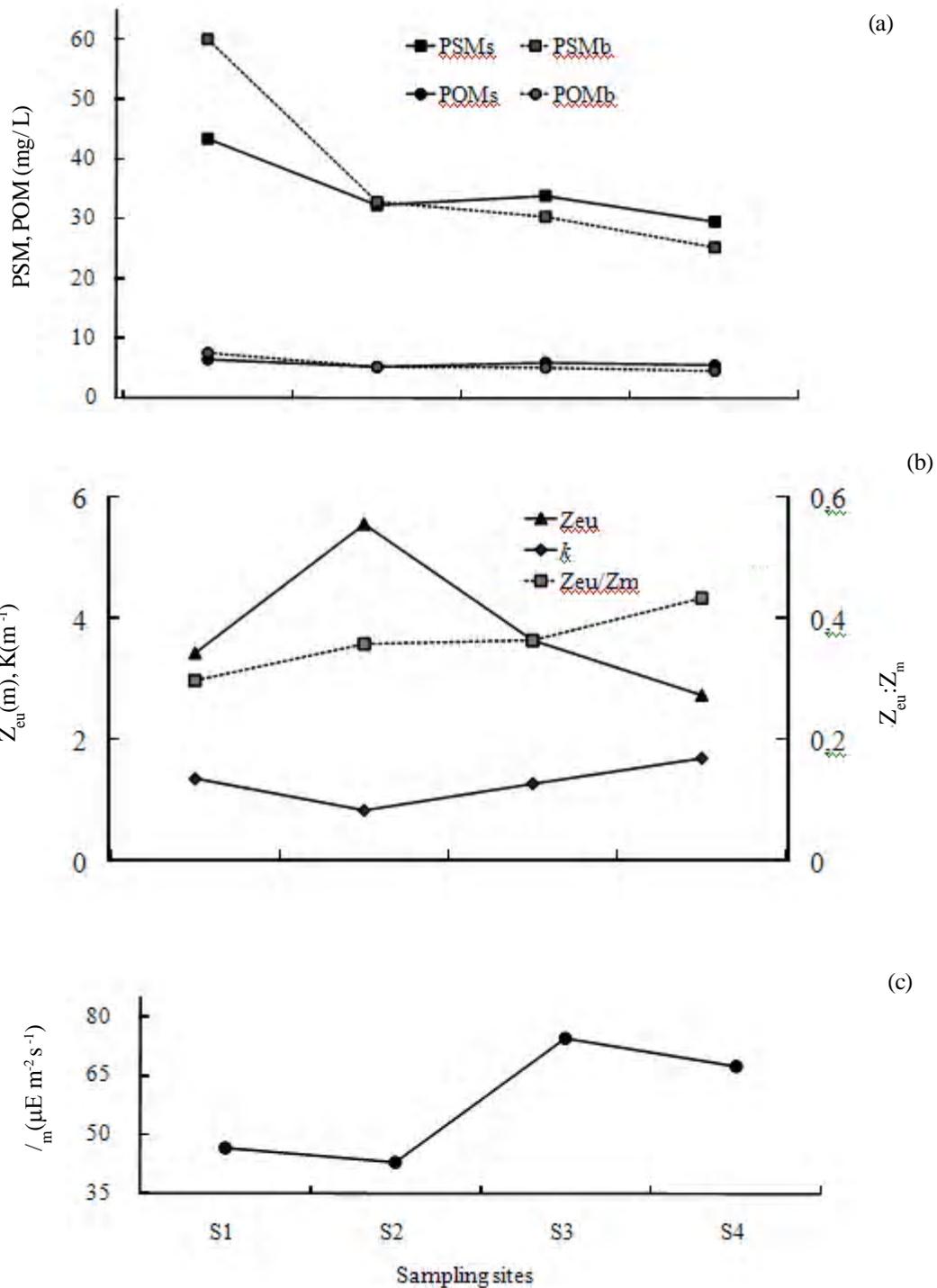


Fig. 3. Variability of a) particulate suspended matter (PSM) and particulate organic matter (POM) concentrations in surface (s) and bottom (b) of the water column; b) depth of the euphotic zone (Z_{eu}), light extinction coefficient (k) and the euphotic zone to mixed zone ratios ($Z_{eu}:Z_m$) and c) mean light intensity in the mixed zone (I_m) in the sampling sites (S1-S4) along the main channel of the Bahía Blanca Estuary

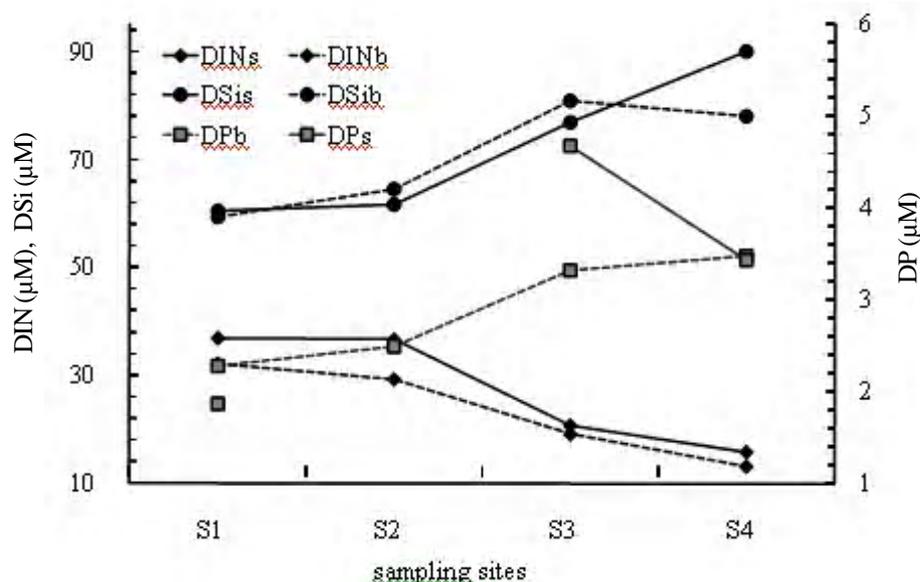


Fig. 4. Variability of dissolved inorganic nutrient concentrations in surface (s) and bottom (b) waters in the sampling stations (S1-S4) along the main channel of the Bahía Blanca Estuary. DSi: dissolved silicates, DP: dissolved phosphate and DIN: dissolved nitrogen (nitrite + nitrate + ammonium). Values of dissolved phosphate concentration in the surface (DP_s) in station 2 (S2) are missing

bottom sediments by turbulence and resuspension processes (Freije *et al.*, 2008; Guinder *et al.*, 2009a). In contrast to other nutrients, no important anthropogenic input of silica exists. Dissolved silica originates from biogeochemical reactions which set free dissolved silica from alkali and aluminum silicate minerals (Correll *et al.*, 2000), whereas seasonal variability is caused by uptake by diatom communities (Struyf *et al.*, 2004). In the inner zone of the Bahía Blanca Estuary, at Puerto Cuatrerros station (S3), the winter diatom bloom is responsible of relatively fast depletion of DIN while there is a lag in phytoplankton assimilation of dissolved silicate and phosphate (Popovich *et al.*, 2008b).

Phytoplankton development (in cellular abundance) was conspicuously higher in the innermost zone of the estuary (S3 and S4), reaching in S4 up to $\sim 1.1 \times 10^6$ cells L^{-1} in the middle of the channel and 1.8×10^6 cells L^{-1} on the coast, around 4-times the cellular density found in S1 (Fig. 5a). In addition, the phytoplankton biomass and the chlorophyll concentration described a smooth trend to increase towards the head of the estuary suggesting that the predominant species in S3 and S4 were of relatively small size. The phaeopigments concentration also increase towards the inner zone of the estuary (Fig. 5b), likely indicating higher zooplankton grazing pressure and/or more intense resuspension processes of bottom sediments, rich in degraded chlorophyll

derived from senescence phytoplankton, microphytobenthos and macrophytes. These findings were consistent with the study of Popovich and Marcovecchio (2008). These authors found an increasing gradient of phytoplankton abundance towards the head of the estuary and concluded that the winter-early spring diatom bloom occurred only in the innermost zone of the estuary. The inner part of the Bahía Blanca Estuary is a semi-closed area with a restricted circulation with low advection and a relatively high residence time (ca. 30 days) (Perillo *et al.*, 2001). Apparently, the particular hydrographic features of the estuary; shallow, funnel-shaped with low water mass intrusions from the ocean and river discharge, allow the accumulation of phytoplankton biomass and nutrients in the internal zone. In addition, low precipitation in the sampling season and in consequence low freshwater run-off (Guinder 2011) could have been a causal factor in the occurrence of the winter bloom in the inner area of the estuary as phytoplankton communities were not “flushed” away by peak discharges (Struyf *et al.*, 2004).

Diatoms were the dominant group in the phytoplankton community in the four sampling stations; phytoflagellates (5-15 μm) became more frequent in the outer ones. Among the most abundant species of diatoms found in the samples were: *Chaetoceros diadema*, *C. debilis*, *C. ceratosporus*, *Thalassiosira* sp. (15-45 μm), *T. eccentrica*, *T. pacifica*

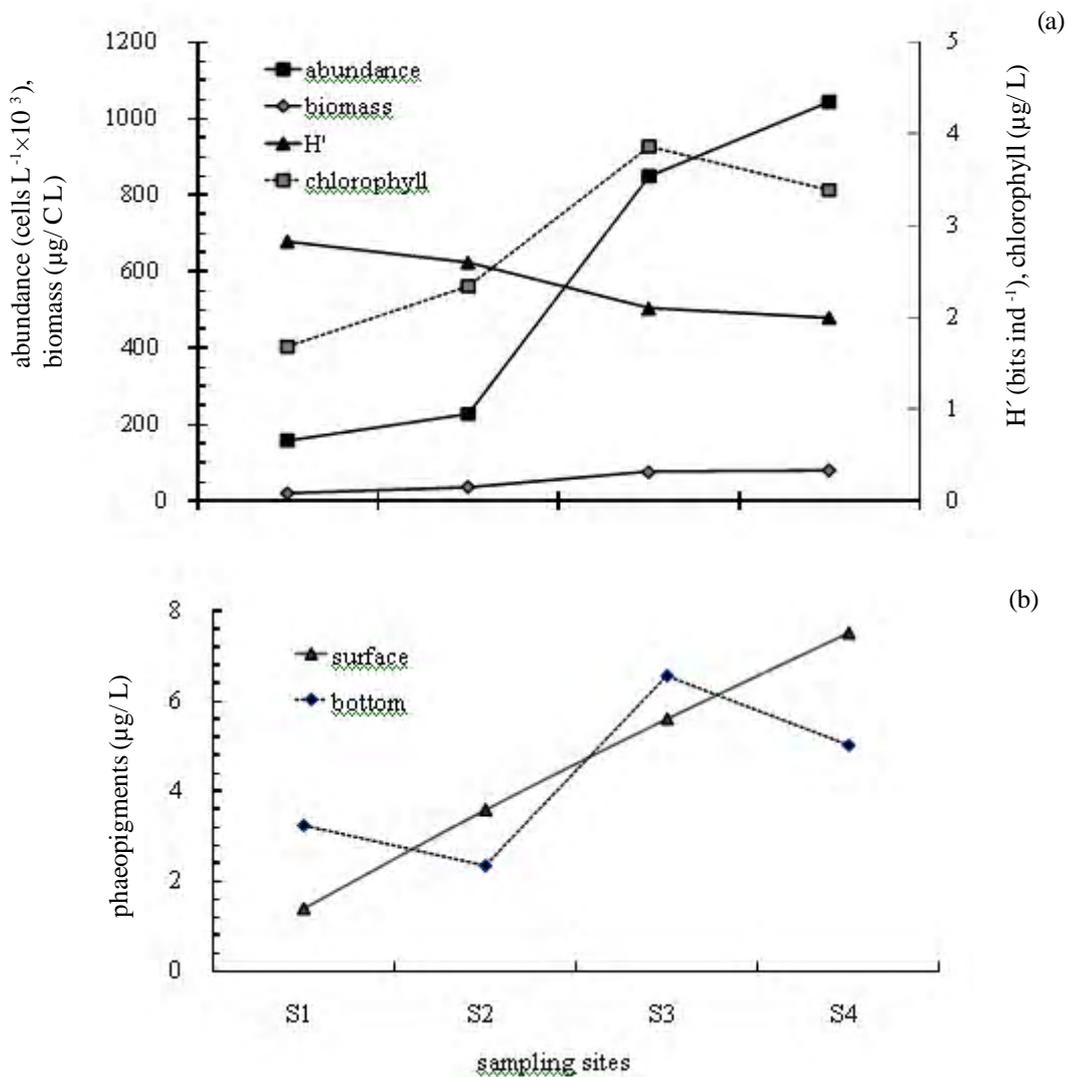


Fig. 5. Variability of a) phytoplankton biomass, cellular densities and diversity index H' and b) chlorophyll a and phaeopigment concentration in the sampling stations (S1-S4) along the main channel of the Bahía Blanca Estuary

and *Cyclotella* aff. *choctawatcheena* (5-15 μm). Other species present with low abundances were: *Guinardia delicatula*, *Leptocylindrus minimus*, *Thalassiosira hendeyi* and *Dityllum brightwellii* (diatoms), the dinoflagellate *Scropsiella trochoidea* and the xantophyceae *Ophiocytium* sp. The diversity of the phytoplankton community was lower in the internal stations (Fig. 5a) as the “winter blooming species” (*sensu* Gayoso, 1998) (e.g. *Thalassiosira* s and *Chaetoceros* s), were dominant in the samples. These phytoplankton species were classified as autochthonous of this area, as they were not present in the adjacent platform (Popovich and Marcovecchio, 2008). In particular, the small diatom *Cyclotella* aff.

choctawatcheena Prasad, was by far the most abundant species in S3. Despite this species has been previously reported in the estuary, it was always present in low abundances and hence it has been characterized as “non-blooming species” (Popovich *et al.*, 2008). Nevertheless, in recent years (2006-2008) this diatom has become frequent and even dominant in the inner zone of the Bahía Blanca Estuary (Guinder *et al.*, 2010; Guinder, 2011) probably related to the shift in the hydro-climate regimen reported in the region (i.e. drier and warmer years and therefore higher water temperatures and salinity) (Guinder *et al.*, 2010). *Cyclotella choctawatcheena* has been identified as a cosmopolitan inhabitant of coastal brackish waters and

saline lakes (Prasad and Nienow, 2006). A key of its successful colonization may be its tolerance for wide ranges of temperature (10-30 °C) and salinity (5.14 - 79.98), inhabiting estuaries and near-shore waters with fluctuating salinities (Prasad and Nienow, 2006 and reference therein). In the Argentinean coast, this diatom species has been recently reported in Río de La Plata Estuary (Prasad and Nienow, 2006), which is located around 700 km from the Bahía Blanca Estuary (see Calliari et al. (2005) and Licursi et al. (2006) for a detailed description of Río de La Plata Estuary).

CONCLUSION

The analysis of the long-term data series collected at Puerto Cuatrerros station (S3) revealed significant changes in the phenology of the phytoplankton in recent years: shifts in the timing, duration and magnitude of the winter-spring diatom bloom together with changes in species composition and size structure of the community (Guinder *et al.*, 2010). During 2006, the phytoplankton annual pattern was particularly different from the recurrent annual cycle, describing biomass peaks spread throughout the year (Guinder, 2011). The dominance of *Cyclotella* aff. *choctawatcheena* in the Bahía Blanca Estuary in recent winters seems to be related with the warmer and saltier characteristics of the estuarine inner zone. The shallow depth allows a close link between water mass properties and atmospheric modifications (Freije and Marcovecchio, 2004). Therefore, the combination of increasing air temperatures and lower precipitations in the region in the last years (Guinder *et al.*, 2010) has potentially induced higher evaporation and further reduction in freshwater inflow into the estuary. Such environmental changes have probably modified the ecological niches of the phytoplankton species and favoured outburst conditions for opportunistic species which eventually established as predominant in the system.

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