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Role of Nutrients in Phytoplankton Development during a Winter Diatom Bloom in a Eutrophic South American Estuary (Bahía Blanca, Argentina)

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ABSTRACT

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The Bahía Blanca Estuary is considered highly eutrophic. Long-term studies have shown that the winter–early spring bloom can be considered the development of a diatom assemblage with *Thalassiosira curviseriata* as the dominant species. Since 2003, several changes have been observed in the annual pattern of nutrients and phytoplankton. To assess the availability of nutrients and their relationship with phytoplankton development, nutrient variability was studied during a winter bloom in 2003 (April 22–September 4). Nitrite, nitrate, ammonium, phosphate, silicates, chlorophyll-*a,* phytoplankton abundance, particulate organic matter, and physicochemical parameters were measured in surface water at Cuatreros Port. In the Sauce Chico River mouth, we determined the concentration of nutrients to estimate the input of this river. The results of this study were compared with those of a previous usual diatom bloom from 2002. In 2003, the bloom was dominated by the *Cyclotella* sp. with high chlorophyll-*a* concentrations (26.5–40.4 $\mu g\ L^{-1}$). *Thalassiosira curviseriata* was present only at three sampling dates, reaching up to 19% of the total abundance. Mean values of dissolved oxygen, nitrate, oxygen saturation percentage, and salinity were significantly higher in 2003 than in 2002, while N:P ratios were significantly lower. Si was never limiting. A shift in the limiting nutrient between the years (N in 2003 and P in 2002) could have lead to a change in species dominance during the blooms. Results suggest that the nutrients levels in the coastal ecosystems at Cuatreros Port play an important role in the control of phytoplankton dynamics during the productive period and partially explain its interannual variability.

ADDITIONAL INDEX WORDS: *Nutrients, diatom bloom, Si:N:P ratios, Cuatreros Port, Argentina.*

INTRODUCTION

Coastal zone and shallow marine areas are among the most productive systems within the world (Mateus, Mateus, and Baretta, 2008). Shallow coastal waters are naturally eutrophic ecosystems, where both the biology and the physical dynamics are strongly influenced by the freshwater runoff from the land, as well as the exchange of water with the adjacent open sea (Borum, 1996; Gobler *et al.,* 2005). Over the last decades, the increase of anthropogenic inputs of nitrogen (N) and phosphorous (P) have led to severe eutrophication problems, inducing an enhancement of phytoplankton primary production in many coastal areas (Buddemeier *et al.,* 2001; Rabalais and Nixon, 2002; Raboubille, Mackenzi, and May Ver, 2001; Ruttenberg, 2005). Cultural or anthropogenic eutrophication is a common thread that links a range of problems in many coastal regions worldwide (Cloern, 2001), resulting in changes in community dominance and species composition (Dixon *et al.,* 2009; Dolbeth *et al.,* 2003; Domingues, Barbosa, and Galvão, 2005; Rocha, Galvão, and Barbosa, 2002). However, the variability of the environmental condition on account of the impacts of climate change can affect the ecology of estuarine environments (Nixon *et al.,* 2009; Pannard *et al.,* 2008; Struyf, Van Damme, and Meire, 2004). In addition, a sudden nutrient supply from rainfall and turbulence typically yields a response from phytoplankton such as diatom (Dixon *et al.,* 2009; Sarthou *et al.,* 2005; Senthilkumar, Purvaja, and Ramesch, 2008). Finally, phytoplankton is an excellent indicator of ecological change (Paerl, Peierls, and Rossignol, 2007).

The Bahía Blanca Estuary is a shallow, well-mixed, highly turbid, temperate, and mesotidal estuarine coastal waters system (Perillo *et al.,* 2001). In the north shoreline of the

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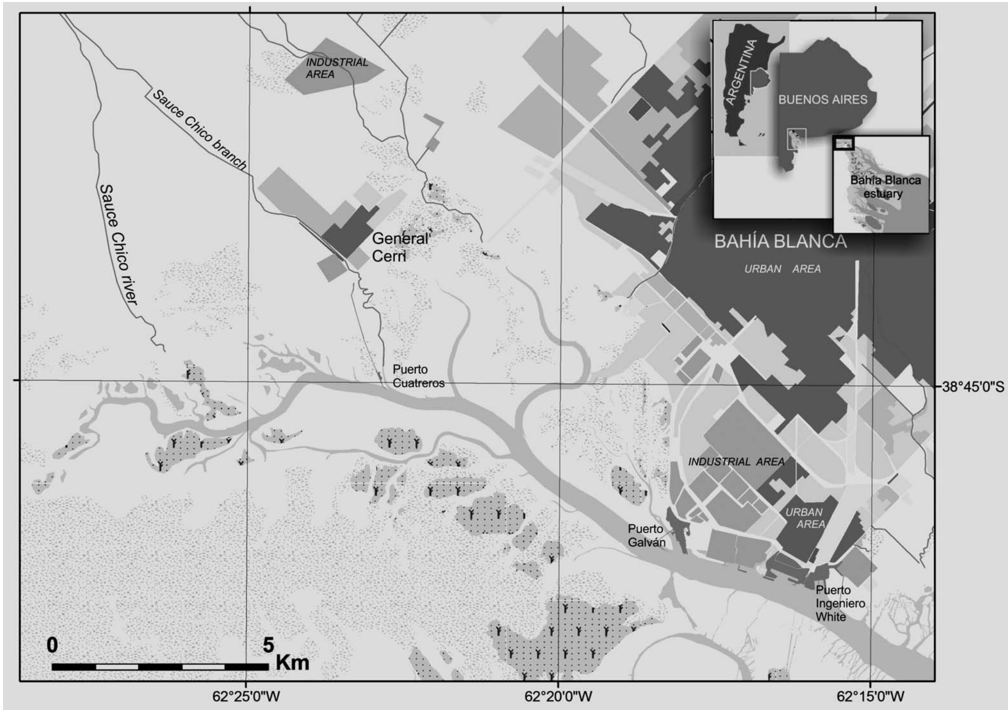

Figure 1. Map showing the inner zone of the Bahía Blanca Estuary, indicating the sampling station, Puerto Cuatreros, and the Sauce Chico River branch.

estuary, the urban pressure is strong: oil, chemical, and plastic factories; two commercial harbors; and a big industrial city with more than 350,000 inhabitants are settled there, and the main soil use is directed to both agricultural and livestock development (Arias *et al.,* 2009; Marcovecchio and Ferrer, 2005; Tombesi, Pistonesi, and Freije, 2000). Two freshwater tributaries enter the estuary from the northern shore: the Sauce Chico River (drainage area of 1600 km^2) and Napostá Grande Creek (drainage area of 1240 km^2) (Figure 1). Both tributaries behave similarly in spring and summer during maximum mean rainfall, but they are out of phase in autumn when the Sauce Chico River has a secondary mode (Piccolo and Perillo, 1990). Although mean annual runoff flows of the Sauce Chico River and Napostá Grande Creek are low (1.5–1.9 and 0.5–0.9 $m^3\ s^{-1}$, respectively), runoff from the Sauce Chico River may peak between 10 and 50 $m^3\ s^{-1}$. The recorded maximum was 106 $m^3\ s^{-1}$ in 1977, although there are other sources that indicate a maximum of 200 $m^3\ s^{-1}$ (Piccolo, Perillo, and Melo, 2008). Besides these major freshwater inputs, the inflows from smaller tributaries into the estuary are intermittent and only significant during periods of high local precipitation. However, the largest input of freshwater, nutrients, and contaminants is provided by the sewage discharges from Bahía Blanca, Punta Alta, and Ingeniero White cities (Piccolo, Perillo, and Melo, 2008).

Historically (1978–2012, database of Chemical Oceanography Area of Instituto Argentino de Oceanografía, or IADO), the inner zone of the estuary is considered highly eutrophic (Freije and Marcovecchio, 2004; Marcovecchio and Freije, 2004) due to its very high nutrient concentrations (mean of 8.02 ± 13.64 μM for nitrate, or NO_3^-; 1.77 ± 1.86 μM for nitrite, or NO_2^-; 32.31 ± 25.78 μM for ammonium, or NH_4^+; 1.83 ± 2.25 for dissolved inorganic phosphorous, or DIP; and 87.30 ± 31.46 μM for dissolved silicates, or DSi) except during the bloom period. At this time, they significantly decline as a result of phytoplankton consumption (Popovich and Marcovecchio, 2008; Popovich *et al.,* 2008; Spetter, 2006).

A general functioning model of the temperate coastal marine environments usually includes a nutrients cycle with the highest levels during winter and a phytoplankton bloom during the spring and summer (Domingues, Barbosa, and Galvão, 2005; Hallegraeff and Jeffrey, 1993; Smayda, 1980). Unlike this, and through long-term studies on physicochemical parameters and phytoplankton dynamics that have been carried out in the Bahía Blanca Estuary, a seasonal pattern was observed that was characterized by high nutrient levels during autumn (Freije and Marcovecchio, 2004; Lara and Pucci, 1983; Spetter, 2006) and a recurrent diatom bloom during winter and early spring (Gayoso, 1998, 1999; Popovich *et al.,* 2008; Popovich, Guinder, and Pettigrosso, 2008). The mentioned winter diatom bloom is the typical event in biomass production and the more important event in the annual cycle of the phytoplankton within this system (Popovich, 2004; Popovich and Marcovecchio, 2008). *Thalassiosira* and *Chaetoceros* species are the most important components during this bloom, and *Thalassiosira curviseriata* has been considered the dominant species (Popovich and Gayoso, 1999; Popovich and Marcovecchio, 2008; Popovich *et al.,* 2008). Historically (1978–

2002) during winter blooms, the levels of abundance, chlorophyll-*a* (Chl-*a*), and primary production reached 12×10^6 cells L^{-1}, 54 μg L^{-1}, and 300 mgC m^{-3} h^{-1}, respectively (Freije and Gayoso, 1988; Freije and Marcovecchio, 2004; Gayoso, 1999; Popovich *et al.,* 2008).

Nevertheless, from 2003 to the present, several significant changes have been observed in the annual pattern of nutrients (Spetter *et al.,* 2008) and phytoplankton, especially in those linked to the presence and abundance of *T. curviseriata* (Guinder, Popovich, and Perillo, *et al.,* 2009; Guinder *et al.,* 2010; Popovich, Guinder, and Pettigrosso, 2008). The presence, or absence, and the abundance of a given species of phytoplankton within a given estuary are influenced by such locally characteristic environmental conditions as temperature, light, nutrients, and salinity, as well as other water quality characteristics (Smayda, 1983). Many studies in estuaries and coastal waters have shown that nutrient enrichment has been responsible for cultural eutrophication, which induces alteration in phytoplankton assemblage (Domingues, Barbosa, and Galvão, 2005; Ferris and Lehman, 2007; Lilleb *et al.,* 2005; Lopes *et al.,* 2007; Maguer *et al.,* 2009; Yin and Harrison, 2008). In the Bahía Blanca Estuary, the effect of the variability of environmental parameters like freshwater discharge and precipitation regimen on nutrient availability and the phytoplankton community has scarcely been documented. To assess the availability of nutrients and their relationship with phytoplankton development, nutrient variability was studied in relation to environmental conditions and phytoplankton structure during a winter diatom bloom in 2003 (considered a turning year). A previous typical winter diatom bloom (developed in 2002), reported by Popovich *et al*. (2008), was compared with the results obtained in this study with the specific objective of detecting differences.

MATERIALS AND METHODS

Study Area and Sampling Site

The Bahía Blanca Estuary (38°45′–39°40′ S, 61°45′–62°30′ W), located in the SW area of the Buenos Aires Province, Argentina (Figure 1), is a mesotidal estuary (Perillo *et al.,* 2001). The mean tidal amplitude ranges from 2.2 to 3.5 m, the spring tidal amplitude ranges from 3 to 4 m, and the highest tidal amplitudes occur near the head of the estuary (Perillo *et al.,* 2001). The estuary is formed by a series of NW- to SE-oriented tidal channels separated by extensive tidal flats, salt marsh patches, and islands (Perillo, 1995). The total surface of the system is approximately 2300 km^2, of which 1150 km^2 is intertidal, 740 km^2 is subtidal, and 410 km^2 is islands.

The sampling site is located at the inner zone of this estuary, in Cuatreros Port (Figure 1), which is considered a chemically representative site of this zone (Freije and Marcovecchio, 2004). Water exchange within the estuary is regulated by a semidiurnal tidal wave cycle, which floods the extended mud flats twice a day. This area has a mean depth of 7 m within a vertically homogeneous water column and high turbidity as a result of the combined effect of both winds and tide currents, which maintain large amounts of suspended matter (Gelós *et al.,* 2004; Piccolo and Perillo, 1990). Strong NW and N winds dominate the typical weather pattern of the region, with a mean velocity of 24 km h^{-1} and gusts of more than 100 km h^{-1} (Piccolo and Perillo, 1990). The annual precipitation of the region is variable; the maximum annual rainfall recorded is 712 mm, and the minimum is 540 mm (Piccolo, 2008). Salinity values of 32.8 ± 3.7 (mean ± standard deviation, or SD, from 1974–2002) and variations between 17.3 and 41.9 have been recorded (Freije and Marcovecchio, 2004; Freije *et al.,* 2008). The Sauce Chico River (Figure 1), whose watershed crosses highly agricultural and cattle breeding lands (drainage area of 1600 km^2), is the main freshwater source, carrying a mean annual runoff of 1.72 m^3 s^{-1} (with a maximum flow rate of 18.32 m^3 s^{-1}), which could increase up to 10 to 106 m^3 s^{-1} during heavy autumn rainfall (Piccolo, Perillo, and Melo, 2008). This tributary proved to be an important source of nutrients to the inner zone of the estuary (Spetter, 2006; Spetter *et al.,* 2008), and a branch of this river outflows close to the sampling station (Figure 1).

Sampling and Analysis

Sampling was performed at Cuatreros Port from April 22 to September 4, 2003, at high tide, close to noon, fortnightly, and twice weekly during the winter bloom. Surface estuarine water samples (~0.50-m depth) were collected with a 2.5-L Van Dorn sampler bottle and directed to determine the concentrations of dissolved inorganic nutrients, Chl-*a*, particulate organic matter (POM), and phytoplankton abundance. All samples were collected assuming that the entire water column was well mixed, had neither thermal nor haline vertical stratification in the inner estuary, and was independent of the season and tidal cycle (Freije, unpublished data; Freije and Marcovecchio, 2004). Simultaneously, temperature, conductivity/salinity, pH, turbidity, and dissolved oxygen (DO) concentration were *in situ* measured using a Horiba U-10 multisensor device, and the oxygen saturation percentage (Ox.S%) was calculated from DO concentration, temperature, and salinity.

Water samples (250 mL) for nutrients and Chl-*a* analysis were filtered through Whatman glass fiber grade C (GF/C, 47 mm diameter and 1.2 μm) membranes. Samples for nutrients determination were frozen (–20°C) in plastic bottles until analysis (Clesceri, Greenberg, and Eaton, 1998). Ammonium was immediately fixed by the addition of specific chemical reagents (Clesceri, Greenberg, and Eaton, 1998), kept in the dark, and frozen. Nitrate (Treguer and Le Corre, 1975a); NO_2^- (Grasshoff, Erhardt, and Kremling, 1983); NH_4^+ (Richards and Kletsch, 1964; modified by Treguer and Le Corre, 1975b), DIP, such as PO_4^{3-} (Eberlein and Kattner, 1987); and DSi (Technicon, 1973) concentrations were determined using an automatized and five-channel upgraded Technicon AutoAnalyzer II. The limit of quantification of methods is 0.10 μM for NO_3^-, 0.02 μM for NO_2^-, 0.01 μM for NH_4^+, 0.01 μM for DIP, and 1.00 μM for DSi. Dissolved inorganic nitrogen (DIN) concentration was calculated as the sum of NO_2^-, NO_3^-, and NH_4^+.

Water samples (250 mL) for POM analysis were filtered through muffled (450–500°C, 1 h) Whatman GF/C membranes, and the filters with the retained suspended organic matter were frozen (–20°C) to determine POM (Clesceri, Greenberg, and Eaton, 1998). Chlorophyll-*a* concentration was measured

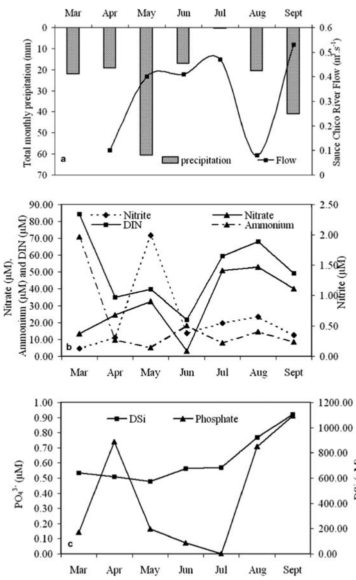

Figure 2. Variation of (a) total monthly precipitation at Bahía Blanca City and freshwater flow of the Sauce Chico River; (b) DIN, NH_4^+, NO_3^-, and NO_2^- concentration; and (c) DIP and DSi concentration in the Sauce Chico River's outlet during March–September 2003.

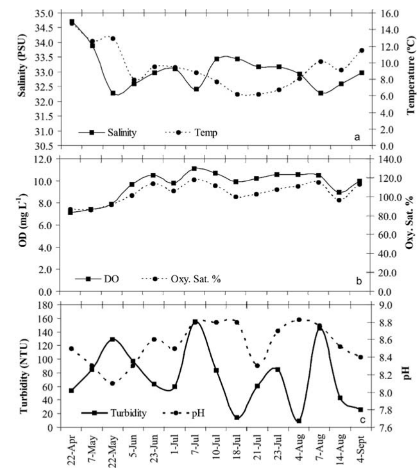

Figure 3. Variation of (a) salinity and temperature, (b) DO and Ox.S%, and (c) turbidity and pH in surface water at Cuatreros Port during April–September 2003.

according to the American Public Health Association method (Clesceri, Greenberg, and Eaton, 1998) (limit of detection of 0.02 mg m^{-3}), and POM concentration was measured following the Strickland and Parsons (1968) method (range of 10–4000 mgC m^{-3}), both using a Beckman DU-II ultraviolet-visible spectrophotometer in the determinations.

For phytoplankton quantitative analysis, subsamples were *in situ* preserved with a few drops of acid Lugol's solution and counted on a Sedgwick-Rafter chamber. The entire camber was examined at 400× magnification using a Nikon Eclipse RE 300 inverted light microscope, and each algal cell was counted as a unit to the species level (McAlice, 1971). For identification purposes, samples were taken with a Nansen net (30-µm mesh) fixed with a 0.4% formaldehyde solution and examined under a Zeiss Standard R microscope equipped with phase contrasted objectives. The size of each cell (diameter) was measured at 400× magnification using a calibrated ocular micrometer under a Zeiss Standard R microscope.

The flow of the Sauce Chico River outlet was monthly *in situ* measured, and the water samples for the determination of the dissolved nutrient concentrations were collected there. Finally, precipitation measurements were obtained from the Argentine National Meteorological Service (database 2002–2003) at Bahía Blanca City.

RESULTS

Precipitation Measurement and Freshwater Inputs

From March to September 2003, the total precipitation in the Bahía Blanca region was 180.9 mm, with a maximum rainfall peak (60 mm) recorded in May (Figure 2a). The Sauce Chico River's flow from April to September 2003 was 0.33 ± 0.19 m^3 s^{-1}. From April to May 2003, an increasing in the freshwater flow (0.1–0.4 m^3 s^{-1}) was registered. From June to September 2003, the freshwater mean flow was 0.47 ± 0.06 m^3 s^{-1}. In August 2003, a significant decreased was registered, achieving a minimum of 0.08 m^3 s^{-1} that followed precipitation absence in July 2003.

Simultaneously, DIN mean concentration in the Sauce Chico River outlet was 45.4 ± 13.9 µM from April to September 2003 (Figure 2b). NO_3^- (34.1 ± 18.5 µM) was the dominant fraction of the DIN. In the same freshwater tributary, PO_4^{3-} and DSi mean concentrations were 0.5 ± 0.4 and 761.4 ± 207.3 µM, respectively (Figure 2c).

Environmental Variables

In the 2003 study period, a surface water temperature mean value of 9.4 ± 2.5°C was registered. The temperature decreased from 14.7°C on April 22 (maximum) to 7.9°C on June 5 and from 9.5°C on June 23 to 6.2°C on July 18 and 21 (minimum) (Figure 3a). Finally, it increased up to 11.5°C on September 4. Salinity (33.1 ± 0.6) decreased from April 22 (34.7) to May 22 (32.3) coincident with the registration of the highest precipitation in the zone for this period.

Dissolved oxygen (9.7 ± 1.3 mg L^{-1}) ranged between 7.1 and 11.1 mg L^{-1}, with oxygen oversaturation percentages recorded from June 5 to July 10 (101–111%), from July 21 to August 7 (102–115%), and on September 4 (113%) (Figure 3b). However, turbidity was extremely variable (58 ± 43 nephelometric turbidity units, or NTU), with values greater than 129 NTU registered on May 22, July 7, and August 7 (Figure 3c). The range of pH varied from 8.1 to 8.8, with the maximum measured in July 7–18 and August 4–7, 2003.

Nutrients Concentration

Concentration of DIN ranged between 8.5 and 92.6 µM (Figure 4a) during the study period, achieving the highest values in July 21 (92.6 µM) and July 23 (57.6 µM). This concentration was dominated by NH_4^+ and showed a pattern

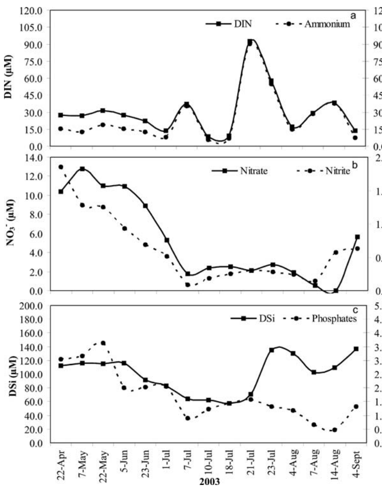

Figure 4. Nutrient concentrations: (a) DIN and NH_4^+, (b) NO_3^- and NO_2^-, and (c) DIP (as PO_4^{3-}) and DSi at Cuatreros Port during April–September 2003.

following the trend of this nutrient (Figure 4a), which during the studied period ranged from 5.9 μM (July 10) to 90.1 μM (July 21).

During 2003, the maximal NO_3^- concentrations were detected between April 22 and June 23 (10.8 ± 1.4 μM), and its minimum was present at the end of the sampling period, on August 14 (not detectable) (Figure 4b). Finally, NO_2^- concentrations were usually less than 1.8 μM throughout the sampling period.

During the 2003 study period, the concentration of PO_4^{3-} varied between 0.5 and 3.6 μM, with the maximum measured at the beginning of sampling (May 22) and the minimum value measured at the end of that period (August 14) (Figure 4c). However, DSi concentrations were high throughout the whole period (100.1 ± 27.0 μM). A minimum value of 57.8 μM was detected on July 18. Since July 23, when an important increase was recorded, the DSi concentration remained high until the end of the sampling period (102.8–194.7 μM).

During the sampling period, a gradual decrease of all analyzed nutrients was observed from May 22 to July 7 with the exception of NH_4^+, which decreased at the beginning of this period but increased from 7.9 to 35.5 μM from July 1 to July 7 (Figure 4a). This diminution in the nutrient concentrations was coincident with the start of the diatom bloom, as discussed later. From July 10 to 18, the concentrations of dissolved nutrients were $NO_2^- \approx 0.2$ μM, $NO_3^- \approx 2.5$ μM, $NH_4^+ = 5.9$–6.6 μM, $PO_4^{3-} \approx 1.3$ μM, and DSi = 57.8–62.3 μM (Figure 4). Since July 18, a recuperation step of the nutrients was observed following a new decrease in the nutrient concentrations that reached minimum values (especially for NO_3^- and PO_4^{3-}) coincident with the collapse of the diatom bloom, as discussed later. Nevertheless, a significant increase of NH_4^+ concentration (6.6–90.1 μM) was measured on July 21, just when this ion presented its maximum level, and then abruptly decreased (15.1 μM) (Figure 4a).

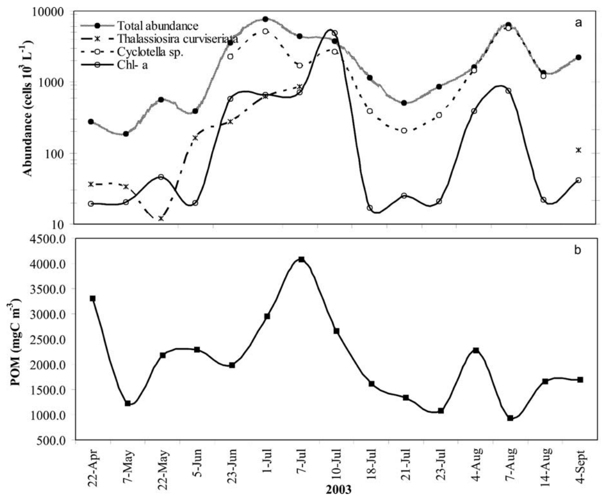

Figure 5. Variation of (a) Chl-*a* concentration, total abundance of phytoplankton, and abundance of *T. curviseriata* and the *Cyclotella* sp. and (b) POM concentration in surface estuarine water in the 2003 winter diatom bloom period in the inner zone of the Bahía Blanca Estuary.

Chl-*a*, Phytoplankton Abundance, and POM

Both total phytoplankton abundance and Chl-*a* concentration in the 2003 sampling period showed the occurrence of a winter diatom bloom (June–August) in the inner zone of the Bahía Blanca Estuary (Figure 5a). Diatoms were always the main component of the phytoplankton community, and the *Cyclotella* sp. (diameter of 8–15 μm) was dominant.

Chlorophyll-*a* concentration reached three important peaks of about 27.2, 40.2, and 28.2 μg L^{-1} in the 2003 period (Figure 5a). The first was observed from June 23 to July 7, coincident with the maximum diatom abundance (7.6×10^6 cells L^{-1} on July 1), where *T. curviseriata* abundance (6.2×10^5 cells L^{-1}) was one order of magnitude lower than that of the *Cyclotella* sp. (5.1×10^6 cells L^{-1}) (Figure 5a). On July 10, the Chl-*a* concentration was the highest, achieving a total phytoplankton abundance of 3.8×10^6 cells L^{-1}, where the *Cyclotella* sp. reached 2.7×10^6 cells L^{-1}. The last Chl-*a* peak was registered from August 4 to August 7, reaching high diatom numbers (6.4×10^6 cells L^{-1}) dominated by the *Cyclotella* sp. (5.8×10^6 cells L^{-1}). Neither in the second nor in third Chl-*a* peak was *T. curviseriata* present.

The range of POM concentrations in the 2003 study period was 928 mgC m^{-3} (August 7) to 4075 mgC m^{-3} (July 7) (Figure 5b). The maximum value of POM was coincident with a higher total abundance of diatoms: 4.4×10^6 cell L^{-1} on July 7, 2003 (Figures 5a and b).

DISCUSSION

Despite the quasiregular, predictable, and well-documented annual pattern that characterized the phytoplankton in the Bahía Blanca Estuary (Gayoso, 1999; Popovich *et al.,* 2008; and references therein), this study shows a strong deviation that represents a contribution to understanding of the nutrients–

phytoplankton relationship in coastal ecosystems such as the Bahía Blanca Estuary.

Change of Dominance Diatom

From 1978 to 2002, the diatom bloom was dominated for the same species: *T. curviseriata, T. hibernalis, T. anguste-lineata, T. rotula, T. pacifica, Chaetoceros similis, C. ceratosporus, C. debilis,* and *C. diadema* (Gayoso, 1999; Popovich and Marcovecchio, 2008). Although interannual variations in the pattern of phytoplankton is a common feature in coastal environments (Baek *et al.,* 2009; Glé *et al.,* 2008; Yamamoto, Inokuchi, and Sugiyama, 2004), as well as in the Bahía Blanca Estuary (Gayoso, 1998), the dominance of *T. curviseriata* during the winter diatom bloom was observed as a recurrent event throughout the long-term phytoplankton studies in the inner zone of the Bahía Blanca Estuary; this species accounted for 60 to 90% of the total cells in all studied diatom bloom periods (Pettigrosso and Popovich, 2009; Popovich and Gayoso, 1999; Popovich and Marcovecchio, 2008; Popovich *et al.,* 2008). Despite this, during the 2003 winter diatom bloom, the dominant species was the *Cyclotella* sp., while *T. curviseriata* was a minor species that disappeared as the bloom developed. From that turning year, this unprecedented behavior was repeated in the following years, indicating a trend of the *Cyclotella* sp. to remain in this environment (Guinder, Popovich, and Perillo, *et al.,* 2009; Guinder *et al.,* 2010). The *Cyclotella* sp.'s dominance in other environments has been attributed to an increase in water temperature, which induces the stratification and the diminution of the nutrient levels in the upper water column (Rühland, Paterson, and Smol, 2008; Winder, Reuter, and Schladow, 2008). During the last 30 years, the mean annual values of water temperature had a positive trend, with an increase up to 1.2°C in the water of the estuary's inner zone (Arias *et al.,* 2011; Beigth, 2007). In addition, noticeable warmer and drier weather conditions during the last decade in the Bahía Blanca area have been associated with recent changes observed in the phenology and structure of phytoplankton within this estuary (Guinder *et al.,* 2010). However, the obtained results, as included in the present study, were not enough to set up a possible cause–effect relationship.

Variability of Nutrients and Environmental Parameters during the Diatom Bloom

The 2003 bloom started with the lowest temperatures of the period (6.2–7.9°C) and was characterized by high DIN concentrations (26.7 μM $\leq$ DIN $\leq$ 31.3 μM, especially $NO_3^- =$ 10.4–12.8 μM), PO_4^{3-} (2.0 μM $\leq PO_4^{3-} \leq$ 3.6 μM), and DSi ($\approx$115 μM). Initial phytoplankton assemblages were composed mainly of the *Cyclotella* sp. and *T. curviseriata*. Immediately, the increase in the abundance of these species coincided with a significant depletion of DIN (especially NO_3^-), PO_4^{3-}, and DSi, as well as with higher Ox.S% and increasing pH. These chemical variations have been observed in many other temperate bays and estuaries during the bloom period (Cabeçadas, Nogueira, and Brogueira, 1999; Cloern, 1996; Twonsend and Thomas, 2002). For example, Cloern pointed out that these large fluctuations in the water indicate a net autotrophic ecosystem (1996), which—for the area of study—typically shows a strong uptake of nutrients by phytoplankton in the first step of the bloom, when both species predominant. Previous reports have shown that *T. curviseriata* has been a pioneer in the diatom bloom in coincidence with the decrease in the nutrients levels, mainly NO_3^- even at high concentrations of NH_4^+ (Popovich *et al.,* 2008). However, from the middle of the 2003 winter bloom, *T. curviseriata* was not present, while the *Cyclotella* sp. dominated until the end of the bloom.

Comparing the obtained results with those from other temperate estuarine systems, all 2003 nutrient concentrations analyzed from Cuatreros Port were much higher than the values described on high tide for the Guadiana Estuary, SW Iberia (Domingues, Barbosa, and Galvão, 2005); Arcachon Bay, France (Glé *et al.,* 2008); San Francisco Bay, California (Dugdale *et al.,* 2007); and Ría de Aveiro, Portugal (Lopes *et al.,* 2007). The seasonal variation of NO_2^-, NO_3^-, PO_4^{3-}, and DSi concentrations in the system during the 2003 sampling period showed the typical trend described for this region of the estuary during the winter diatom bloom (Gayoso, 1999; Lara and Pucci, 1983; Popovich *et al.,* 2008; Spetter, 2006). In contrast, NH_4^+ showed the lower concentrations from the beginning of the study up to July 1, 2003 (13.9 $\pm$ 3.7 μM), with respect to the historical records (32.3 $\pm$ 25.8 μM) (Freije and Marcovecchio, 2004; Freije *et al.,* 2008). In general, NH_4^+ was the dominant fraction of DIN (46–97%). Ammonium plays an important role in bloom dynamics by limiting NO_3^- uptake; in addition, high NH_4^+ inputs and concentrations can suppress the access to NO_3^- by phytoplankton and may change the occurrence of bloom (Dugdale *et al.,* 2007). The fading of the bloom pointed out the occurrence of two processes: (1) low rates of phytoplankton NH_4^+ uptake and (2) regeneration of the bloom-produced organic N by grazing or by bacterial action at the sediment surface (Caffrey, 1995); the combination of both processes resulted in NH_4^+ concentrations returning to levels that inhibit NO_3^- uptake (Dugdale *et al.,* 2007). In regard to the 2003 bloom period, it can be hypothesized that when the NH_4^+ concentration increased in the surface water, at stable NO_3^- levels ($\sim$2 μm) the *Cyclotella* sp. quickly took up NH_4^+, increasing its abundance. This fact could have contributed to the high turbidity levels as a result of low sedimentation rates. However, Guinder, Popovich, and Perillo (2009) maintained that the decrease of suspended particulate matter concentration in the water column with a concomitant increase in the penetration of solar radiation—at the beginning of the bloom—seemed to be one of the main drivers to the development of recent phytoplankton winter blooms in the Bahía Blanca Estuary. Studies by Saito and Tsuda (2003) in the Oyashio region confirmed that the light limitation changes the uptake ratio of nutrients and the elemental composition of diatoms.

Comparison of Nutrients and Environmental Variables during Blooms: 2003 vs. 2002

A previous typical winter diatom bloom (developed in 2002) reported by Popovich *et al*. (2008) was compared with the present results. The comparison showed that surface water temperature, salinity, DO, Ox.S%, turbidity, POM, NO_2^-, NO_3^-, PO_4^{3-}, DSi, and silicon/nitrogen ratio (Si:N) ratio mean values were higher in the 2003 than in the 2002 bloom period (Table 1). Highly significant differences were determined for salinity ($p < 0.0001$) and Ox.S% ($p < 0.0005$), and significant

Table 1. *Average ± standard deviation, minimum and maximum values of the environmental variables, POM, Chl-*a*, and nutrient concentrations and ratios (N:P and Si:N) in the surface water at Cuatreros Port during the 2003 winter diatom bloom.*

	2002			2003		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Temperature (°C)	8.3 ± 2.0	5.1	11.9	9.4 ± 2.5	6.2	14.7
Salinity (psu)*	31.2 ± 1.1	28.1	32.8	33.1 ± 0.6	32.3	34.7
DO (mg L^{-1})†	8.6 ± 1.2	6.8	10.5	9.7 ± 1.3	7.1	11.1
Ox.S%*	89 ± 10	74	108	104 ± 10	87	118
pH	8.6 ± 0.4	8.0	9.5	8.5 ± 0.2	8.1	8.8
Turbidity (NTU)	58 ± 43	13	147	74 ± 50	10	155
POM (mgC m^{-3})	1719 ± 708	627	3244	2086 ± 883	928	4085
Chl-*a* (μg L^{-1})	16.9 ± 10.5	3.9	44.3	15.1 ± 12.4	3.3	40.4
NO_2^- (μM)	0.4 ± 0.4	0.05	1.60	0.6 ± 0.5	0.1	1.8
NO_3^- (μM)†	2.0 ± 2.9	ND	10.8	5.3 ± 4.4	ND	12.8
NH_4^+ (μM)	43.2 ± 34.2	14.3	102.8	24.3 ± 22.9	5.9	90.4
DIN (μM)	45.6 ± 36.0	14.7	105.3	30.1 ± 21.5	8.5	92.6
DIP (μM)	1.0 ± 0.4	0.3	1.6	1.7 ± 0.9	0.5	3.6
DSi (μM)	96.3 ± 37.5	35.0	194.6	100.1 ± 27.0	57.8	136.7
N:P†	45.3 ± 26.3	11.4	100.2	24.4 ± 23.8	6.4	82.8
Si:N	3.2 ± 1.8	1.0	5.8	4.6 ± 2.5	0.8	10.1

* Highly significant difference ($p < 0.0001$ or $p < 0.0005$).
† Significant difference ($p < 0.05$).
ND, not detectable.

differences ($p < 0.05$) in DO values, concentration of NO_3^-, and N:P ratios were detected. The lower concentrations of DIN and the higher concentrations of PO_4^{3-} and DSi in the 2003 bloom period reflect the lower N:P ratios and the higher Si:N ratios (Table 1). Ammonium was the dominant fraction of DIN in both bloom periods, but in 2002 it represented a higher percentage (82–100%) than in 2003. In this comparison, the highest NO_3^- concentration and the lowest NH_4^+ concentration recorded during the studied period, with respect to 2002, were the principal factors governing the DIN difference.

Nutrient Ratios

Niraula *et al*. (2007) have shown that the availability of N determines the diatom community composition if Si and P are not deficient. The nutrient limitation effect on phytoplankton growth has been discussed considering the optimal molar ratios among Si, N, and P, *i.e.,* Si:N:P = 16:16:1 (Brzezinski, 1985; Redfield, Ketchum, and Richards, 1963) for marine phytoplankton growth. In this sense, the nutrient ratios among DIN, P, and silicate concentrations measured during the highly productive period (*i.e.,* April 22–September 4, 2003, and May 3–August 26, 2002) were compared against the Redfield ratios to better define a potential nutrient control mechanism within the studied system. For the 2003 sampling period, the Si:N ratios were higher than 1 except on July 21 (day 10 within Figure 6a), when the ratio presented its minimum (0.8) linked to a N:P ratio higher than 16 (N:P = 58.6). From the beginning of the study to July 1 (days 1–6), the N:P ratios were less than 13.8 (mean = 9.6). Then, on July 7 (day 7), the N:P ratio was more than 16 (N:P = 41.5); however, from July 10 to 18 (days 8 and 9, respectively), the N:P ratios were about 6.7. From July 21 to August 14 (days 10–14), the N:P ratios were more than 43 with the exception of August 4 (day 12), when this value was less than 16 (14.7). Finally, at the end of the sampling period, the N:P ratios were about 10.

In the 2002 bloom period, data showed Si:N ratios of at least 1 (Figure 6b). At the beginning of this bloom period

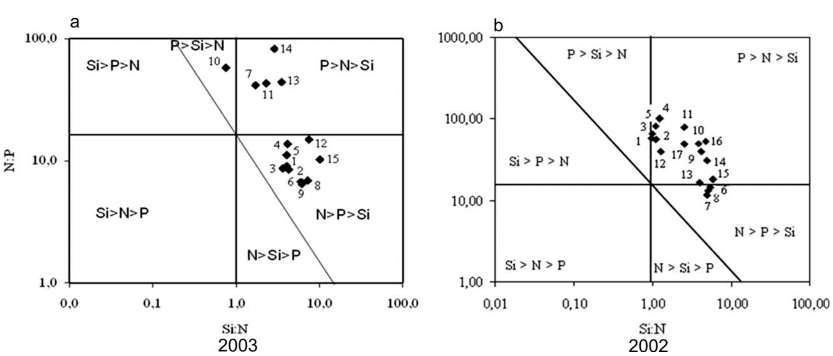

Figure 6. Representation of the Si:N:P molar ratios in the water column at Cuatreros Port from (a) 22 April (day 1) to 4 September (day 15), 2003, and (b) 3 May (day 1) to 26 August (day 17), 2002. Molar quotients between the concentrations of potentially limiting nutrients are delimited in this logarithmic plot (log Si:N vs. log N:P) by Si:N = 1 lines, N:P = 16 lines, and Si:P = 16 lines, which define six areas within the plots, with each characterized by potentially limiting nutrients in order of priority (Rocha, Galvão, and Barbosa, 2002).

Table 2. *Average ± standard deviation of the flow of the Sauce Chico River and the nutrients concentrations at the Sauce Chico River outlet during the 2002 and 2003 winter diatom bloom.*

	2002 Mean ± SD	2003 Mean ± SD
Flow (m^3 s^{-1})	0.57 ± 0.37	0.33 ± 0.19
NO_2^- (μM)	0.9 ± 0.9	0.7 ± 0.6
NO_3^- (μM)	23.0 ± 7.5	34.1 ± 18.5
NH_4^+ (μM)	65.0 ± 42.7	10.7 ± 4.8
DIN (μM)	96.8 ± 39.6	45.4 ± 16.9
DIP (μM)	0.3 ± 0.5	0.5 ± 0.4
DSi (μM)	661.8 ± 272.5	761.4 ± 207.3

(days 1–5 within Figure 6b), the Si:N ratios were about 1, whereas the N:P ratios ranged between 55.1 and 100.2. From June 28 to July 4 (days 6–8), the Si:N ratios were more than 1 (4.9–5.5) and the N:P ratios were less than 16 (11.4–14.6). From July 12 to August 26 (days 9–17), the Si:N ratios were more than 1 (1.3–5.8) and the N:P ratios were more than 16 (16.4–77.5). Thus, the relative proportions of available nutrients in the inner zone of the estuary during both the 2002 and the 2003 bloom periods (Figures 6a and b) suggested that there was a large excess of silicates in both periods. On the contrary, a P (and secondarily N) deficiency was observed in 2002 and a N (and secondarily P) deficiency was seen in 2003. When Si:N and Si:P ratios are relatively high and Si is adequately available, the growth of diatoms is favored (Domingues, Barbosa, and Galvão, 2005; Rocha, Galvão, and Barbosa, 2002). In conclusion, we suggest that a shift in the potential limiting nutrient between the examined years (N in 2003 and P in 2002) could have lead to a change in species dominance during the winter blooms.

Nutrient Variability in Relation to Freshwater Inputs

The main factors controlling the concentration of inorganic N species in the water column of shallow coastal marine ecosystems are inputs arising from fluvial discharges and those resulting from exchange across the sediment–water interface (Dugdale and Goering, 1967; Herbert, 1999). Seven years of DIN data in the Sauce Chico River's mouth indicate a mean concentration on the order of 60 μM (Freije, unpublished data) and PO_4^{3-} concentrations up to 7.3 μM after high rainfall (Spetter, 2006). The Sauce Chico River influences both the salinity and the nutrient concentrations in the inner zone of the Bahía Blanca Estuary (Lara and Pucci, 1983; Marcovecchio *et al.,* 2009; Martinez *et al.,* 2006; Spetter, 2006). In 2003, salinity presented small variation with high values (>32), reflecting the effect of reduced freshwater inputs to Cuatreros Port due to lower precipitation. A decrease in the salinity value was recorded in May 2003, when the highest rainfall probably promoted an increase in the freshwater flow and consequently forced a dilution effect. Although no significant differences were detected, DIN mean concentration in the Sauce Chico River outlet was lower in 2003 than in 2002. The composition of DIN was also different; NO_3^- was the dominant fraction of DIN in 2003, and NH_4^+ dominated in 2002 (Table 2). Thus, the Sauce Chico River's inputs of NO_3^-, NH_4^+, DIP, and DSi were probably reflected in the surface estuarine water in Cuatreros Port. However, the potentiality of groundwater as a nutrient source to the estuary might be considered. In this sense, and even though it was not fully quantified, a high input of NO_3^- from groundwater to the estuary has been proposed in the intermediate zone of the Bahía Blanca Estuary (Negrín *et al.,* 2011). Consequently, it is suggested that the groundwater may be another important source of NO_3^- for the inner zone in the Bahía Blanca Estuary. Accordingly, the reduction of the Sauce Chico River flow and the decrease in the estuarine water dilution by lower rainfall would influence the supply of nutrients to the inner zone of the estuary, mainly in DIN, which could produce changes in phytoplankton productivity or composition, as reported in other environments (Baek *et al.,* 2009; Boyer *et al.,* 2009; Maotian *et al.,* 2007; Xu *et al.,* 2008).

Considerations about Nutrient Uptake by Phytoplankton of Different Size

Bury *et al*. (2001) showed that both microplankton (20–200 μm) and nanoplankton (2–20 μm) assimilated proportionately more NO_3^- than NH_4^+, and they agree that a substantial part of new production may occur in the small phytoplankton size range with resultant low sedimentation rates. Consequently, we suggest that the high account of NO_3^- linked to the lower concentration of NH_4^+ at the beginning of the bloom and the high turbidity could have been the chemical drivers, which can explain the growth of the smaller diatoms.

Despite not having data on the iron (Fe) concentration in the study period, the concentration of dissolved Fe from 1997 to 2001 (0.10–38.27 μg L^{-1}) was higher than in the 2004–06 period (3.17–10.37 μg L^{-1}) (Andrade, 2001; Botté, 2005). As the coastal diatoms need further requirement of Fe than do the ocean diatoms (Sunda and Huntsman, 1995) and small coastal diatoms uptake Fe more quickly because they have a bigger surface–volume relationship (Sunda and Huntsman, 1995), it can be postulated that with a lower concentration of Fe, the growth of small diatoms would be favored.

The high Fe uptake and the storage capacity in the coastal diatoms allow these species to accumulate excess Fe during periods of high availability, low growth rate, or both, which then can be drawn upon later in periods of high growth rate during blooms (Sunda and Huntsman, 1995). However, cells growing on NO_3^- should require 40 to 50% more Fe than cells supplied with NH_4^+ (Morel, Rueter, and Price, 1991; Raven, 1986). Even though the Fe values described earlier do not reach limiting levels, the effect of N source availability on Fe uptake could be an important factor that affects the bloom species succession during the bloom.

Zooplankton Grazing and Excretion

The collapse of the winter diatom bloom that occurred on August 14, 2003, presumably might be related to NO_3^- and DIP depletion due to a rapid consumption by phytoplankton, as well as an increase in grazing impact consequently promoting an increase in NH_4^+ concentration by zooplankton excretion. Grazing pressure could also explain differences in the winter diatom bloom. The regeneration of N by zooplankton grazing and excretion was recognized as a main source of NH_4^+ to sustain algal growth in shallow marine–coastal environments (Dagg and Breed, 2003; Dugdale, 1985; Zehr and Ward, 2002), such as the inner zone of the Bahía Blanca Estuary. Besides, during grazing, most of the excreted NH_4^+ is presumably taken up again by the phytoplankton (Dagg and Breed, 2003; La

Roche, 1983; Pettigrosso and Popovich, 2009; Sakshuang and Olsen, 1986), as observed in this study. During the studied period, Pettigrosso (unpublished data) registered a marked microzooplankton (predominantly ciliates) abundance peak strongly related to a decrease in the biomass of smaller diatoms—a kind of relationship that is in agreement with those observed by many other authors (Barría de Cao, Pettigrosso, and Popovich, 1997; Niraula *et al.,* 2007; Pettigrosso, Barría de Cao, and Popovich, 1997). Moreover, previous studies on zooplankton from this region reported the occurrence of large pulses of *Eurytemora americana,* an invasive herbivore copepod, from the end of winter up to spring (Berasategui *et al.,* 2009; Hoffmeyer, 1983, 2004a, 2004b). Thus, zooplankton pressure could be partly responsible for the end of the phytoplankton bloom.

The nutrients dynamic in Cuatreros Port and the phytoplankton community composition are strongly related. The interannual variability of the nutrients and phytoplankton pattern depends not only on the environmental changes but also on factors such as zooplankton grazing and excretion, tidal energy, atmospheric transport, and benthic fluxes that should be evaluated in future studies of the Bahía Blanca Estuary.

CONCLUSION

(1) The nutrient dynamics and winter phytoplankton bloom are strongly related in the inner zone of the Bahía Blanca Estuary.
(2) During the 2003 winter, an atypical diatom bloom occurred, with the *Cyclotella* sp. as the dominant species.
(3) It is strongly suggested that a shift in the potential limiting nutrient between different years can lead changes in species dominance during the winter blooms.
(4) A strong interannual variability has been well documented in the studied system, which has been characterized historically by a recurrent phytoplankton bloom, with *T. curviseriata* as the dominant species.
(5) The available nutrients' standing stock for the development of this bloom appears to be highly dependent on precipitation events and the Sauce Chico River's inputs, as well as seemingly influenced by phytoplankton uptake and zooplankton grazing and excretion.
(6) The nutrients levels in coastal ecosystems such as Cuatreros Port play an important role in the control of phytoplankton dynamics during the productive period and partially explain its interannual variability.

Thus, a long-term evaluation of all these factors should be made in future studies to contribute to understanding of the mechanisms involved in the recent phytoplankton bloom changes in this land–estuary boundary.

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□ RESUMEN □

El estuario de Bahía Blanca es considerado altamente eutrófico. Los estudios anteriores han demostrado que la floración invernal del fitoplancton se caracteriza por el desarrollo de una asociación de diatomeas con *Thalassiosira curviseriata* como la especie dominante. Desde el 2003, se han observado cambios en el patrón anual de nutrientes y fitoplancton. La variabilidad de los nutrientes fue estudiada en Puerto Cuatreros durante una floración invernal (22 Abril–4 Septiembre 2003) con el fin de evaluar la disponibilidad de nutrientes y su relación con el desarrollo del fitoplancton. Se determinó la concentración de nitrito, nitrato, amonio, fosfato, silicatos, clorofila *a,* materia orgánica particulada, la abundancia del fitoplancton y los parámetros fisicoquímicos en agua superficial. Para estimar el aporte del Río Sauce Chico se determinaron las concentraciones de los nutrientes en su desembocadura. Los resultados de este estudio se compararon con los de una floración típica previamente estudiada durante el 2002. En 2003, la floración estuvo dominada por *Cyclotella* sp. presentando altas concentraciones de clorofila *a* (26.5–40.4 $\mu g\ L^{-1}$). *Thalassiosira curviseriata* alcanzó el 19% de la abundancia total y se detectó solo en tres fechas del muestreo. Los valores promedios de oxígeno disuelto, nitrato, porcentaje de saturación de oxígeno, y salinidad fueron significativamente mayores en 2003 que en 2002, mientras que la relación N:P fue significativamente menor. El Si nunca fue limitante. Se sugiere que un cambio en el nutriente limitante (N en 2003 y P en 2002) puede haber conducido a un cambio en la dominancia de la especies durante la floración invernal. Los resultados evidencian que los niveles de nutrientes en sistemas costeros como Puerto Cuatreros cumplen un rol importante en el control de la dinámica del fitoplancton durante un período productivo y podrían explicar en parte su variabilidad interanual.