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Use of phytoplankton assemblages to assess the quality of coastal waters of a transitional ecosystem: Río de la Plata estuary

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A R T I C L E I N F O

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A B S T R A C T

Among the estuarine ecosystems under anthropogenic stress, the Río de la Plata can represent a case study to help identify phytoplanktonic species diagnosing and warning about water quality changes. The freshwater tidal zone on the coast of Argentina is used for several purposes, including recreational and navigational activities and the provision of drinking water. We analyzed the relationship between the abundance of the phytoplanktonic species, changes in water quality (linked to enrichment with nutrients and organic matter) and the land use on the coast of Argentina. A canonical correlation analysis (CCA) allowed us to identify two environmental gradients, one related to anthropogenic activities, where the most influential factors were BOD_5, DIN, PO_4^{3-} and DO, and a second gradient related to turbidity and conductivity. The relative abundances of 24 species were significantly correlated with the deterioration of the water quality. This set of tolerant species is mostly composed of taxa considered C-strategists, and the most represented group was the Chlorococcalean algae. The percentage of this group can provide an early warning indicator of the impairment of the water quality; its abundance exceeded 30% at those sites with a bad water quality (reaching 19000 cell mL^{-1}), and were less than 15% (300 cell mL^{-1}) in sites with a good water quality.

The use of a reduced group of species constitutes a potential tool for monitoring, complementing another common indicators such as chlorophyll *a* or the total density of phytoplankton. Considering that most of these tolerant species are widely distributed it is possible to employ them as a biomonitor in other freshwater zones of temperate estuaries.

1. INTRODUCTION

Estuaries are among the most productive, resourceful, and dynamic aquatic ecosystems on Earth. These watersheds support more than 75% of the human population and are sites of large increases in nutrient loading associated with urban and agricultural expansion. They process much of the world's riverine and coastal watershed discharge. As such, they receive a large share of land based nutrients and other pollutants entering through surface runoff, atmospheric deposition, groundwater discharge, and much of it delivered via rivers draining urban centers and agricultural watersheds (Howarth et al., 1996; Jaworski et al., 1997; Paerl, 1997; Paerl et al., 2002; Borja et al., 2011). The impacts of human pollution and habitat alteration are most evident and of greatest concern at the microbial level, where a bulk of production and nutrient cycling takes place. Therefore microbial bioindicators play a major role in detecting and characterizing changes in water quality (Paerl et al., 2003).

Because of their direct link and sensitivity to nutrient loading, phytoplankton growth is considered a direct effect and one of the primary symptoms indicative of eutrophication (Bricker et al., 2003) and, as such, phytoplankton represents a good indicator for nutrient-related impacts. Typically, measurements of chlorophyll *a* are used to represent phytoplankton biomass in coastal systems, but other measures, such as the abundance and species composition, and the changes in the frequency and duration of blooms are also informative of the condition and health of the estuaries (Andersen and Laamanen, 2009; Bricker et al., 2003; Ferreira et al., 2007; Souchu et al., 2000; EPA, 2005). While the analysis of the composition and abundance of phytoplankton requires expertise in identifying species, the information provided is comprehensive, and allows for the recognition of their ecological preferences, their strategies of life and identifying actual and future changes in water quality.

Among the estuarine ecosystems under anthropogenic stress, the Río de la Plata, a temperate large coastal plain estuarine system with an

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area of 35,500 km^2, naturally rich in nutrients and trophically dominated by plankton (López Laborde and Nagy, 1999), represents a case study to identify phytoplanktonic species capable of diagnosing and warning about water quality changes.

The main urban centers of Argentina and Uruguay are set along the shores of the Río de la Plata, where 12.8 million people live in Buenos Aires city and its metropolitan area (INDEC, 2010). The high urbanization and industrialization level that concentrates on the inner zone of the estuary generates an input of pollutants that pose a threat to both the biota and human health. Among these contaminants nutrients and organic matter enter the system through the waterways that cross the coastal cities and the poorly treated effluent sewage. In addition, dredging and modification of the hydrological regime of coastal wetlands, among other human interventions, have altered the morphology of the coast, interfering with the integrity of both the physical habitat and the natural biological processes (FREPLATA, 2005; Gómez and Cochero, 2013).

In this regard the phytoplankton community is affected by constantly increasing anthropogenic and climatic pressures; the global changes have been much more pronounced in the last decades (Nagy, 2006; Gómez, 2014), leading to changes in the structure of the phytoplankton, such as diversity, density and biomass (Sathicq et al., 2015). This water source is used for several purposes, including recreational and navigational activities and the provision of drinking water, approximately 6.000.000 m^3/day (www.aguasbonaerenses.com.ar; www.aysa.com.ar). Therefore it is valuable to recognize phytoplanktonic indicators, to help assess the water quality and ultimately contributing to the decision making process required to improve the environmental quality.

We hypothesized that phytoplankton composition would change in relation to increasing concentration of organic matter and inorganic nutrients in the water, favoring a group of species that indicate the deterioration in water quality. To achieve this goal we analyzed the relationship between the abundance of the phytoplanktonic species and the natural and anthropogenic factors in the study area, represented as changes in water quality (linked to enrichment with nutrients and organic matter) and the land use on the coast. In order to characterize some morpho-functional aspects of the species, their biological forms, size and life strategies were analyzed.

2. MATERIALS AND METHODS

2.1. Study area

The sampling area covered 170 km of the freshwater tidal zone of the Franja Costera Sur of the Río de la Plata, between the mouth of the Luján river (DL) and Punta Piedras (PP) (Fig. 1). 31 surveys were conducted between 2005 and 2012, where 360 samples were collected between the shoreline and the 3000 m line. Sampling was conducted at 36 sites, 18 of which were sampled by land, and the other 18 sites were sampled by using the hydrographic survey ship “ARA Cormorán" from the Servicio de Hidrografía Naval Argentino. All sampling sites were located below the 5 m isobath. A land use map was extracted from Gómez and Cochero (2013) that considers the land uses in an area of 10 km around each sampling site (Fig. 1).

The sites located in the uppermost sector of the study area (DL, SI, Pal, R, Sar, SD, Ber) are directly exposed to the impact of the city of Buenos Aires (with almost 3 million of inhabitants), where great port activities are held, and domestic and industrial effluents are discharged. At the site Bz the sewage discharge from the city of Buenos Aires (2500 m. from the coast) is located, and at the site Bag the sewage discharge from the city of La Plata (with 654000 inhabitants), a few meters away from the shoreline. It is also important to mention that within the sampling area there are three water intakes (at Pal, Ber and downstream of PL) which provide about 89% of the drinking water for many urban centers, including Buenos Aires and La Plata. Located downstream of the Bal site, recreational activities and small-scale fishing dominate, and the sites are exposed to rural activities that take place in the surroundings. The PI and PP sites are located near the maximum turbidity front of the estuary.

2.2. Sample collection

In each sampling site 125 mL of subsurface water were collected by triplicate for the quantitative analysis of phytoplankton. Qualitative samples were also collected with a plankton net of 35 μm pore, for the taxonomic analysis of phytoplankton. The samples were fixed in the field with formalin (final concentration 2% [v/v]). For the dissolved nutrients analysis (soluble reactive phosphorus, nitrate, nitrite and ammonium nitrogen) 200 mL of water were filtered through glass fiber filters (Whatman G / FC, 1.2 μm pore). For the biological oxygen demand analysis, 250 mL of water were also collected, and all samples were transported in coolers (4ºC) to the laboratory.

2.3. Physicochemical parameters

Temperature (° C), pH, conductivity (μS cm^{-1}), turbidity (NTU) and dissolved oxygen (mg L^{-1}) were measured in the field with a multiparametric sensor Horiba U-50.

2.4. Analytical methods

Soluble reactive phosphorus ($P\text{-}PO_4^{3-}$), nitrite ($N\text{-}NO_2$) and ammonia nitrogen ($N\text{-}NH_4^{+}$) were determined colorimetrically; nitrate ($N\text{-}NO_3^{-}$) was reduced to nitrite before colorimetric measurements. All these determinations were made according to Mackereth et al. (1978). Dissolved Inorganic Nitrogen (DIN) was calculated by adding the measured fractions of nitrogen. BOD_5 was determined by incubation for 5 days at 20°C and subsequent measurement of the dissolved oxygen (Clesceri et al., 1998).

2.5. Phytoplankton analysis

Quantitative phytoplankton analysis was performed according to Lund et al. (1958), using an inverted microscope Olympus IX51, with magnifications of 400X and 600X, using sedimentation chambers of 5 or 10 mL according to the amount of phytoplankton and suspended solids. The samples were settled for at least twelve hours, with the addition of acetic lugol (Prygiel and Leitao, 1994). The sampling error was calculated by Lund et al. (1958) and did not exceed 20%. Taxonomic determinations were carried out using specific bibliography for different taxonomic groups. For the detailed analysis of diatoms, samples were oxidized, washed and mounted with Naphrax® (Stevenson and Bahls, 1999). An Olympus BX 51 microscope with phase contrast and Nomarski interference, at 1000X magnification was used for the identification of phytoplankton.

From all the identified species, we made a selection considering only those with euplanktonic habits, and those taxa that had more than 5% frequency in all the samples and more than 1% of relative abundance in at least one sample. The exclusion of rare taxa is a common practice since it complicates the output of the analysis, masking any possible effects on the more abundant taxa, due to random chance (Marchant, 2002).The species were classified according to their biological forms (unicellular, filamentous, coenobium or colonial). In the same manner, they were classified in C, S and R life strategies according to Reynolds (1988, 2006). The C strategy corresponds to Competitors or invasive algae with rapid exploitation of the resources available. The S strategy corresponds to Stress-tolerant (low nutrient levels) species that efficiently match their demand to the limited supply of nutrients. R-strategists are disturbance-tolerant (Ruderals) that thrive in transient habitats. The size classification in picoplankton (0.2-2 μm), nanoplankton (2-20 μm), microplankton (20-

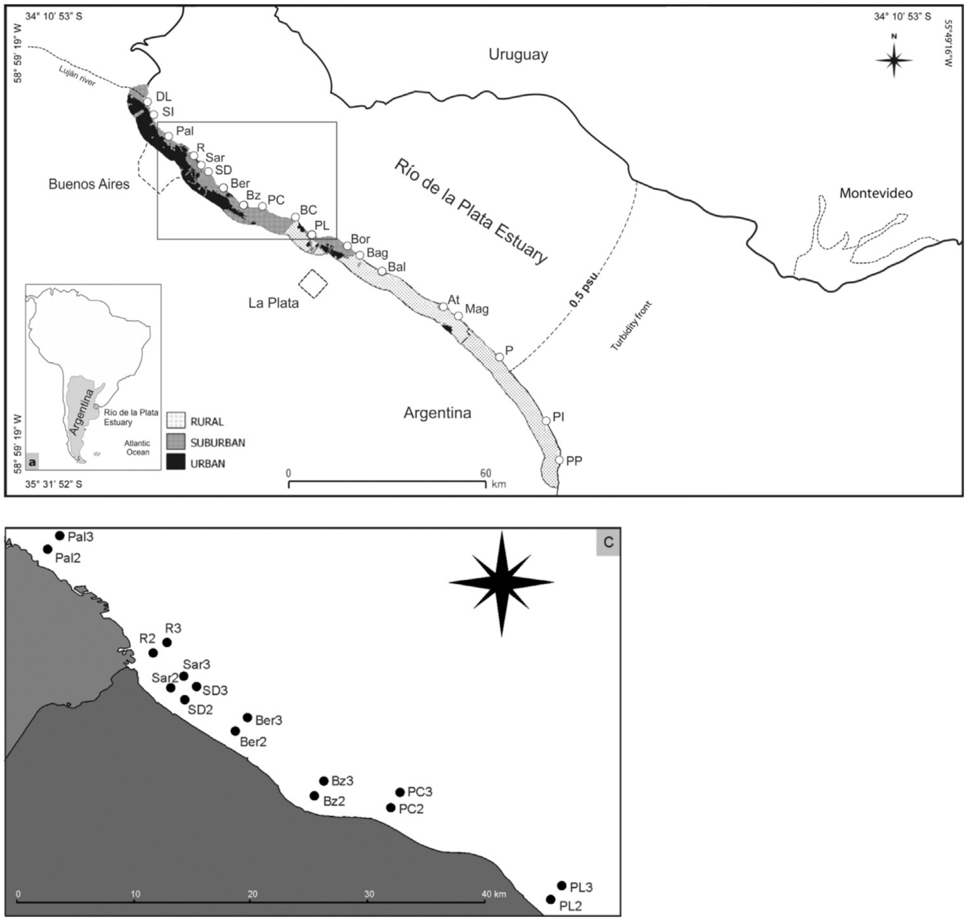

Fig. 1. a. Location of the Río de la Plata estuary in South America. b. Study area showing the shoreline sampling sites and the land uses corresponding to the surrounding area (Modified of Gómez et al., 2012). c. Detail of the rectangle in figure b. showing the sampling sites away from the shoreline (1500 m: 2, 3000 m: 3).

200 µm) and mesoplankton (200 µm-2 mm) was done according to Sieburth et al. (1978)

2.6. Classification of the sampling cases

The sampling cases were classified by their water quality, considering the DO concentration, DIN, PO_4^{3-} and BOD_5, according to Gómez et al. (2012); a value is assigned to each study case according to the following scale: 5 (good water quality), 3 (moderate water quality) and 1 (bad water quality) (Table 1).

2.7. Statistical analysis

A canonical correspondence analysis (CCA) was used to explore the relationship between the environmental variables and the selected species. Prior to this analysis, a detrended correspondence analysis (DCA) was performed to know the length of the gradient (ter Braak and Smilauer, 1998), and only those variables with an inflation factor < 10 were retained, since a higher value would indicate multicolinearity between variables (ter Braak and Verdonschot, 1995). The overall significance of the ordination and the significance of the first axis were tested with a Monte Carlo permutation test ($P < 0.01$) using restricted permutations.Non parametric Spearman correlations (R_S) were used to explore the relationship between each of the euplanktonic frequent species and the categories of water quality, and also between the relative abundance of the entire group of tolerant species and the percentage of coastal urbanization. Only those species that significantly correlated with the categories of water quality ($p < 0.05$) were included in the group of tolerant species.

Table 1
Concentration ranges of physicochemical variables to define water quality on the coast of Río de la Plata according to Gómez et al. (2012).

Assigned value	DO (mg L^{-1})	NID (mg L^{-1})	PO_4^{3-} (mg L^{-1})	BOD_5 (mg L^{-1})
5	> 8.2	< 0.55	< 0.13	< 3
3	8.2 - 5	0.55 – 1.64	0.13 – 0.32	3 - 10
1	< 5	> 1.64	> 0.32	> 10

3. RESULTS

3.1. Environmental characteristics

The physicochemical characteristics of the sampling sites are shown in Table 2. Of all the cases analyzed, 11% had a bad water quality, 50% a moderate water quality and the remaining 39% a good water quality. Sar, SD, Ber Bag and R were among the coastal sites (< 500 m) with the worst water quality, while Bal, Mag, PI, P and PP were the sites with best water quality.

A progressive improvement in the water quality is noticeable on the sites as they are further away from the shore. At 1500m from the shore only two sites present cases with poor water quality, SD2 and PL2, while at 3000m from the shore no cases of bad water quality were observed.

The sites located further south of the study area have a strong influence from the maximum turbidity front (Mag, P, PI and PP) as evidenced by a marked increase in conductivity and turbidity.

Table 2
Mean values (± standard deviation) of physical chemical variables of each sampling site.

	Cond [μS cm^{-1}]	pH	Temp [°C]	Turbidity [NTU]	DO [mg L^{-1}]	BOD_5 [mg L^{-1}]	DIN [mg L^{-1}]	P-PO_4^{3-} [mg L^{-1}]
DL	533 ± 759	7.8 ± 0.8	22 ± 3.2	228 ± 214	7.9 ± 2	2 ± 1	0.98 ± 0.42	0.25 ± 0.3
SI	314 ± 63	7.6 ± 0.3	21.6 ± 4	113 ± 146	7.2 ± 0.7	4 ± 2	1.46 ± 0.25	0.24 ± 0.1
Pal	468 ± 696	7.8 ± 0.5	23.1 ± 3.9	213 ± 175	7.4 ± 2.5	4 ± 2	1.39 ± 0.42	0.13 ± 0.05
Pal2	165 ± 41	7.5 ± 0.3	18.8 ± 5.4	103 ± 96	8.1 ± 1.2	2 ± 2	0.51 ± 0.11	0.08 ± 0.09
Pal3	154 ± 38	7.5 ± 0.2	19.7 ± 5.2	98 ± 129	8.2 ± 0.9	2 ± 1	0.5 ± 0.19	0.06 ± 0.04
R	409 ± 66	7.4 ± 0.3	20.5 ± 5	81 ± 42	5.6 ± 4	8 ± 6	1.47 ± 0.69	0.31 ± 0.35
R2	209 ± 59	7.4 ± 0.3	18.9 ± 5.3	101 ± 92	7.2 ± 1.2	3 ± 2	0.83 ± 0.26	0.1 ± 0.11
R3	163 ± 80	7.7 ± 0.3	18.5 ± 5.3	97 ± 87	8.1 ± 1	2 ± 1	0.64 ± 0.36	0.07 ± 0.05
Sar	660 ± 190	7.7 ± 0.7	20.5 ± 5.6	133 ± 98	5.6 ± 2.4	16	4.1 ± 0.18	0.42 ± 0.18
Sar2	260 ± 72	7.6 ± 0.2	18.8 ± 5.2	112 ± 134	6.5 ± 1.4	4 ± 2	1.22 ± 0.6	0.2 ± 0.19
Sar3	170 ± 46	7.6 ± 0.2	18.4 ± 5.3	123 ± 156	8 ± 0.9	2 ± 1	0.67 ± 0.27	0.09 ± 0.08
SD	862 ± 379	7.6 ± 0.5	23.1 ± 5.6	182 ± 147	4 ± 2.7	13 ± 1	2.17 ± 1.13	1.03 ± 0.88
SD2	284 ± 74	7.4 ± 0.2	19.4 ± 5.8	95 ± 60.4	6.3 ± 1.6	6 ± 4	1.07 ± 0.6	0.22 ± 0.19
SD3	175 ± 60	7.6 ± 0.3	18.6 ± 5.3	118 ± 137	8.1 ± 0.7	2 ± 1	0.59 ± 0.19	0.08 ± 0.07
Ber	562 ± 202	8.1 ± 0.5	23.6 ± 6.4	188 ± 215	7.8 ± 2.8	34 ± 47	1.41 ± 0.98	0.38 ± 0.28
Ber2	249 ± 56	7.5 ± 0.3	18.8 ± 5	126 ± 108	6.4 ± 0.9	5 ± 4	1.16 ± 0.5	0.16 ± 0.13
Ber3	160 ± 40	7.6 ± 0.3	18.3 ± 5	123 ± 155	8.2 ± 0.9	2 ± 1	0.6 ± 0.1	0.1 ± 0.1
Bz	523 ± 191	8.3 ± 0.4	23.7 ± 6.3	163 ± 150	8.7 ± 1.8	9 ± 6	1.86 ± 0.9	0.45 ± 0.29
Bz2	318 ± 94	7.3 ± 0.2	19.5 ± 4.9	88 ± 70	5.8 ± 1.7	6 ± 5	1.4 ± 0.6	0.19 ± 0.12
Bz3	198 ± 71	7.6 ± 0.3	18.8 ± 5.4	100 ± 96	7.5 ± 1.4	6 ± 4	0.87 ± 0.4	0.13 ± 0.09
PC	361 ± 78	7.9 ± 0.7	22.7 ± 7.5	215 ± 37	8.9 ± 1.7	4	1.38 ± 0.6	0.3 ± 0.14
PC2	187 ± 52	7.3 ± 0.4	18.5 ± 5	109 ± 117	6.9 ± 1.1	4 ± 4	0.98 ± 0.5	0.13 ± 0.2
PC3	150 ± 36	7.6 ± 0.3	18.7 ± 5.6	123 ± 159	8.3 ± 0.7	3 ± 3	0.56 ± 0.19	0.1 ± 0.1
BC	342 ± 70	8.4 ± 0.7	24.2 ± 5.6	120 ± 95	9 ± 1.5	5 ± 4	1.16 ± 0.33	0.24 ± 0.06
PL	360 ± 154	8.5 ± 1.1	22.2 ± 7.7	146 ± 110	9.7 ± 2.6	8 ± 6	1.43 ± 0.77	0.26 ± 0.13
PL2	242 ± 42	7.3 ± 0.4	19.2 ± 5.2	83 ± 67	5.3 ± 1.5	8 ± 5	1.42 ± 0.5	0.15 ± 0.1
PL3	186 ± 47	7.5 ± 0.2	18.2 ± 5.2	88 ± 82	7.4 ± 1.3	3 ± 4	0.79 ± 0.53	0.08 ± 0.07
Bor	550 ± 89	8.3 ± 0.4	23.6 ± 1.2	299 ± 118	9.5 ± 0.5	5 ± 1	2 ± 0.49	0.34 ± 0.03
Bag	573 ± 241	7.7 ± 0.3	25.2 ± 7.8	79 ± 67	7.7 ± 1.9	16 ± 9	1.03 ± 0.81	0.65 ± 0.44
Bal	400 ± 139	8.4 ± 0.6	22.3 ± 7.4	193 ± 146	9.7 ± 2.6	8 ± 6	0.47 ± 0.35	0.14 ± 0.06
At	732 ± 452	8.2 ± 0.2	25.3 ± 8	245 ± 328	8.6 ± 0.5	8 ± 4	0.72 ± 0.33	0.12 ± 0.05
Mag	1491 ± 1021	8.4 ± 0.7	22.3 ± 5.4	779 ± 266	8.8 ± 0.9	8 ± 7	0.58 ± 0.49	0.14 ± 0.07
P	1858 ± 1430	8.6 ± 0.5	23.6 ± 5.6	630 ± 252	9.1 ± 0.7	5 ± 5	0.54 ± 0.57	0.14 ± 0.07
PI	5313 ± 4573	8.2 ± 0.5	22.9 ± 5.1	490 ± 366	8.4 ± 1	7 ± 6	0.38 ± 0.3	0.14 ± 0.07
PP	6452 ± 4245	8.4 ± 0.9	21.4 ± 4.2	772 ± 291	8.9 ± 0.7	6 ± 5	0.43 ± 0.33	0.11 ± 0.05

3.2. Composition

A total of 438 taxa were identified, of which 60 were euplanktonic frequent species. Of this selection of species 10% was represented by cyanobacteria, 60% by chlorophytes, 7% by euglenophytes and the remaining 23% by diatoms. (Table 3)

3.3. Phytoplankton and environmental variables

The result of canonical correlation analysis (CCA) conducted to explore the relationship between environmental variables and the density of the different species allowed us to identify two environmental gradients, one related to anthropogenic activity, that ordered those species related to BOD_5, DIN, PO_4^{3-} and DO (increases towards the left upper quadrant), and a second gradient that responds to the natural variability of the estuary, conformed by turbidity and conductivity (increases towards the right upper quadrant). The first gradient was related to species such as *Dictyosphaerium ehrenbergianum, Coelastrum microporum, Tetrastrum glabrum, Crucigeniella rectangularis, Scenedesmus nanus, Schroederia setigera, Kirchneriella obesa, Actinastrum hantzschii, Pandorina morum, Monoraphidium tortile, Monoraphidium minutum, Scendesmus acuminatus, Didimocystis bicelullaris, Eutetramorus fotii* and *Cyclotella striata*. The second gradient was associated to species of diatoms such as *Thalassiosira rudolfii, Skeletonema potamos, Skeletonema subsalsum, Actinocyclus normanii* and *Aulacoseira distans* (Fig. 2)

The correlation between water quality categories (Table 1) and the relative abundances of the selected species demonstrated that a group of 24 species (hereafter referred as tolerant species) obtained a significant correlation with the deterioration of the water quality ($p < 0.05$), showing that as water quality worsens the proportion of these species in the phytoplankton assemblage increases.

The contribution percentage of the group of tolerant species to the phytoplankton assemblage was obtained from the sum of the relative abundances of these 24 species. This group of tolerant species was represented in 79.2% by chlorophytes, especially the chlorococcalean group, with some cyanobacteria and euglenophytes; the principal life form was the coenobium (> 60%), followed by the unicellular and filamentous forms. The dominant life strategy was the C (> 80%), and the most important size, with more than 80% abundace, was the nanoplanktonic fraction (< 20 um) (Fig. 3). In those samples with a bad water quality the species' richness from this subgroup varied between 1 and 19. The group of taxa tolerant to water quality impairment exceeded 30% at those sites with bad water quality (reaching 19000 cell mL^{-1}), and were less than 15% (300 cell mL^{-1}) in sites with good water quality). Considering the distribution of these species according to the different water qualities we observed that *P. agardhii, D. subsolitarium, S. potamos, E. acus, P. morum, E. limnophila, R. mediterranea*, and *C. microporum* reached an average relative abundance of over 70% in poor water qualites (Fig. 4).

A significant positive correlation was also observed between the relative abundance of this species' group and the percentage of coastal urbanization ($p < 0.001$). In this regard it was observed that the highest percentage of tolerant species (> 45%) were located at sites Sar, R and SD, on the shoreline. On the other hand, their percentage diminished with increasing distance from the coast, in correspondence with a better water quality, reaching values of 10% in sites Pal3 and R3, located at 3000 m (Fig. 5)

4. DISCUSSION

Being estuaries areas that are naturally rich in organic matter, the

Table 3
Selected species with their corresponding acronyms, life forms, life strategies and size categories. Between parentheses the amount of species from each group considered in the correlations is shown. Asterisk after the acronyms shows a significant correlation with the deterioration of water quality ($p < 0.05$).

Species	Acronym	Life forms	Life Strategies	Size class
Cyanobacteria (6 species)				
Merismopedia glauca (Ehr.) Kütz.	MGLA	colonial	C	N
M. tenuissima Lemm.	MTEN	colonial	C	P
Microcystis aeruginosa Kütz.	MAER	colonial	S	N
Planktothrix agardhii (Gom.) Anagn. et Kom.	PAGA*	filamentous	R	N
Pseudanabaena catenata Lauterborn	PCAT	filamentous	R	N
Raphidiopsis mediterranea Skuja	RMED*	filamentous	R	N
Chlorophyta (36 species)				
Actinastrum fluviatile (Schröder) Fott	AFLU	coenobium	C	Mic
A. hantzschii Lagerh.	AHAN*	coenobium	C	N
Binuclearia eriensis Tiffany	BERI	filamentous	R	N
Closterium acutum Brébisson	CACU	unicell	C	Mic
C. acutum var. *variabile* (Lemm.) Krieger	CAVA	unicell	R	Mic
C. ceratium Perty	CCER	unicell	R	Mic
C. parvulum Nägeli	CPAR	unicell	R	Mic
Coelastrum microporum Näg.	CMIC*	coenobium	C	N
Crucigenia quadrata Morr.	CQUA*	coenobium	C	N
Crucigeniella rectangularis (Näg.) Gay	CREC*	coenobium	C	N
Dictyosphaerium ehrenbergianum Näg.	DEHR*	coenobium	C	N
D. pulchellum Wood	DPUL*	coenobium	C	N
D. subsolitarium Van Goor	DSUB*	coenobium	C	P
Didymocystis bicellularis (Chod.) Kom.	DBIC*	coenobium	C	N
Eutetramorus fotti (Hind.) Kom.	EFOT*	coenobium	C	N
Kirchneriella contorta (Schmidle) Bohl.	KCON	unicell	C	N
K. obesa (W. West) Schmidle	KOBE*	unicell	C	N
Monoraphidium arcuatum (Korš.) Hind.	MARC	unicell	C	Mic
M. contortum (Thuret) Kom.-Legn.	MCON	unicell	C	N
M. griffithii (Berk.) Kom.-Legn.	MGRI*	unicell	C	Mic
M. komarkovae Nyg.	MKOM	unicell	C	Mic
M. minutum (Näg.) Kom.-Legn.	MMIN*	unicell	C	N
M. tortile (W. & G. S. West) Kom.-Legn.	MTOR	unicell	C	N
Oocystis borgei Snow	OBOR*	coenobium	C	N
Pandorina morum (Mull.) Bory	PMOR*	coenobium	C	N
Planctonema lauterbornii Schmidle	PLAU	filamentous	R	N
Scenedesmus acuminatus (Lagerh.) Chod.	SACU*	coenobium	C	Mic
S. acutus Meyen	SCAC	coenobium	C	N
S. ecornis (Ehr.) Chod.	SECO	coenobium	C	N
S. intermedius Chod.	SINT*	coenobium	C	N
S. intermedius var. *acaudatus* Hortobagyi	SIAC	coenobium	C	N
S. nanus Chodat	SNAN*	coenobium	C	N
S. opoliensis P. Richt.	SOPO	coenobium	C	N
S. quadricauda (Turp.) Bréb. sensu Chod.	SQUA*	coenobium	C	N
Schroederia setigera (Schröder) Lemm.	SSET	unicell	C	Mic
Tetrastrum glabrum (Roll) Ahlstr. & Tiff.	TGLA*	coenobium	C	N
Euglenophyta (4 species)				
Euglena acus Ehr.	EACU*	unicell	R	Mes
E. limnophila Lemm.	ELIM*	unicell	R	Mic
E. viridis (Müll.) Ehr.	EVIR	unicell	R	Mic
Trachelomonas volvocina (Ehr.) Ehr.	TVOL	unicell	R	N
Bacillariophyta (14 species)				
Actinocyclus normanii (Greg. ex Grev.) Hust.	ANOR	unicell	R	Mic
Aulacoseira islandica (Müll.) Simonsen	AUIS	filamentous	R	N
A. distans (Ehr.) Sim.	AUDI	filamentous	R	N
A. granulata (Ehr.) Sim.	AGRA	filamentous	R	N
A. granulata var. *angustissima* (Müll.) Sim.	AGAN	filamentous	S	N
Cyclotella atomus Hustedt	CATO	unicell	S	N
C. atomus var *gracilis* Genkal et Kiss	CAGR	unicell	S	N
C. meneghiniana Kütz.	CMEN	unicell	R	Mic
C. striata (Kütz.) Grunow	CSTR	unicell	R	Mic
Nitzschia acicularis (Kütz.) Smith	NACI	unicell	R	Mic
N. gracilis Hantzsch	NGRA	unicell	S	Mic
Skeletonema potamos (Weber) Hasle	SKPO*	filamentous	R	N
S. subsalsum. (Cleve-Euler) Bethge	SKSU	filamentous	R	N
Thalassiosira rudolfii (Bachmann) Hasle	TRUD	colonial	R	N

biota often show similarities to areas receiving organic matter from anthropogenic activity (Borja et al., 2012), this feature supports the concept of “estuarine quality paradox” (Dauvin, 2007; Elliott and Quintino, 2007) and makes difficult to isolate the stress inflicted by human activities on the estuary.

In the Río de la Plata estuary, a NW-SE gradient is recognized, where salinity, pH and dissolved oxygen increase downstream, that is inherent to the estuary dynamic. A second gradient is also recognizable heading in the opposite direction, where organic matter content and nutrient concentrations increase due to a greater anthropogenic activity (Gómez et al. 2004, FREPLATA 2005). Related to the first gradient, the distribution of the phytoplankton fits the two ecocline

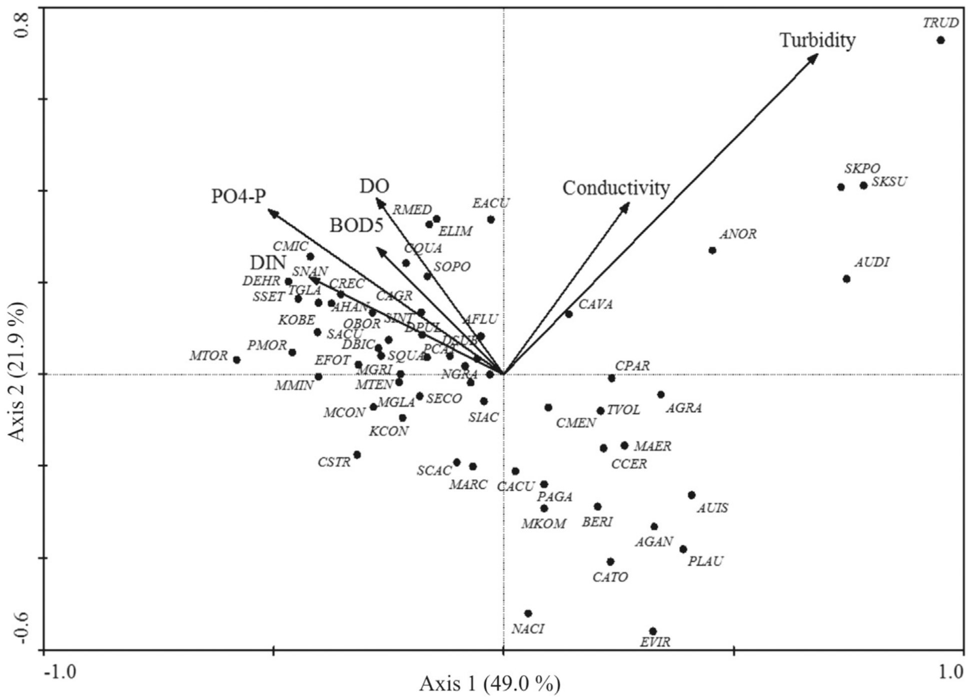

Fig. 2. Biplot of axis 1 (49% of the variance) and axis 2 (21.9% of the variance) of the CCA, showing the physical chemical variables and the species. For abbreviations of the species' names, see Table 3.

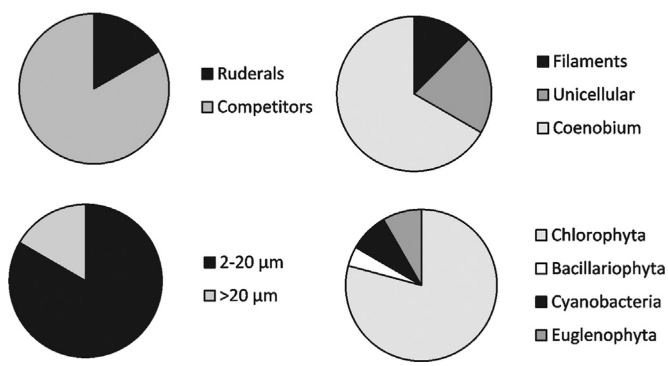

Fig. 3. Distribution of different ecological traits of the tolerant species (Taxonomical groups, Life forms, Life strategies and Size).

model proposed by Attrill and Rundle (2002), one in the direction of the freshwater-mixohaline zone and the other in the direction of the marine-mixohaline zone (Gómez et al., 2004).

In this study we focused on the freshwater zone of the estuary, where we observed an overlap on the distribution of species that responds to natural factors and anthropogenic stress, and noticed that with the deterioration of water quality, as a result of increased anthropogenic pressure and the input of organic matter and nutrients, some phytoplanktonic species increased their proportion in the assemblage. We recognized that 24 species, common in the coastal phytoplankton of the freshwater zone of the estuary (Gómez and Bauer, 1997; Gómez and Bauer, 1998; Gómez et al., 2002; Gómez et al., 2004; Gómez, 2014; Sathicq et al., 2014), were able to thrive under such conditions. The percentage of this group of tolerant species allowed us to have an indicator capable of detecting the impairment of the water quality in different sites of the study area. Percentages > 30% were linked to coastal sites with large amounts of organic matter, mainly located between the sampling sites R and Ber. This area is influenced by the sewage discharge of the city of Buenos Aires, pouring into the estuary 18.2 $m^3\ s^{-1}$ of poorly treated sewage with an average BOD of 7740 kg h^{-1}. Also in this area some highly polluted rivers - Matanza-Riachuelo river (R), Sarandí (Sar) and Santo Domingo (SD) channels- discharge more than 9000 kg h^{-1} BOD. A similar pattern is seen in sites Bor and Bag, which receive the sewage discharge from La Plata city, with a BOD greater than 300 kg h^{-1} (Menéndez et al., 2011). On the other hand, in the sites further offshore and with better water quality (evidenced by the decrease in the concentrations of nutrients and BOD), a diminished percentage of these species was observed.

This set of tolerant species is mostly composed of taxa considered C-strategists growing under favorable resource-replete conditions, rapid in the conversion of resources to biomass and a high frequency of cell division and recruitment of subsequent generations (Reynolds, 2006). Taxonomically, the most represented group in this selection is the Chlorococcalean algae, recognized in the literature as a common element in eutrophic systems (eg: Brook, 1965; Padisák and Reynolds, 1998), with high metabolic rates, low sink rates and shortest generational time than other groups (Litchman et al., 2007). Also, Chlorococcalean of small size are considered efficient carbon fixers in open and turbulent waters and good competitors for resources when coexisting with diatoms (Happey-Wood, 1988). The prevalence of this group of chlorophytes (mostly small coenobia forms) in our study area led to changes in the structure and strategies of phytoplankton, displacing the diatoms forming chains (eg. *Aulacoseira* sp.) and filamentous chlorophytes (eg. *Ulothrix* sp. and *Binuclearia eriensis*) reported in previous works as a characteristic phytoplanktonic assemblage in the freshwater area of the estuary (Gómez et al., 2004; Calliari et al., 2005; Gómez, 2014). It is also frequent the development of *Planktothrix agardhii* under poor water quality, a potentially toxic cyanobacterium with a great physiological and morphological flexibility, abundant in rich nutrient and turbid mixed layers (Chorus and Bartram, 1999).

Further knowledge of the competitive advantages of the species included in the tolerant group can allow their use as a water quality indicator. The appropriate conditions for their development imply their rapid proliferation, causing issues in the water intakes and in the drinking water production due to the presence of potentially toxic species as *P. agardhii*, or favoring the eutrophication process with hazardous consequences in the coastal zone. However, no complete replacement of species, according to the different water qualities, was

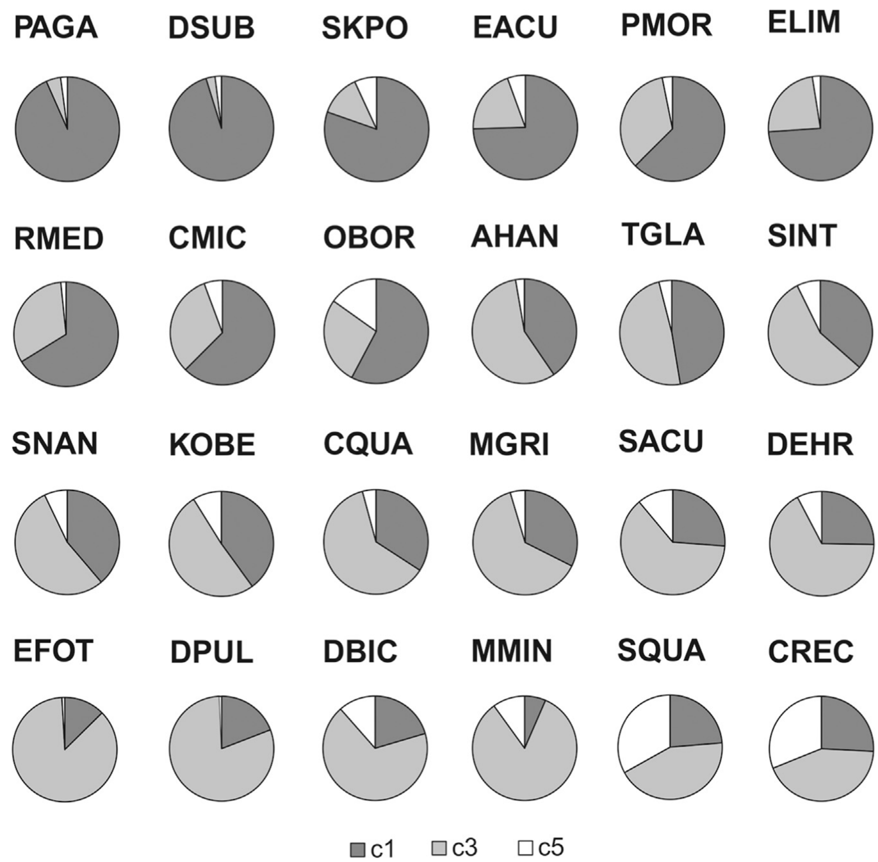

Fig. 4. Relative abundance of the species considered as tolerant according to the correlation analysis with water quality categories (C5: good water quality, C3: moderate water quality, C1: bad water quality).

observed probably due to the inherent natural variability of the Río de la Plata. Also, the adaptive features of this group of species confer an indicator that does not get masked by the ENSO phenomenon in the ecosystem, since there is no significant turnover of species between periods or exclusive assemblages for the different cycles (neutral, La Niña, El Niño), and the species in the tolerant group are present under both conditions of the phenomenon (Sathicq et al., 2015).

The species' composition found in the freshwater tidal zone of the Río de la Plata was similar to the assemblages observed in the lower basin of the Uruguay and Paraná rivers, and in general in the phytoplankton typical of large rivers. (O'Farrell, 1994; Reynolds and Descy, 1996; O'Farrell et al., 1998). Taking into account that most of these tolerant species are also widely distributed it is possible to employ them as biomonitors in another freshwater zones of temperate estuaries.

An important part of determining the ecological integrity in an ecosystem is the measurement of the biological integrity, while counting and identifying the phytoplanktonic species is a time consuming task that requires a specialist, the information that provides is valuable and of common use in multiple water quality indices (Prygiel et al., 1999). The advantage of the tool presented in this article is the use of a reduced group of well known, easily identifiable species. Knowing the proportion of only a few species it is possible to contribute to the characterization of water quality related to the enrichment with nutrients and organic matter, adding information to other common indicators such as chlorophyll *a* or phytoplankton total density.

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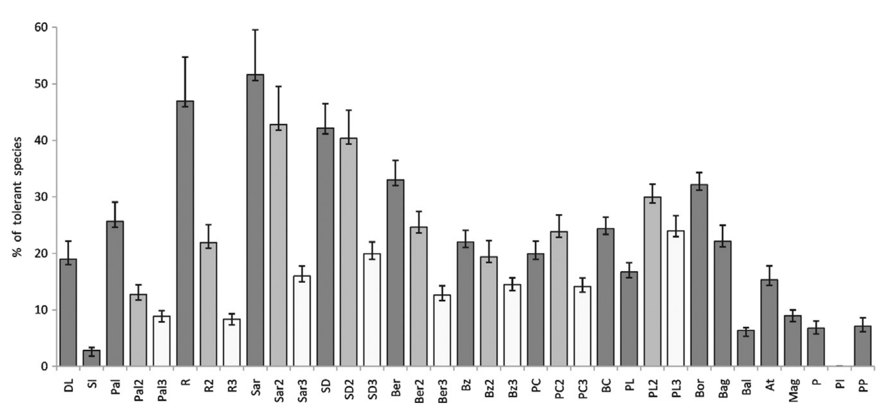

Fig. 5. Relative abundance (average and standard deviation) of the group of species considered tolerant, in all the sampling sites. Dark grey bars are for shoreline sites, medium grey are for sites at 1500m and light grey are for sites at 3000m.

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