

# Biofilms coating *Schoenoplectus californicus* as indicators of water quality in the Río de la Plata Estuary (Argentina)

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**Abstract** Community composition, structure, biomass and tolerance to pollution of biofilms growing on *Schoenoplectus californicus* were assessed, together with physico-chemical parameters of the water, to evaluate their usefulness as water quality indicators along the Argentinian shore of the freshwater zone of the Río de la Plata Estuary. Monthly sampling was carried out at three sites, one of which, located in a nature reserve, was considered as the reference site while the others were affected by sewage outlets and port activity. The reference site had higher dissolved oxygen and turbidity values, the polluted sites greater conductivity, oxygen demands, phosphate, ammonium and nitrite values. Diatoms dominated the biofilms at the reference site; ciliates reached higher densities at the polluted sites. Significant differences were found in species numbers, species diversity index, biomass, tolerance to pollution of the taxa, and the Pampean Diatom Index, between the reference and the impacted sites. This study shows that the

biofilms growing on *Schoenoplectus californicus* are good indicators of the changes in the water quality of their environment.

**Keywords** Biofilms · Río de la Plata Estuary · *Schoenoplectus californicus* · Water quality indicator

## Introduction

Physical, chemical and bacteriological variables commonly form the basis of water quality monitoring because of their ability to detect changes in the environment in a rapid and straightforward manner. These traditional measurements, however, provide no information as to the effects of such changes on the biological communities, which are ultimately affected by altered environmental conditions (Vis et al. 1998). Several studies have demonstrated that many periphyton characteristics are related to water quality (Ács et al. 2003, 2004, 2006; Cattaneo 1987; Ector and Rimet 2005; Kiss et al. 2002; Lowe and Pan 1996; McCormick and Stevenson 1998; Szabó et al. 2004, 2005) but the use of epiphytic communities presents a number of methodological problems especially when the community is used for monitoring purposes. However, when the substratum is largely covered by hydrophytes the epiphytic organisms on their surfaces are often important and should be considered, therefore they can be used to assess water quality (Cazaubon 1996).

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Because of their hydrodynamics and their extensive riparian human settlements, estuaries are particularly susceptible to anthropogenic impacts and their integrity is currently at risk worldwide (Kiddon et al. 2003). The implementation of proper management strategies is important from these ecosystems.

The Río de la Plata Estuary has an extensive basin, with a surface area of 3,170,000 km<sup>2</sup>, which drains to the Atlantic Ocean. This is a microtidal estuary located on the East Coast of South America between 34°09'–36° 20' S and 55°09'–58° 30' W. It is 320 km long and its width varies from 38 km in the upper zone to 230 km at the mouth (Urien 1972). The annual mean freshwater discharge is 22,000 m<sup>3</sup> s<sup>-1</sup>. The tidal regime is predominantly semidiurnal (two high and two low tides per day) and its amplitude ranges from 30 to 100 cm (Guerrero et al. 1997). Agriculture, cattle rearing, industrial and port activities, and the urban areas impact on the estuary affecting the water quality.

*Schoenoplectus californicus* (C. A. Meyer) Soják (Cyperaceae), commonly called bulrush, is tolerant to a wide range of salinities (0.2–25‰) and is widely distributed along the shore of the Río de la Plata. Its density has been recorded as 136–175 stems m<sup>-2</sup> (Tur and Rossi 1976). According to Gómez et al. (2003) irregularities which occur on the stem surfaces of *S. californicus* favour the establishment and development of the biofilm. The studies on communities coating the stems of bulrush in this estuary have explored their taxonomic composition, spatial patterns of abundance, diversity, life-form strategies and their trophic and saprobic preferences (Claps 1981, 1984, 1987; Gómez et al. 2002, 2003). However, their potential use as indicators of the water quality has not previously been investigated.

The aim of this study was to assess the usefulness of the biofilms coating *S. californicus* as indicators of the water quality along the Argentinian shore of the freshwater tidal zone of the Río de la Plata. For this purpose the taxonomic composition, density, biomass, diversity and tolerance of the taxa to the organic pollution and eutrophication were investigated and the relationships of the taxa to the environmental variables have been explored.

The approach used in this study could provide a valuable baseline for future water quality assessment efforts on temperate estuaries.

## Material and methods

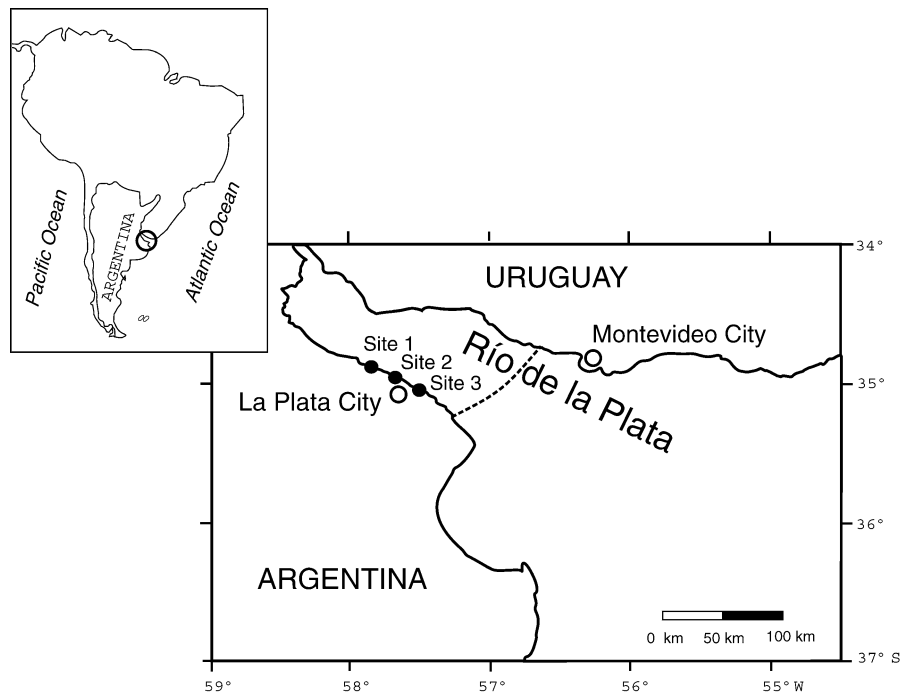
Three sampling sites (Fig. 1) were established in the freshwater tidal zone of the estuary where the salinity is below 0.5 psu (Gómez et al. 2004). This part of the estuary is exposed to severe anthropogenic impacts therefore it was not possible to find a reference site with good water quality. The reference site in this study, site 1, is subject to less anthropogenic impact than the other two sites and has an acceptable water quality; it is located in the nature reserve “Selva Marginal de Punta Lara.” Site 2 is 32 km downstream from site 1 and is impacted by two sources of pollution, the port activity and the domestic effluents of Berisso City. Moreover, due to the coastal morphology, this site is located at an area with lower hydrodynamic energy than the others. Site 3 is 25 km downstream from site 2 and is impacted by the sewage outlet of La Plata City.

Biofilm samples were taken monthly between December 2002 and October 2003. Fifteen stems of bulrush (average diameter 1.1±0.33 cm) were cut randomly, the bottom 20 cm were kept and stored in flasks, with distilled water in the bottom to create a humid atmosphere, until arrival at the laboratory. Five stems were used for the estimation of ash free dry weight (AFDW) and ten for taxonomic identifications and counts of the epiphytic organisms. Conductivity (Lutron 4303-CD), dissolved oxygen (Oxymeter 600-ESD), turbidity (Turbidity meter 800-ESD), temperature and pH (Hanna HI 8633) were measured *in situ* with portable meters. Water samples were collected to be analysed for NH<sub>4</sub><sup>+</sup> – N, NO<sub>3</sub><sup>-</sup> – N, NO<sub>2</sub><sup>-</sup> – N, PO<sub>4</sub><sup>3-</sup> – P, BOD<sub>5</sub> and COD (Mackereth et al. 1978) and were stored at 4°C until arrival at the laboratory.

In the laboratory the biofilm was removed by brushing. The diameters and lengths of the stems were measured to calculate the total colonised areas. Subsamples for counts were preserved with formalin (final concentration 4%) and the microorganisms counts were made using a Sedgwick–Rafter chamber and expressed per cm<sup>2</sup> (Clesceri et al. 1998, pp. 10–33).

Subsamples used for diatom identifications were cleaned with H<sub>2</sub>O<sub>2</sub>, washed thoroughly using distilled water, mounted on microscope slides with Naphrax® and then examined with an Olympus BX 50 microscope with phase contrast and Normarski DIC optics. The references used for taxonomic identification of the diatoms were: Frenguelli (1941), Krammer and

**Fig. 1** Map showing the location of the Río de la Plata Estuary (circle) in South America and the Estuary with the sampling sites along the coast. The dashed line shows the isohaline of 0.5 psu



Lange-Bertalot (1986, 1988, 1991a, 1991b) and Patrick and Reimer (1966, 1975). The old nomenclature was updated according to Kusber and Jahn (2003).

The biomass of the biofilm was calculated as ash-free dry weight (AFDW) according to Clesceri et al. (1998), pp. 10–34. Species diversity was calculated using the Shannon & Wiener index according to Shannon and Weaver (1949) index; saprobic and trophic species preferences were established according to Sládeček (1973); Lange-Bertalot (1979), Lowe (1974), Gómez (1998), Gómez and Licursi (2001) and Licursi and Gómez (2002). A total of 300 diatom valves were counted in each sample to calculate the Pampean Diatom Index (IDP) for the assessment of organic pollution and eutrophication status (Gómez and Licursi 2001).

Canonical correspondence analysis (CCA) was used to explore the relationships between taxa composition and environmental variables. This weighted averaging technique was applied because unimodal species response curves could be expected according to the results of a preliminary detrended correspondence analysis (ter Braak and Verdonschot 1995). Taxa with abundances greater than 1% in at least one sample and present in three or more samples were included in this analysis. Taxa data were  $\log(x+1)$  transformed before analysis to obtain a normal distribution. Environmental data that were not normally

distributed were normalised. Only environmental variables with a variance inflation factor <10 were retained in the analysis, because a greater value would indicate multicollinearity among variables (ter Braak and Verdonschot 1995). A Monte Carlo permutation test was performed to check the axis significance.

To determine statistically significant differences between sites a one way analysis of variance was employed (Zar 1996). When the data did not meet the normality and homo-scedasticity assumptions they were analysed by Kruskal-Wallis one way ANOVA on ranks (turbidity, pH, COD, BOD<sub>5</sub>, PO<sub>4</sub><sup>3-</sup> - P, NH<sub>4</sub><sup>+</sup> - N, NO<sub>3</sub><sup>-</sup> - N, H', density of major taxonomic groups, density of sensitive, less tolerant and more tolerant taxa). A multiple comparison procedure, the Student–Newman–Keuls method (for all pairwise multiple comparisons), was used to isolate the site, or sites, that differ from the others with  $p < 0.05$ .

## Results

### Physico-chemical characteristics

The main physico-chemical characteristics of the sampling sites are shown in Table 1. Site 1, with low anthropogenic impact, was characterized by the highest

**Table 1** Geographic locations of the three sampling sites on the Río de la Plata Estuary and average values of physico-chemical variables (SD)

|   | Site 1      | Site 2      | Site 3        |
|---|-------------|-------------|---------------|
| Latitude S  | 34°45'39"   | 34°52'05"   | 34°52'29"     |
| Longitude W   | 58°01'49"   | 57°51'35"   | 57°49'19"     |
| Temperature (°C)  | 20.7 (6.8)  | 20.2 (5.7)  | 21.0 (5.6)    |
| Dissolved oxygen (mg l <sup>-1</sup> )                  | 7.4 (0.9)   | 1.4 (0.9)   | 4.9 (1.7)     |
| Conductivity (μS cm <sup>-1</sup> )                     | 415 (99)    | 905 (158)   | 676 (77)      |
| Turbidity (NTU)   | 50.4 (21.9) | 10.0 (3.9)  | 41.0 (22.2)   |
| PH  | 7.4 (0.6)   | 7.1 (0.3)   | 7.4 (0.5)     |
| COD (mg l <sup>-1</sup> )                               | 47.6 (33.8) | 56.1 (67.6) | 120.7 (165.8) |
| BOD <sub>5</sub> (mg l <sup>-1</sup> )                  | 10.1 (17.6) | 10.2 (8.7)  | 33.8 (32.6)   |
| PO <sub>4</sub> <sup>3-</sup> - P (mg l <sup>-1</sup> ) | 0.35 (0.14) | 0.98 (0.52) | 0.76 (0.29)   |
| NH <sub>4</sub> <sup>+</sup> - N (mg l <sup>-1</sup> )  | 0.19 (0.21) | 1.74 (1.14) | 1.70 (1.12)   |
| NO <sub>2</sub> <sup>-</sup> - N (mg l <sup>-1</sup> )  | 0.04 (0.04) | 0.05 (0.03) | 0.11 (0.04)   |
| NO <sub>3</sub> <sup>-</sup> - N (mg l <sup>-1</sup> )  | 1.49 (1.97) | 1.14 (1.26) | 2.14 (3.72)   |

(For details of the sites see text).

values of dissolved oxygen and the lowest values of PO<sub>4</sub><sup>3-</sup> - P and NH<sub>4</sub><sup>+</sup> - N. Site 2, impacted by port activity and domestic effluents, presented the highest values of conductivity, NH<sub>4</sub><sup>+</sup> - N and PO<sub>4</sub><sup>3-</sup> - P and the lowest values of dissolved oxygen and turbidity. High conductivities at site 2 are due to the pollution, not to the influence of marine water, as recorded further downstream. The highest values of BOD<sub>5</sub>, COD, NO<sub>2</sub><sup>-</sup> - N and NO<sub>3</sub><sup>-</sup> - N, and high values of PO<sub>4</sub><sup>3-</sup> - P and NH<sub>4</sub><sup>+</sup> - N were recorded at site 3, which is exposed to the sewage outlet of La Plata City. The results of the ANOVA and post hoc multiple comparisons are shown in Table 2.

### Community structure

A total of 158 taxa were identified in the 33 samples collected. Significant differences in taxa richness were found between the reference site and the other two

sites, but not between sites 2 and 3. The maximum numbers of species were recorded at the reference site and the minima at sites 2 and 3 (Fig. 2a).

Species diversity varied between 1.79 and the extreme value 4.03 bits ind<sup>-1</sup> at the reference site, between 0.05 and the extreme value 2.64 bits ind<sup>-1</sup> at site 2, and between 0.12 and the extreme value 2.60 bits ind<sup>-1</sup> at site 3. Significant differences in the mean values of species diversity were found between the reference site and the polluted sites, but not between the latter (Fig. 2b).

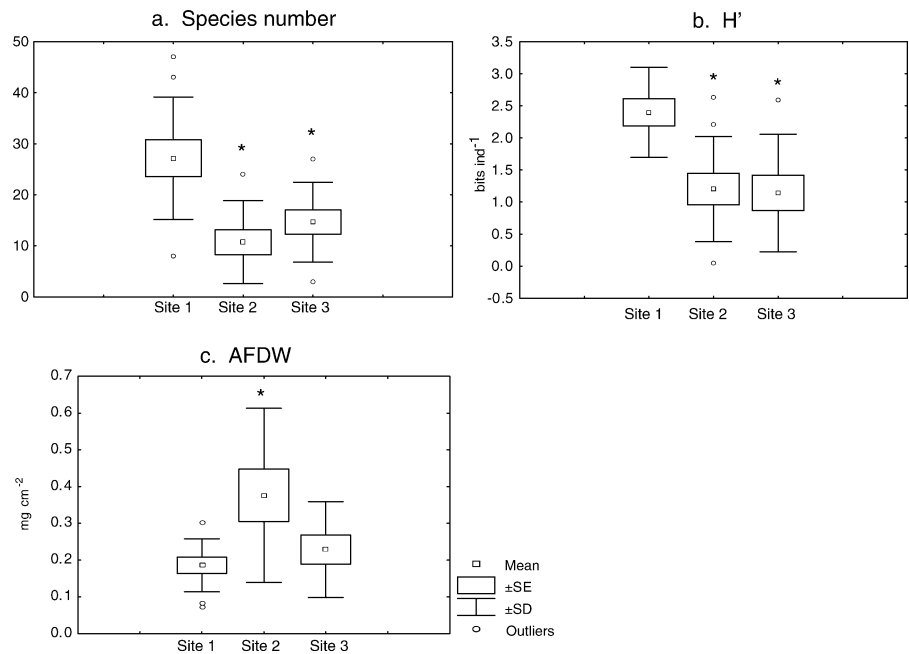
AFDW values ranged between 0.07 and 0.30 mg cm<sup>-2</sup> at site 1, between extreme values of 0.10 and 0.95 mg cm<sup>-2</sup> at site 2, and between 0.08 and the extreme value 0.54 mg cm<sup>-2</sup> at site 3. Mean values of AFDW were higher at the polluted sites, and significant differences were found between the reference site and site 2 and between sites 2 and 3, but not between sites 1 and 3 (Fig. 2c).

**Table 2** Results of the one way analysis of variance and all pairwise multiple comparisons for environmental variables at three sites on the Río de la Plata Estuary (for details of the sites see text)

|                                   | P      | Post hoc multiple comparisons |            |            |
|-----------------------------------|--------|-------------------------------|------------|------------|
|                                   |        | S 1 vs S 2                    | S 1 vs S 3 | S 2 vs S 3 |
| Temperature                       | n. s.  | —                             | —          | —          |
| Dissolved oxygen                  | <0.001 | *                             | *          | *          |
| Conductivity                      | <0.001 | *                             | *          | *          |
| Turbidity                         | <0.001 | *                             | —          | *          |
| PH                                | n. s.  | —                             | —          | —          |
| COD                               | n. s.  | —                             | —          | —          |
| BOD <sub>5</sub>                  | <0.05  | —                             | *          | —          |
| PO <sub>4</sub> <sup>3-</sup> - P | <0.001 | *                             | *          | —          |
| NH <sub>4</sub> <sup>+</sup> - N  | <0.001 | *                             | *          | —          |
| NO <sub>2</sub> <sup>-</sup> - N  | <0.001 | —                             | *          | *          |
| NO <sub>3</sub> <sup>-</sup> - N  | n. s.  | —                             | —          | —          |

\*indicates significant differences with  $p < 0.05$ .

**Fig. 2** Species number, Shannon diversity index ( $H'$ ) and ash free dry weight (AFDW) of the analysed biofilms. \* indicates significant differences ( $p < 0.05$ ) between the marked site and the reference site (site1)



The greatest densities of autotrophs were recorded at the reference site (maximum: 4,300 cell  $\text{cm}^{-2}$ ). Diatoms and chlorophytes were the dominant organisms in the biofilm at this site, and statistically significant differences were found between diatom densities recorded at this site and those recorded at the two polluted sites, but not between the latter (Fig. 3a, b). Cyanophytes were more abundant at site 2, where a greater density of euglenoids was also observed (Fig. 3c, d). Diatoms dominated the autotrophic assemblage at site 3.

Regarding the heterotrophs, their highest density values were recorded at the polluted sites and they reached a maximum of 7,600 org  $\text{cm}^{-2}$  at site 3. The most important group at sites 2 and 3 was the ciliates (Fig. 3e). Rotifers were more abundant at site 1, but always in very low densities (Fig. 3f). Nematodes reached their highest densities at site 2 (Fig. 3g).

Community composition and environmental variables

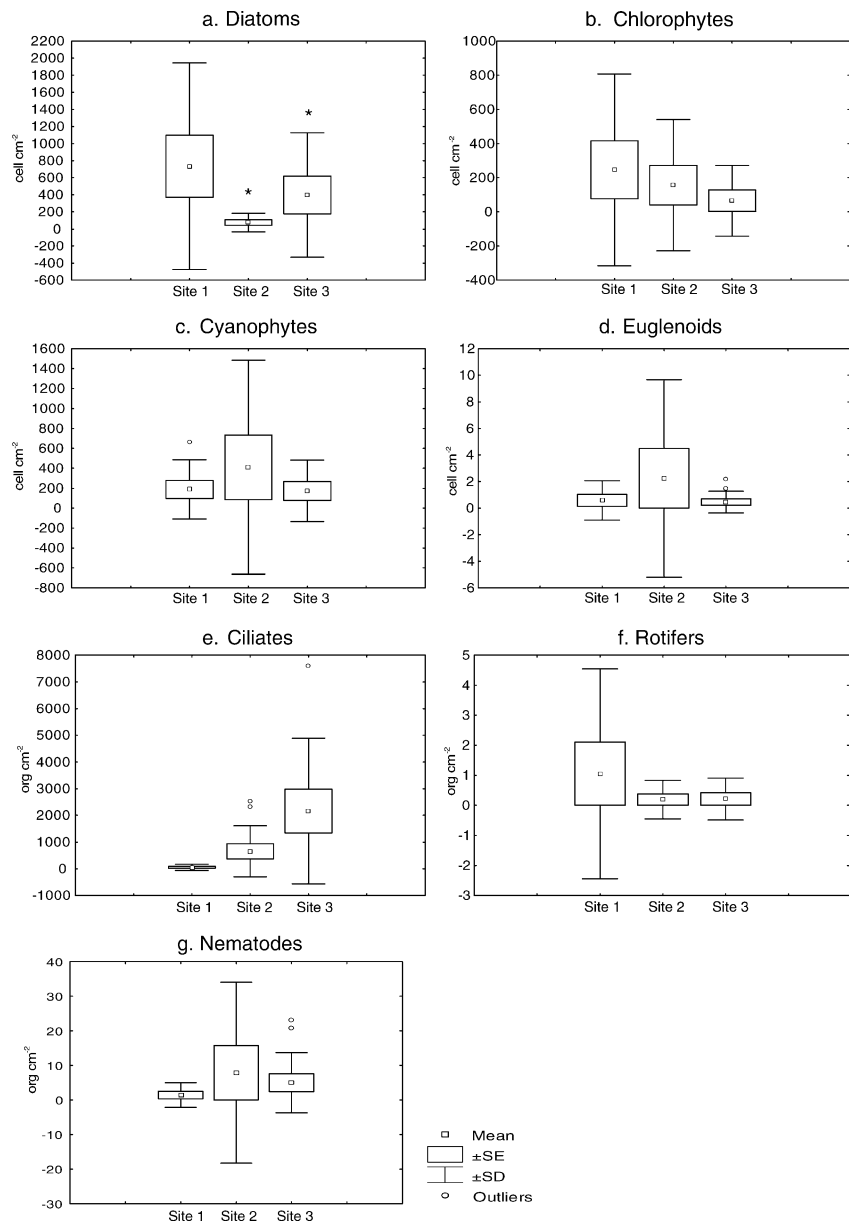
Canonical correspondence analysis (CCA) and Monte Carlo permutation tests showed that physico-chemical variables explained a statistically significant amount of the variation in the composition of the biofilms. The first two axes explained 48.5% of the sum of all canonical eigen values and were selected for graphical

representation (Fig. 4). The 55 taxa selected (Table 3) could be separated into two groups along the first axis ( $p = 0.015$ ). One group (right side of the figure) was related to the highest values of turbidity (average  $50 \pm 22$  NTU), dissolved oxygen (average  $7.5 \pm 1$  mg  $\text{l}^{-1}$ ) and pH (average  $7.4 \pm 0.5$ ). This group included mainly species characteristic of the reference site, among them sensitive species such as *Encyonema silesiacum*, *Navicula erifuga*, *N. rhynchocephala*, *Neidium dubium*, *Nitzschia fonticola*, *N. nana*, *Placoneis clementis* and *Pleurosira laevis* and less tolerant species such as *Gomphonema augur*, *Luticola ventricosa* and *Nitzschia brevissima*. The other group of taxa (left side of the figure), was related to high values of conductivity (average  $740 \pm 200$   $\mu\text{S cm}^{-1}$ ),  $\text{BOD}_5$  (average  $22 \pm 26$  mg  $\text{l}^{-1}$ ), COD (average  $87 \pm 120$  mg  $\text{l}^{-1}$ ), and  $\text{NH}_4\text{-N}$  (average  $1.7 \pm 1.1$  mg  $\text{l}^{-1}$ ),  $\text{NO}_2^- \text{-N}$  (average  $0.08 \pm 0.04$ ) and  $\text{PO}_4^{3-} \text{-P}$  (average  $0.87 \pm 0.42$  mg  $\text{l}^{-1}$ ) concentrations. This group included mainly the taxa characteristic of polluted sites such as the more tolerant *Nitzschia palea*, *Epistylis plicatilis*, *Pseudoglaucoma* sp. and *Chroococcus* sp. among others.

Species tolerance of pollution

Exploring the tolerance to pollution of the taxa, we observed that sensitive and less tolerant taxa were

**Fig. 3** Density of major taxonomic groups estimated for the biofilms analysed at the three sampling sites. \* indicates significant differences ( $p < 0.05$ ) between the marked site and the reference site (site1)



better represented at site 1 than in the other sites; while more tolerant taxa were dominant at site 3 (Fig. 5a, b, c). Significant differences were found between the reference site and the polluted sites for sensitive taxa and for more tolerant taxa, but not between the latter.

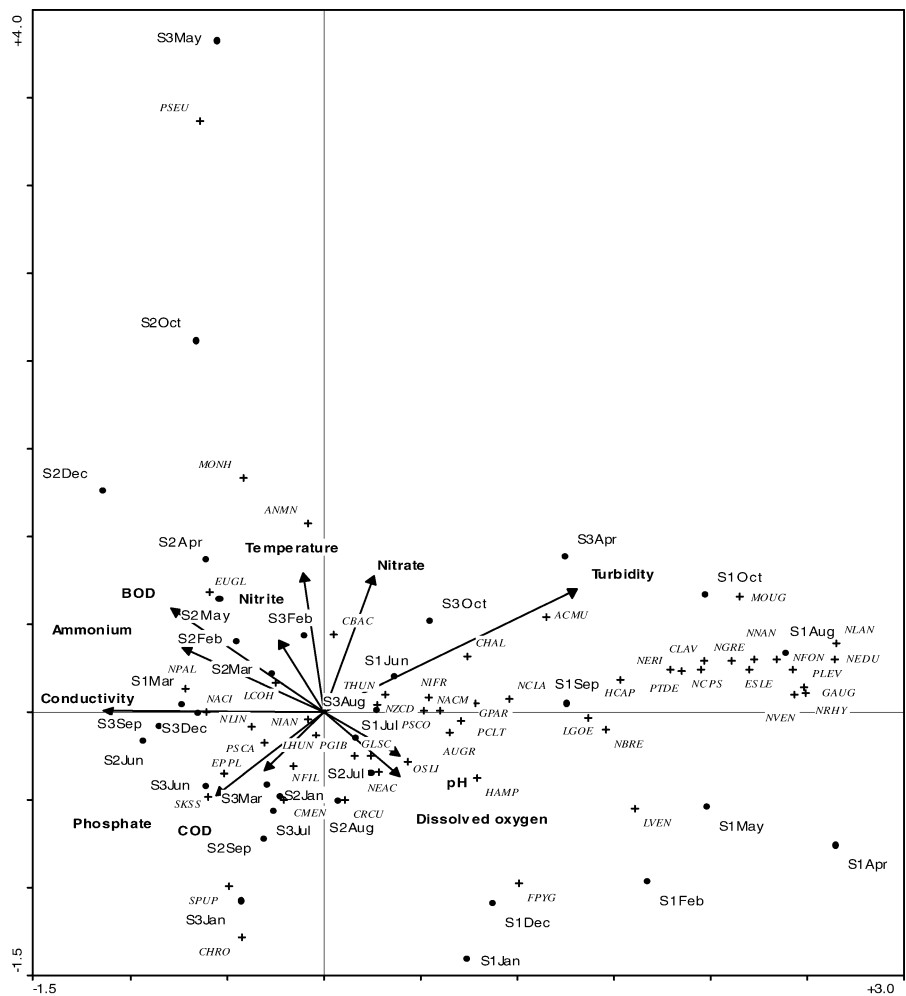
The mean Pampean Diatom Index (IDP) value at the reference site was within the range  $>1.5$ – $2$ , values of IDP corresponding to an acceptable water quality. The means at sites 2 and 3 were within the range  $>2$ – $3$ , values of IDP corresponding to bad water quality.

The mean IDP value was significantly different between the reference site and sites 2 and 3, but not between the latter (Fig. 5d).

## Discussion

The taxa composition of the biofilm was clearly related to the changes in water quality in the freshwater tidal zone of the Río de la Plata Estuary. The canonical correspondence analysis allowed us to

**Fig. 4** Ordination diagram displaying the first two axes of the CCA constrained with environmental variables. For acronyms of the taxa, see Table 3. Sampling sites are represented by the letter *S* and the corresponding number followed by the first three letters of the month



distinguish a group of taxa related to poor water quality, characteristic of the polluted sites, where the more tolerant taxa were best represented. Another group of taxa was related to a better water quality recorded at the reference site, with a predominance of less tolerant and sensitive species.

Density of algae found in the biofilms of the reference site was higher than that found at the polluted sites. The decrease of algal density in sites exposed to sewage outlets is in agreement with the observations of Villanueva et al. (2000) and Biggs (1989) who stated that the input of organic matter causes a reduction of algal biomass despite the increase of phosphorus. Villanueva et al. (2000) pointed out that the shading effect due to the deposition of organic matter limits the development of periphytic algae.

No seasonal patterns were observed in those descriptors of the biofilms analysed, except for the

dominance of major algal taxonomic groups at the reference site. There, diatoms were the dominant group, except in summer, when chlorophytes dominated. The same seasonal pattern was reported by Gómez et al. (2002, 2003) in sites along the Río de la Plata with good water quality. In this study the density of cyanophytes increased at the human impacted site 2. Similarly, Vis et al. (1998), working on the St Lawrence River, noted an increase in relative abundance of filamentous cyanophytes with an increasing amount of urban wastewater. Giorgi and Malacalza (2002) reported, in a tributary of the Río de la Plata, a decrease in the number of diatoms and a greater abundance of cyanophytes and euglenoids downstream of an industrial discharge which showed a positive correlation with phosphate and organic matter content and a negative correlation with oxygen concentration. Nevertheless, according to our results, a qualitative change rather than a quantitative one occurred at the

**Table 3** List of the taxa selected for the CCA (preceded by the acronyms) from those recorded at three sites on the Río de la Plata Estuary

| Acronym         | Taxon   | Site 1 | Site 2 | Site 3 |
|-----------------|---|--------|--------|--------|
| Bacillariophyta |   |        |        |        |
| ANMN            | <i>Actinocyclus normanii</i> (Gregory ex Greville) Hustedt                  | *      |        | *      |
| AUGR            | <i>Aulacoseira granulata</i> (Ehrenberg) Simon                              | *      | *      |        |
| CBAC            | <i>Caloneis bacillum</i> (Grunow) Cleve                                     | *      |        | *      |
| CRCU            | <i>Craticula cuspidata</i> (Kützing) Mann                                   |        | *      | *      |
| CHAL            | <i>C. halophila</i> (Grunow ex Van Heurck) Mann                             | *      | *      | *      |
| CMEN            | <i>Cyclotella meneghiniana</i> Kützing                                      |        | *      | *      |
| ESLE            | <i>Encyonema silesiacum</i> (Bleisch in Rabh.) D.G. Mann                    | *      |        | *      |
| FPYG            | <i>Fallacia pygmaea</i> (Kützing) Stickle & Mann                            | *      | *      | *      |
| GAUG            | <i>Gomphonema augur</i> Ehrenberg   | *      | *      | *      |
| GPAR            | <i>G. parvulum</i> Kützing  | *      | *      | *      |
| HAMP            | <i>Hantzschia amphioxys</i> (Ehrenberg) Grunow                              | *      |        | *      |
| HCAP            | <i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot Metzeltin & Witkowski | *      |        | *      |
| LHUN            | <i>Lemnicola hungarica</i> (Grunow) Round & Basson                          | *      | *      |        |
| LCOH            | <i>Luticola cohnii</i> (Hilse) D.G. Mann                                    | *      | *      |        |
| LGOE            | <i>L. goeppertiana</i> (Bleisch in Rabenhorst) D.G. Mann                    | *      | *      | *      |
| LVEN            | <i>L. ventricosa</i> (Kützing) D. G. Mann                                   | *      |        | *      |
| NERI            | <i>Navicula erifuga</i> Lange-Bertalot                                      | *      | *      | *      |
| NGRE            | <i>N. gregaria</i> Donkin   | *      |        | *      |
| NLAN            | <i>N. lanceolata</i> (Agardh) Ehrenberg                                     | *      | *      | *      |
| NRHY            | <i>N. rhynchocephala</i> Kützing  | *      |        |        |
| NVEN            | <i>N. veneta</i> Kützing  | *      |        | *      |
| NEAC            | <i>Neidium affine</i> (Ehrenberg) Pfitzer fo. <i>Constricta</i> Krasske     | *      | *      | *      |
| NEDU            | <i>N. dubium</i> (Ehrenberg) Cleve  | *      |        |        |
| NZCD            | <i>Nitzschia acicularioides</i> Hustedt                                     | *      | *      | *      |
| NACI            | <i>N. acicularis</i> (Kützing) W. M. Smith                                  | *      |        | *      |
| NACM            | <i>N. acuminata</i> (W. M. Smith) Grunow                                    | *      |        | *      |
| NIAN            | <i>N. angustata</i> Grunow  | *      | *      | *      |
| NBRE            | <i>N. brevissima</i> Grunow   | *      | *      | *      |
| NCLA            | <i>N. clausii</i> Hantzsch  | *      | *      | *      |
| NCPS            | <i>N. compressa</i> (J. W. Bailey) Boyer                                    | *      | *      |        |
| NFIL            | <i>N. filiformis</i> (W. M. Smith) Van Heurck                               | *      | *      | *      |
| NFON            | <i>N. fonticola</i> Grunow in Cleve et Möller                               | *      |        |        |
| NIFR            | <i>N. frustrulum</i> (Kützing) Grunow                                       | *      | *      | *      |
| NLIN            | <i>N. linearis</i> (Agardh) W. M. Smith                                     | *      | *      | *      |
| NNAN            | <i>N. nana</i> Grunow in Van Heurck   | *      | *      |        |
| NPAL            | <i>N. palea</i> (Kützing) W. Smith  |        | *      | *      |
| PGIB            | <i>Pinnularia gibba</i> Ehrenberg   | *      | *      |        |
| PSCA            | <i>P. cf. subcapitata</i> Gregory   | *      | *      | *      |
| PCLT            | <i>Placoneis clementis</i> (Grunow) Cox                                     | *      | *      | *      |
| PTDE            | <i>Planothidium delicatulum</i> (Kützing) Round & Bukhtiyarova              | *      |        |        |
| PLEV            | <i>Pleurosira laevis</i> (Ehrenberg) Compère                                | *      | *      | *      |
| SPUP            | <i>Sellaphora</i> aff. <i>pupula</i> (Kützing) Mereschkowksy                |        |        | *      |
| SKSS            | <i>Skeletonema subsalsum</i> (Cleve-Euler) Bethge                           | *      |        | *      |
| THUN            | <i>Tryblionella hungarica</i> (Grunow) D.G. Mann                            | *      | *      | *      |
| Euglenophyta    |   |        |        |        |
| EUGL            | <i>Euglena</i> sp. Ehrenberg  |        | *      | *      |
| Chlorophyta     |   |        |        |        |
| CLAV            | <i>Closterium acutum</i> var. <i>variabile</i> (Lemm.) Krieger              | *      | *      | *      |
| MOUG            | <i>Mougeotia</i> sp. C. A. Agardh   | *      |        | *      |



**Table 3** (continued)

| Acronym    | Taxon  | Site 1 | Site 2 | Site 3 |
|------------|--|--------|--------|--------|
| Cyanophyta |  |        |        |        |
| PSCO       | <i>Pseudoanabaena constricta</i> (Szafer) Lauterborn | *      | *      | *      |
| CHRO       | <i>Chroococcus</i> sp. Nägeli                        | *      |        | *      |
| OSLI       | <i>Oscillatoria limosa</i> Agardh ex Gomont          | *      | *      | *      |
| Ciliata    |  |        |        |        |
| ACMU       | <i>Acropisthium mutabile</i> Perty                   | *      |        | *      |
| EPPL       | <i>Epistylis plicatilis</i> Ehrenberg                | *      | *      | *      |
| GLSC       | <i>Glaucoma scintillans</i> Ehrenberg                | *      |        | *      |
| PSEU       | <i>Pseudoglaucoma</i> sp. Kahl                       | *      | *      | *      |
| Nematoda   |  |        |        |        |
| MONH       | <i>Monhystera</i> sp. Bastian                        | *      | *      | *      |

The presence of the taxa in the sampling sites is indicated with \*. (For details of the sites see text).

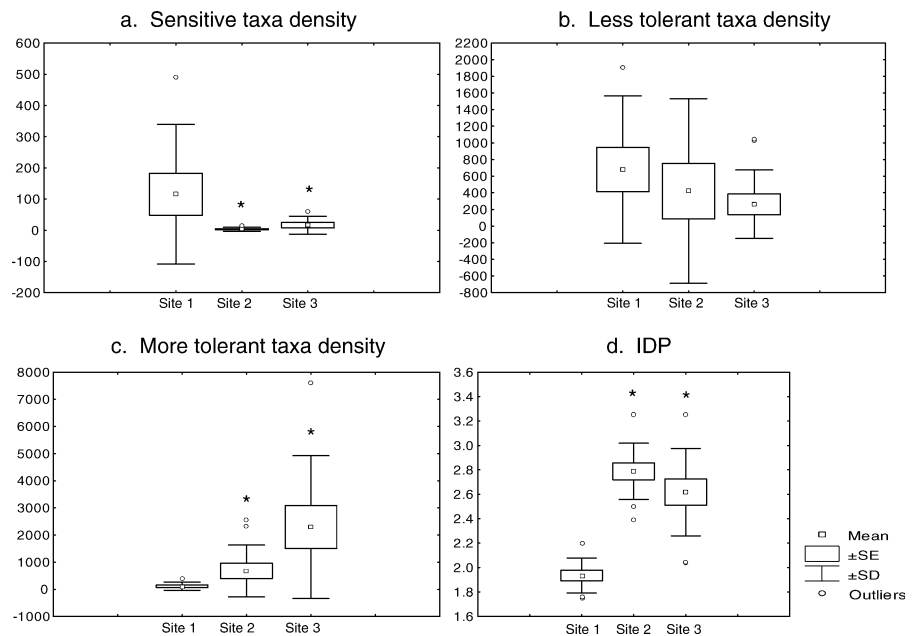
other polluted site. Although cyanophytes densities were similar to that recorded at the reference site, *Oscillatoria chalybea*, a good indicator of organic pollution (Sládeček 1973), was frequent at site 3.

The highest heterotroph densities were recorded at the polluted sites, specially at site 3, and they were mainly accounted for ciliates characteristic of  $\alpha$ -mesosaprobic and polisaprobic environments (Sládeček 1973; Heinz and Dieter 1987). Nematodes reached their highest densities at site 2 and were important principally for their great biomass. Besides the dominance of autotrophs, the highest rotifer densities were recorded at the reference site but in very low numbers, they were

represented by species of the genera *Keratella* and *Trichocerca* indicating oligosaprobic and  $\beta$ -mesosaprobic environments (Sládeček 1973). The shift from autotrophic toward heterotrophic conditions, due to the input of organic matter, has been observed by Biggs (1989), Sládeček (1973) and Villanueva et al. (2000).

In agreement with Biggs (1989) and Cosgrove et al. (2004), in this study the total organic matter of the biofilm (as AFDW) was higher at sites impacted by wastewater discharges. The difference was statistically significant at site 2 where the biomass accrual was favoured by a lower hydrodynamic energy of the estuary.

**Fig. 5** Tolerance to pollution of the taxa and Pampean Diatom Index (IDP) found in the analysed biofilms. \* indicates significant differences ( $p < 0.05$ ) between the marked site and the reference site (site1)



The number of species and the diversity index varied among the sampling sites showing the lowest values in the polluted sites. According to Symoens et al. (1988) the alterations that occur in communities exposed to contamination are as diverse as changes in the number of species, the number of organisms of each species and the total density of organisms. High species richness is assumed to indicate biotic integrity because many species are adapted to the conditions present in the habitat. Species richness is predicted to decrease with increasing pollution because many species are stressed. Species diversity, despite the controversy surrounding it, has historically been used with success as an indicator of organic (sewage) pollution (Stevenson and Bahls 1999). In the present study the number of species and the diversity ( $H'$ ) showed significant differences between the reference site and the two polluted sites, decreasing in the latter. Similarly, the tolerance of the taxa to the organic pollution and eutrophication, and the values of IDP, also marked the impairment of the water quality. The Pampean Diatom Index allowed us to characterise the reference site as a site with moderate eutrophication and organic pollution and acceptable water quality, whereas sites 2 and 3 were sites with bad water quality. The IDP did not show significant differences between these last two sites reflecting the absence of significant differences between them in  $BOD_5$ ,  $PO_4^{3-}-P$  and  $NH_4^+-N$ . However, IDP values were slightly higher at site 2 and lower density of sensitive species is coincident with a worse water quality at this site, evidenced by the lowest oxygen content, the highest conductivity and phosphorus content.

The data set contained several outliers and extreme values, indicating a high variability of those descriptors of the biofilm analysed, but the composition and structure of the biofilms, nevertheless, showed significant differences related to the input of wastewater discharges. Several authors (Biggs 1989; Cosgrove et al. 2004; Economou-Amilli 1980; Kiss et al. 2002; McCormick and Stevenson 1998; Stevenson 1984; Villanueva et al. 2000; Watanabe et al. 1988) have reported, by means of different descriptors, that biofilms thus indicate the ecological state of the environment and the changes that are occurring.

This study shows that the biofilms growing on *S. californicus* along the Argentinian shore of the freshwater tidal zone of the Río de la Plata Estuary are

good indicators of changes in the water quality of this environment. However, according to Gómez et al. (2003) it is also necessary to consider the spatial heterogeneity in the morphology of the coast, due to the fact that biofilms can tolerate different degrees of exposure to winds, waves and turbidity that influence their structural descriptors.

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