ABUNDANCE AND SPECIES COMPOSITION OF PLANKTONIC CILIOPHORA FROM THE WASTEWATER DISCHARGE ZONE IN THE BAHÍA BLANCA ESTUARY, ARGENTINA

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ABSTRACT

The specific composition and abundance variation of the ciliate community from a wastewater discharge zone in the Bahía Blanca estuary, Argentina, were studied all throughout a year, from June 1995 to May 1996. The polluted area exhibited high values of particulate organic matter and nutrients, particularly phosphates. Aloricate ciliates were represented by 15 species belonging to the genera *Strombidium* Claparède & Lachmann, 1859; *Strombidinopsis* Kent, 1881; *Cyrtostrombidium* Lynn & Gilron, 1993; *Strobilidium* Schewiakoff, 1983; *Lohmmanniella* Leegaard, 1915 and *Tontonia* Fauré-Fremiet, 1914. Tintinnids were represented by nine species belonging to the genera *Tintinnidium* Kent, 1881, *Tintinnopsis* Stein, 1867 and *Codonellopsis* Jörgensen, 1924. The total abundance of aloricate ciliates reached a peak of 1,800 ind. l^{-1} and the total abundance of tintinnids reached a peak of 9,400 ind. l^{-1}. *Tintinnidium balechi* Barría de Cao, 1981 was the most abundant ciliate in the community. Considerations on the presence and abundance of ciliates are made in relation to physicochemical and biochemical parameters.

KEYWORDS. Tintinnina, Ciliophora, wastewater, estuaries.

INTRODUCTION

The major source of organic pollution in the world is domestic sewage (CURDS, 1982). In coastal areas, it is common for untreated sewage to be passed directly to the sea. Such is the case in Bahía Blanca City, Argentina. Sewage contains large amounts of organic matter, which, when utilized by bacteria, reduces the dissolved oxygen levels in the aquatic environment. This fact can cause great damage to marine organisms. The disposal of sewage in the sea is considered to be the major source of addition of nitrogen and phosphorous compounds to the aquatic environment. Wastes discharged into the sea cause eutrophication, which modifies the planktonic populations in coastal waters

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both quantitatively and qualitatively. Some planktonic ciliates can live in highly eutrophic waters; hence, they can be used as indicators of organic pollution. Bacterivorous ciliates can reach the highest standing stocks among the pelagic ciliates in some estuarine zones, and should be able to consume one third of annual bacterial production (ARNDT *et al.*, 1990), so they play an important role in regulating the growth of bacteria populations (RIVIER *et al.*, 1985).

The subject of planktonic ciliates in relation to organic pollution has not been studied in the coasts of Argentina, with the exception of the Río de La Plata coast (RIVES, 1997).

The aim is to analyze the composition and abundance variation of the planktonic ciliate community in the wastewater discharge zone of the Bahía Blanca estuary in relation to physicochemical and biochemical parameters.

MATERIAL AND METHODS

The studied area is located in the inner part of the Bahía Blanca estuary (38,8°S, 62,2°W) (fig. 1). To compare results, sampling was carried out at two fixed stations, one in the wastewater discharge zone (WWDS) and the other (Boya 31) in the principal channel of navigation. The wastewater discharge zone is affected by untreated sewage from Bahía Blanca, a city with a population of approximately 350,000 inhabitants (80% of them within the sanitation network).

Samples were collected from 16 June 1995 to 20 May 1996 at two fixed stations approximately every 15 days with a 30 µm mesh plankton net and a Van-Dorn bottle and then fixed with 4 % formaldehyde solution and Lugol's solution, respectively. Observations were made with a contrast-phase microscope, and ciliates were counted with an inverted microscope following the Utermöhl method (HASLE, 1978).

Water temperature, salinity, turbidity, particulate organic matter (POM), nutrients (ammonia, nitrite, nitrate, phosphate, and silicate), Chlorophyll "a", and phaeopigments were measured at both stations on each sampling date.

Nutrients were determined using a Technicon Auto Analyzer II, according to TREGUER & LE CORRE (1975), EBERLEIN & KATTNER (1987) and GRASSHOFF (1983). Chlorophyll "a" and phaeopigments were measured after LORENZEN (1967). Other determinations were performed according to STRICKLAND & PARSONS (1968). A correlation test was done between the total abundance of tintinnids, the total abundance of aloricate ciliates, the abundance of *Tintinnidium balechi* at WWDS, and the physicochemical and biochemical parameters.

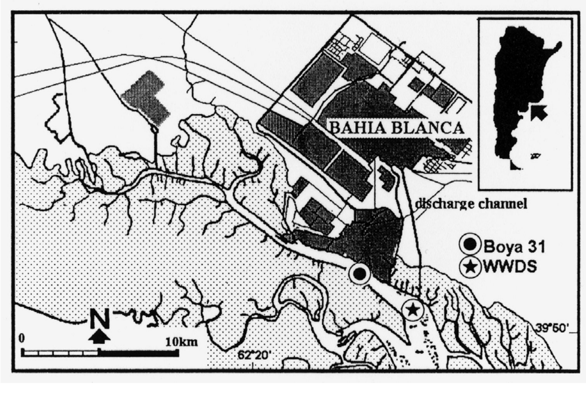


Fig. 1. Bahía Blanca estuary showing the sampling stations: wastewater discharge station (WWDS); Boya 31.

RESULTS

The data obtained for physico-chemical parameters (tab. I) showed main differences on phosphate and silicate values, which were always higher at WWDS (figs. 2, 4). Registered POM data were rather erratic but nearly always higher at WWDS. Chlorophyll "a" and phaeopigments showed a similar trend at both sampling stations (figs. 3, 5) with maximal values in winter.

During the study, the aloricate ciliates group was represented by Choreotrichida and Oligotrichida, and the species found belong to the genera: *Strombidium* Claparède & Lachmann, 1859; *Strombidinopsis* Kent, 1881; *Cyrtostrombidium* Lynn & Gilron, 1993; *Strobilidium* Schewiakoff, 1983; *Lohmmanniella* Leegaard, 1915 and *Tontonia* Fauré-Fremiet, 1914 (figs. 6-9).

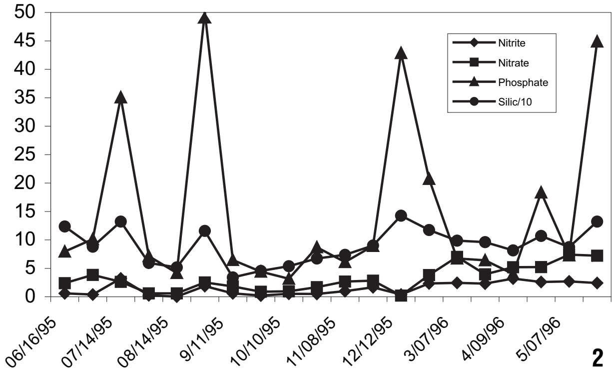
The estimated total abundance of aloricate ciliates varied between a minimum of 100 ind. l^{-1} and a maximum of 3,700 ind. l^{-1}, the latter being registered at the Boya 31 Station, where the total number of individuals was always higher than that at WWDS. Values registered at WWDS varied from 100 ind. l^{-1} to 1,800 ind. l^{-1} (fig. 12).

Strombidinopsis and *Strombidium* were the most widely represented genera, and both were present at the two stations during the sampling period. *Strombidium* reached the highest abundance levels at both stations, representing 37% of the total abundance, while *Strombidinopsis* reached 30%. The other genera showed relative abundances that were slightly higher at WWDS, with the exception of *Cyrtostrombidium*, which was found to have twice the number of individuals at Boya 31 (fig. 13).

Eleven species of tintinnids were encountered in the principal channel of navigation (Boya 31) of the Bahía Blanca estuary: *Tintinnidium balechi* Barría de Cao, 1981; *T.* **aff**. *semiciliatum*; *Tintinnopsis baltica* Brandt, 1896; *T. beroidea* Stein, 1867; *T. brasiliensis* Kofoid & Campbell, 1929; *T. glans* Meunier, 1919; *T. gracilis* Kofoid & Campbel, 1929; *T. levigata* Kofoid & Campbell, 1929; *T. parva* Merkle, 1909; *T. parvula* Jörgensen, 1912 and *Codonellopsis lusitanica* Jörgensen, 1924. Nine species were sporadically represented at the wastewater discharge station (figs. 9-11): *Tintinnidium balechi, T.* **aff**. *semiciliatum*, *Tintinnopsis baltica, T. beroidea, T. brasiliensis, T. glans, T. gracilis, T. parva* and *Codonellopsis lusitanica*. The total amount of tintinnids reached a peak of 9,400 ind. l^{-1} at WWDS, and the highest value observed was of 8,200 ind. l^{-1} at Boya 31 in spring (fig. 14). These peaks were almost exclusively due to *T. balechi*, which was the most conspicuous component of the tintinnid community and was present throughout the sampling period at WWDS. *Tintinnidium balechi* reached percentages over 90% of the total abundance of tintinnids at this station (fig. 14) while it never exceeded 51% of the total abundance of tintinnids at Boya 31.

Table I. Physicochemical and biochemical parameters measured at the wastewater discharge zone (WWDS) and a reference station (Boya 31). Mean values and S.D. (in brackets).

Parameter	Units	WWDS	B 31
Salinity	psu	31.95 (± 5.46)	36.18 (± 1.48)
Temperature	° C	14.3 (± 5.24)	13.4 (± 5.91)
Diss.Oxigen	mg.l^{-1}	6.0 (± 2.70)	7.5 (± 1.5)
Sat. Oxigen	%	67.7 (± 27.6)	87.5 (± 8.41)
Secchi Disk	m	0.51 (± 0.10)	0.67 (± 0.20)
Chlorophyll “a”	µg.l^{-1}	5.48 (± 3.24)	6.69 (± 3.96)
Phaeopigments	µg.l^{-1}	5.23 (± 5.22)	6.02 (± 5.89)
P.O.M	µg.l^{-1}	3554 (± 1898)	1620 (± 594)
Ammonia	N-µM	179.6 (± 88.8)	22.69 (± 14.33)
Nitrite	N-µM	1.51 (± 1.10)	1.42 (± 1.30)
Nitrate	N-µM	3.17 (± 2.2)	3.87 (± 3.2)
Phosphate	P-µM	15.2 (± 14.8)	2.06 (± 0.68)
Silicate	Si-µM	89.96 (± 30.8)	72.80 (± 26.63)



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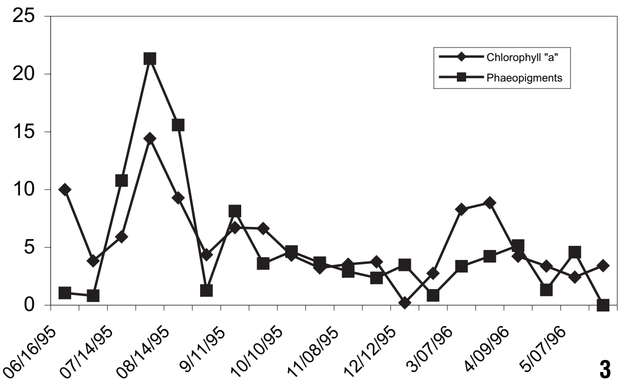

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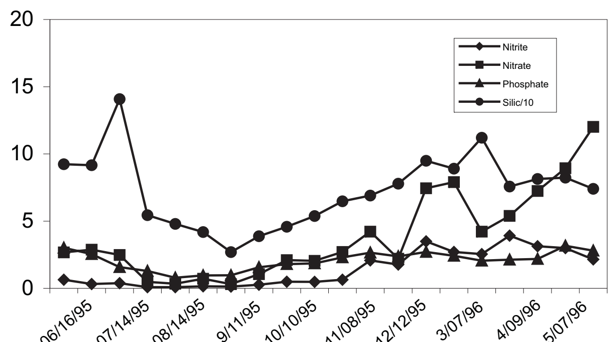

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Figs. 2-4. 2, Nutrients variation at WWDS (wastewater discharge station); 3, chlorophyll "a" and phaeopigments variation at WWDS; 4, nutrients variation at Boya 31 station.

Significant correlation values were found between the abundance of tintinnids, the abundance of the aloricate ciliates, and the abundance of *Tintinnidium balechi*, and some of the physicochemical parameters (tab. II).

DISCUSSION

In general, the total abundance values of aloricate ciliates registered at Boya 31 Station were similar to the values observed in other areas of the estuary (PETTIGROSSO *et al.*, 1997).

Analysis of seasonal variation of ciliate abundances showed that values from both stations were low in winter and tended to increase in spring, reaching the highest peak in summer. The lowest abundances during winter coincided with the annual period of phytoplankton bloom. This behavior has been formerly observed in the inner part of the estuary (PETTIGROSSO *et al.*, 1997). The total abundance of aloricate ciliates presented significant positive correlation values with salinity and negative correlation with POM and phosphate. The constant presence of *Strombidium* and *Strombidinopsis* with high abundance values at

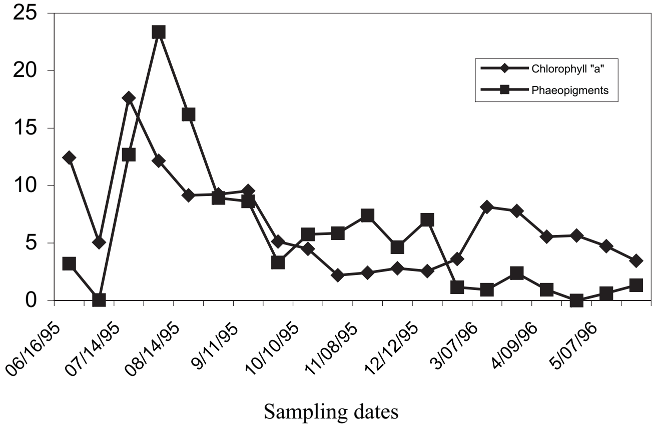
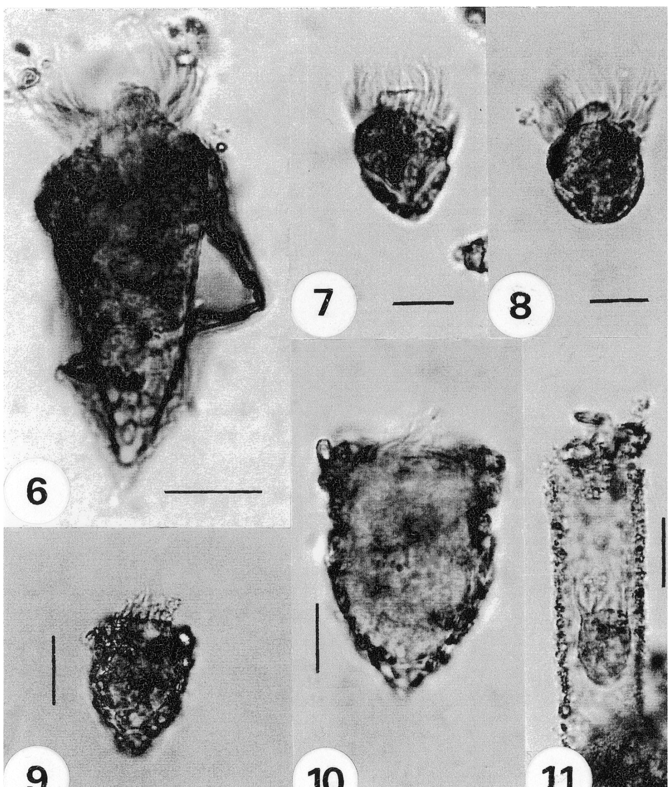
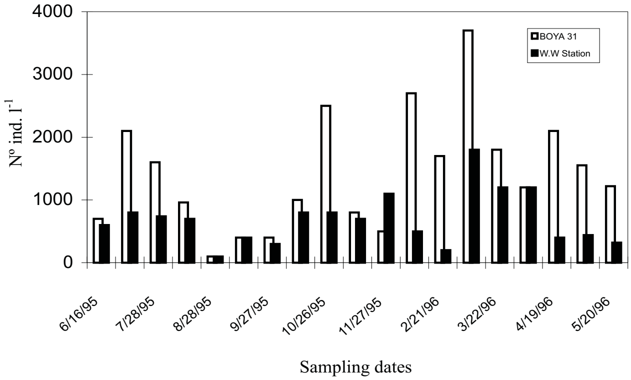


Fig. 5. Chlorophyll “a” and phaeopigments variation at Boya 31 station.



Figs. 6-11. 6, *Strombidinopsis* sp.; 7, *Strombidium* sp.1; 8, *Strombidium* sp.2; 9, *Tintinnopsis parva*; 10, *Tintinnopsis baltica*; 11, *Tintinnidium balechi*. Scale bar: 20 µm.



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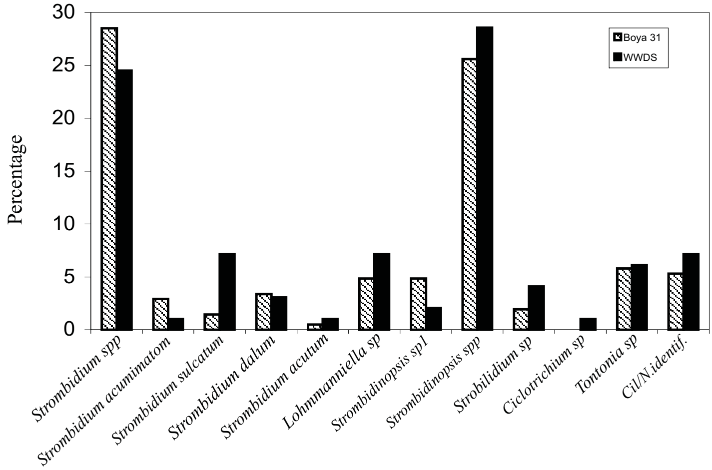

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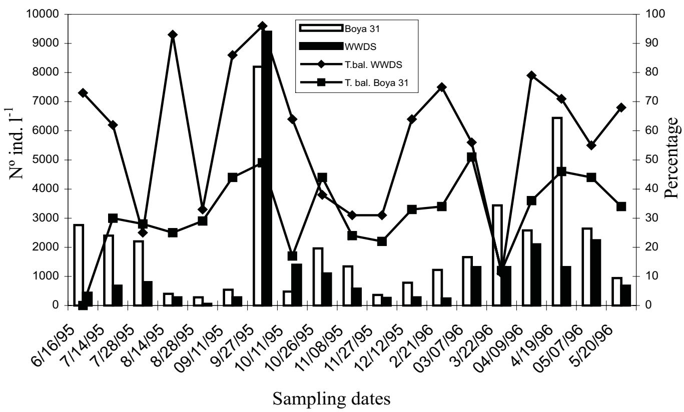

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Figs. 12-14. 12, Total abundance of aloricate ciliates; 13, relative abundance in percentage of aloricate ciliates species; 14, total abundance of tintinnids and relative abundance in percentage of *T. balechi*.

Table II. Significant correlation values (p < 0.5, n=19) between the total abundance of tintinnids (AT), aloricate ciliates (AAC), *Tintinnidium balechi* at WWDS (ATb), and some of the physicochemical parameters.

Tintinnids	
WWDS	Boya 31
AT/Salinity, r=0.522	AT/POM, r=-0.558
AT/POM, r=-0.606	
AT/Phosphate, r=-0.513	
Aloricate ciliates	
WWDS	Boya 31
AAC/Salinity, r=0.550	AAC/Salinity, r=0.540
AAC/POM, r=-0.517	AAC/POM, r=0.555
	AAC/Sil, r=0.507
	AAC/Temp., r=0.506
T. balechi at WWDS	
ATb/Salinity	
ATb//POM, r=-0.605	
ATb/Phosphate, r=-0.509	

both stations most of the time could indicate certain tolerance of these genera to high levels of organic matter and salinity variations. Even though represented by a smaller number of individuals, the genera *Strobilidium*, *Lohmmanniella*, and *Tontonia* also appear to tolerate variations in the parameters considered in this study. The exception was *Cyrtostrombidium*, whose abundance was decreased by 50% at WWDS, demonstrating its high sensitivity to high organic matter levels and low salinity values.

Reports on tintinnids in relation to organic pollution are very scarce in the world. CURDS (1982) referred to tintinnids as organisms highly sensitive to the presence of sewage. It was observed the presence of 9 species of tintinnids at WWDS, an area where the average of organic matter was 3,554 $\mu g. l^{-1}$.

Most of the species found at both stations belonged to *Tintinnidium* and *Tintinnopsis*, which is a characteristic feature of the species composition of the tintinnids community in the Bahía Blanca estuary (BARRÍA DE CAO, 1992). The total abundance of tintinnids was nearly always higher at Boya 31 than at WWDS. Positive correlation values were observed between the abundance and salinity values at WWDS, but their abundance was negatively correlated with POM and phosphate. The peak of tintinnids observed on 27 September 1995 at WWDS was due nearly exclusively to *Tintinnidium balechi*. The most remarkable characteristic of the tintinnid community at WWDS was the constant presence and high abundance values of *T. balechi*, although its abundance was negatively correlated with POM and phosphate. One explanation for the remarkable occurrence of *T. balechi* at WWDS could be its specific food requirements. *Tintinnidium balechi* is a small species with a mean oral lorica diameter of 17.3 µm and a mean peristome diameter of only 12 µm (BARRÍA DE CAO *et al.*, 1997); therefore, its specific diet must consist of very tiny preys. The peaks of chlorophyll “a” at both stations were found during a phytoplankton bloom during the winter. Phytoplankton bloom in the Bahía Blanca estuary is chiefly composed of nanophytoplankton species, but these species, which are chain forming and projection bearing diatoms, could represent only a part of the diet of *T. balechi*, as they are not suitable food for tintinnids in general (BARRÍA DE CAO *et al.*, 1997).

The physicochemical and biochemical characteristics at WWDS would favor the existence of an adequate source of food such as bacteria and heterotrophic microflagellates. Both terrestrial and marine heterotrophic aerobic bacteria develop well in this area (CABEZALÍ & BURGOS, 1988), and coliform bacteria are also very abundant (BALDINI & CABEZALÍ, 1988).

Although there are no references on saprobity in tintinnids from marine waters, it is known that two species of *Tintinnidium*, *T. fluviatile* Kent, 1882 and *T. fluviatile* **v.** *emarginatum* Maskell, 1887 and one species of *Tintinnopsis*: *T. cylindrata* Kofoid & Campbell, 1929, have been considered as oligo-beta-mesosaprobic in freshwater systems (SLADECEK, 1973; FOISSNER, 1988).

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REFERENCES

ARNDT, H.; JOST, G. & WASMUND, N. 1990. Dynamics of pelagic ciliates in eutrophic estuarine waters: importance of functional groups among ciliates and responses to bacterial and phytoplankton production. **Arch. Hydrobiol. Beih. Ergebn. Limnol.**, Stuttgart, **34**:239-245.

BALDINI, M. D. & CABEZALÍ, C. B. 1988. Distribución de *Escherichia coli* en aguas del estuario de Bahía Blanca, Argentina. **Revta lat. am. Microbiol.**, Mexico D.F., **30**:229-234.

BARRÍA DE CAO, M. S. 1992. Abundance and species composition of Tintinnina (Ciliophora) in Bahia Blanca estuary, Argentina. **Estuarine Coastal Shelf Sci.**, London, **34**:295-303.

BARRÍA DE CAO, M. S.; PETTIGROSSO, R. E. & POPOVICH, C. 1997. Planktonic ciliates during a phytoplankton bloom in Bahía Blanca estuary, Argentina. II. Tintinnids. **Oebalia**, N. Ser., Toronto, **23**:21-31.

CABEZALÍ, C. B. & BURGOS, M. A. 1988. Distribución espacial y temporal de bacterias aerobias en el estuario de Bahía Blanca, Argentina. **Revta argent. Microbiol.**, Buenos Aires, **20**:77-86.

CURDS, C. 1982. Pelagic protists and pollution. A review of the past decade. **Annls Inst. oceanogr.**, Paris, **58**(S):117-136.

EBERLEIN, K. & KATTNER, G. 1987. Automatic method for the determination of orthophosphate and total dissolved phosphorous in the marine environment. **Fresenius Z. analyt. Chem.**, Berlin, **326**:354-357.

FOISSNER, W. 1988. Taxonomic and nomenclatural revision of Sladecek list of Ciliates (Protozoa: Ciliophora) as indicator of water quality. **Hidrobiologia**, Dordrecht, **166**:1-64.

GRASSHOFF, K. 1983. **Methods of Seawater analysis**. New York, CEIME Verlag. 317p.

HASLE, G. R. 1978. Settling. The inverted-microscope method. *In*: SOURNIA, A. ed. **Phytoplankton Manual**. Monographs on oceanographic methodology. Paris, UNESCO. v.6, p.88-96.

LORENZEN, C. L. 1967. Determination of chlorophyll and pheopigments. Spectrophotometric equations. **Limnol. Oceanogr.**, Lawrence, **12**:343-346.

PETTIGROSSO, R. E.; BARRÍA DE CAO, M. S. & POPOVICH, C. 1997. Planktonic ciliates during phytoplankton bloom in Bahía Blanca estuary, Argentina. I. Aloricate ciliates. **Oebalia**, N. Ser., Toronto, **23**:3-19.

RIVES, C. V. 1997. Protozoos como indicadores de contaminación. *In*: **Calidad de las aguas de la franja costera sur del Río de La Plata (San Fernando-Magdalena)**. Buenos Aires, Sec. Obras Públicas. p.113-119.

RIVIER, A.; BROWNLEE, D. C. *et al.* 1985. Growth of microzooplankton: a comparative study of bactivorous zooflagellates and ciliates. **Mar. Microbial Food Webs**, Paris, **1**:51-60.

SLADECEK, V. 1973. System of water quality from the biological point of view. **Arch. Hidrobiol. Beih. Ergebn. Limnol.**, Stuttgart, **7**:1-218.

STRICKLAND, J. D. & PARSONS, T. R. 1968. **A practical handbook of seawater analysis**. Ottawa, Fisheries Research Board of Canada. 311p. (Bulletin, 167).

TREGUER, P. & LE CORRE, P. 1975. **Manuel d'analisis des sels nutritifs**. Brest, Université de Bretagne Occidentale. 110p.

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