RIVER RESEARCH AND APPLICATIONS

River Res. Applic. **27**: 1264–1269 (2011)

Published online 1 June 2010 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/rra.1421

LIMNOLOGICAL ASPECTS OF HUMIC SUBSTANCES IN CHUBUT RIVER (PATAGONIA-ARGENTINA)

M. DEL C. SCAPINI,[a]* V. H. CONZONNO,[b] J. D. ORFILA,[a] J. SARAVIA,[a] V. T. BALZARETTI[a] and A. F. CIRELLI[c]

[a] *Facultad de Ciencias Naturales, Sede Trelew, Universidad Nacional de la Patagonia San Juan Bosco, Roca 115, CP 9100, Trelew, Chubut, Argentina*

[b] *Laboratorio de Investigación en Sistemas Ecológicos y Ambientales, Facultad de Ciencias Naturales y Museo, CONICET, La Plata, Argentina*

[c] *Centro de Estudios Transdisciplinarios del Agua, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, CONICET, Avda. Chorroarín 280, Buenos Aires, Argentina*

ABSTRACT

Humic Substances (HSs) of the Chubut River (Patagonia-Argentina) have been investigated, during two consecutive years and different climatic seasons, in order to obtain a first approach at their spatial and temporal distribution, as well as their origin and relation with environmental characteristics. Absorbance at 250 nm and limnological variables were measured and the data processed by different statistical tools. We found that the processes developed in the lower sections of Chubut River are dominated by those produced in the Florentino Ameghino Reservoir. The riverine HSs are present in very low concentrations, have mainly autochthonous origin, exhibit spatial homogeneity and temporal variability similar to nutrients (nitrates and soluble reactive phosphorus). Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: humic substances; acuagenic origin; spatial and temporal distribution

Received 5 June 2009; Revised 30 March 2010; Accepted 20 April 2010

INTRODUCTION

The origin of Chubut River is located in the Andean Patagonian Cordillera and attending to the ecological conditions it is possible to argue that this river exhibits three sections. The first covers the extension from the cordillera until it is dammed to form the Florentino Ameghino Reservoir (Figure 1). In this section, the river receives the affluent Gualjaina River and after that, no significant contribution takes place over its running across the Patagonian plateau. The second starts in the reservoir which was built with the purpose of hydroelectric generation, irrigation and the supply of drinking water to the cities of Dolavon, Gaiman, Trelew, Rawson and Madryn (about 350 000 inhabitants). Therefore, this section, ending at the beginning of the estuary, is very important in relation to the human activities. The estuary constitutes the third section and is the way by which the river reaches the Atlantic Ocean.

Previous studies were done about the river (Helbling *et al*., 2005; Pasquini *et al*., 2005; Miserendino, 2006) and particularly in the estuary Santinelli and Esteves (1993) reported episodes of toxic algae blooms and founded cell and cysts of the dinoflagellate *Alexandrium excavatum*. None of these include humic substances (HSs) studies, in spite of the importance that they have in the transport of organic and inorganic compounds, in the influence of the growth of algae and bacteria (Conzonno and Fernandez Cirelli, 1996; Hertkorn *et al*., 2002) and in the generation of toxic products during chlorination process (Frimmel *et al*., 2004). The properties of HSs are strongly dependent on their origin and the environmental characteristics. The transformation of a lotic to a lentic environment in the reservoir has the consequence of the improvement of phytoplankton production, with the possibility of the increase of metabolic compounds and other decomposition products derived from algae that constitute a source for the generation of aquagenic HSs. In a previous work, it has been reported that HSs in the second section are primarily fulvic acids of mainly aquagenic origin (Scapini *et al*., 2004). The aim of the present paper is to relate the HSs with the limnological parameters in the second section to obtain a first approach of their temporal and spatial distribution and to confirm results of previous work about their origin.

METHODOLOGY

Sampling sites

Samples were taken from July 2002 to June 2004 in the points marked in the map (Figure 1) and described in Table I. These consisted of one station in the reservoir (Murallón)

*Correspondence to: M. del C. Scapini, Facultad de Ciencias Naturales, Sede Trelew, Universidad Nacional de la Patagonia San Juan Bosco, Roca 115, CP 9100, Trelew, Chubut, Argentina.
E-mail: mariadelcarmenscapini@yahoo.com.ar

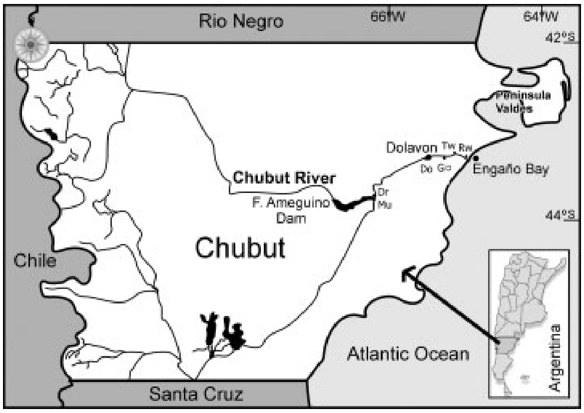

Figure 1. Map of Chubut Province (Patagonia-Argentina) showing locations of sampling sites

and five in the river (Dique Río, Dolavon, Gaiman, Trelew and Rawson) named Mu, Dr, Do, Ga, Tw and Rw.

Analytical methods

Temperature (*T*, graduated 0.5°C mercury thermometer) and Secchi disk transparency were determined *in situ*. The following parameters were measured on unfiltered samples: Conductivity (χ) at 20°C (WTW LF90 conductimeter); pH (Hanna Instruments, 8519 Model, H113329 electrode); Suspended Solids (SS) by Standard Methods 2540D (APHA, 1998); Dissolved Oxygen (DO, Winkler method) in the sample previously fixed *in situ* (Conzonno, 1995); Biochemical Oxygen Demand (BOD, 5 days, 20°C, Winkler method) and Total Phosphorus (Pt, molybdenum blue method) after digestion of the sample to orthophosphate with persulfate in sulphuric acid medium (Conzonno, 1995). On the other hand, samples filtered by 0.45 μm pore size (Millipore membrane filters previously washed to cero absorbance between 200 and 400 nm, 10 cm path length) were used to evaluate the following parameters: absorbance at 250 nm (A_{250}) and at 365 nm (A_{365}) in a Metrolab 1700 UV–VIS spectrometer provided with software SF170 (10 cm path length, quartz cells); Chemical Oxygen Demand (COD) by Standard Methods 5220B (APHA, 1998) and Nitrate (NO_3-N), Soluble Reactive Phosphorus (Pr) and Silica (SiO_2) in an Autoanalyzer II Technicon ^{R} according to Treguer and Le Corre (1975).

Chlorophyll (Chlor) and Pheopigments (Pheo) were measured on samples filtered by glass membrane filter Whatman GF/C by spectrophotometric method 7.8.2 (Golterman *et al*., 1978) after extraction with acetone 90%.

River Discharge (Qr) and Turbidity (t) data were provided by Hidroelectrica Ameghino SA and Cooperativa Electrica de Trelew, respectively.

The data were processed by Excel 2003 and InfoStat (2008).

Table I. Name and location of sampling sites

Name	Location
Murallón (Mu)	43° 41’ 58” S—66° 28 ’57” W
Dique Río (Dr)	43° 41’ 56” S—66° 28’ 42” W
Dolavon (Do)	43° 21’ 08” S—65° 43’ 09” W
Gaiman (Ga)	43° 17’ 32” S—65° 30’ 03” W
Trelew (Tw)	43° 16’ 33” S—65° 16’ 25” W
Rawson (Rw)	43° 17’ 28” S—65° 09’ 49” W

RESULTS AND DISCUSSION

HSs and limnological parameters: contents and spatial–temporal distribution

Results obtained from statistical analysis (Table II) showed that these waters are low in salinity, slightly alkaline, saturated in oxygen and poor in pigments, nutrients and organic matter, with percentages of variation coefficients generally high. The range of water temperature is typical of the semi-arid regions at these latitudes. Fluctuations of plankton biomass may account for the high variations found in the concentration of pigments and nutrients. The variations observed in the discharge are in accord with the irrigation and energy requirements.

UV-visible absorbance is commonly used as an indicator of HSs concentrations especially at 250 and 365 nm (Conzonno and Fernández Cirelli, 1987/1988, 1988; Bloom and Leenheer, 1989; Fooken and Liebezeit, 2000). The general average of A_{250} (0.539, 10 cm path length) indicates waters with low organic matter concentration and/or structures with little absorption at this wavelength. Similar values were found by Martin-Mousset *et al.* (1997) in the Charente river (France), while Conzonno and Fernandez Cirelli (1987) reported notoriously different values in pampasic ponds as in Chascomús Pond where the average is 0.286 (1 cm path length) which is in relation to the high productivity of the surrounding soils.

Taking under consideration all the sampling sites to evaluate spatial and temporal variations, A_{250} values obtained, put on evidence that there are not significant differences among them along the river as may be observed in the Figures 2 and 3, where the values of the stations are superposed (Figure 3), while they differ from each other in the campaigns performed (Figure 2). These facts suggest that A_{250} had spatial homogeneity and temporal variation that was confirmed by Analysis of Variance since only the differences between the sampling dates were significant (Table III). Therefore, HSs did not present significant variations between the sampled waters from the reservoir and along the river. This fact suggests that the reservoir

DOI: 10.1002/rra

Table II. Descriptive statistical analysis of limnological parameters

	Media	CV (%)	n	Minimum	Maximum	$t.s/\sqrt{n}$ (P 95%)
Qr ($m^3 s^{-1}$)	54	27	48	35	82	4.3
T (°C)	11.2	50	48	4.5	20.5	1.6
χ 20°C ($\mu S\,cm^{-1}$)	260	15	48	190	340	11.3
SS ($mg\,L^{-1}$)	14	74	48	2	52	3.0
z (cm)	88	57	48	35	340	14.6
Turbidity (UNT)	10	57	39	1.7	23	1.9
A_{250}	0.539	20	48	0.340	0.789	0.032
A_{365}	0.067	64	48	0.012	0.190	0.013
NO3-N ($\mu mol\,L^{-1}$)	0.5	91	36	0.3	1.8	0.14
PO_4-Pr ($\mu mol\,L^{-1}$)	0.7	62	48	0.0	2.3	0.13
PO_4-Pt ($\mu mol\,L^{-1}$)	2.4	41	48	0.7	4.9	0.29
SiO_2 ($mg\,L^{-1}$)	24	18	20	15.8	31.2	2.0
Chlorophyll ($\mu g\,L^{-1}$)	2.9	97	48	0.2	15.0	0.8
Pheopigments ($\mu g\,L^{-1}$)	8.8	89	48	1.7	39.2	2.3
pH	7.66	2	48	7.31	8.06	0.05
DO (%S)	91	10	48	78	108	2.7
BOD ($mg\,L^{-1}$)	1.7	28	48	0.8	2.6	0.11
COD ($mg\,L^{-1}$)	8.9	38	48	4.0	16	1.0

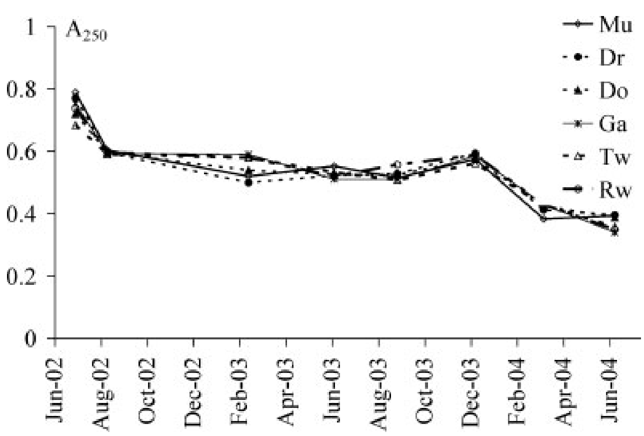

Figure 2. A_{250} spatial distribution in each sampling date

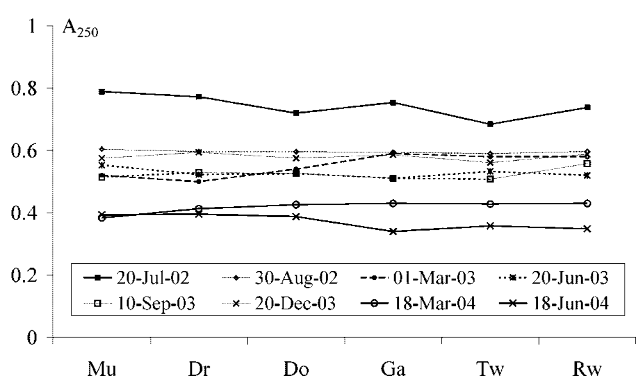

Figure 3. A_{250} temporal distribution in each sampling site

processes may control HSs characteristics in the river waters. This result is in coincidence with Parks and Baker (1997), who postulated that reservoirs are major contributors to total watershed DOC production in arid region.

The low A_{365} average showed a high proportion of compounds that had low absorption in the visible spectrum. The ratio between absorbance at both wavelengths (A_{250}/A_{365}) is indicator of fulvics acids (FA) and humic acids (HA) proportion (Conzonno and Fernández Cirelli, 1996). In the present study, an average value of 14 was obtained for this ratio suggesting a higher proportion of FA. Also this value is even higher to those found for Chascomús Pond (9.3), Vitel Stream (7.4) and Samborombón River (5.0), values over which Conzonno and Fernández Cirelli (1987/1988) emphasized the predominance of fulvic acids

Table III. ANOVA for limnological parameters

Parameter	Origin of variation	F experimental	Probability
A_{250}	Dates	127	0.00
	Sites	0.33	0.89
PO_4-Pr	Dates	22.6	3E-11
	Sites	1.0	0.44
PO_4-Pt	Dates	29.6	7E-13
	Sites	2.0	0.11
NO_3-N	Dates	27	3E-09
	Sites	0.33	0.89
Chlorophyll	Dates	14	2E-08
	Sites	0.6	0.68
χ	Dates	7.8	1.1E-05
	Sites	26	7.0E-11

DOI: 10.1002/rra

proportion in these aquatic environments.Other limnological parameters like phosphorus were considered as soluble reactive phosphorus *(*Pr*)* and total phosphorus (Pt). Phosphorus is relatively poor in the hydrosphere, except in polluted areas where fertilizer and/or urban (sewage, surfactants) contributions can occur. This is not the case of the study area where anthropogenic activities are poor and begins from Dolavon site (Figure 1). Another phosphorus sources can be the runoff of the drainage basin and the recycled sediments (Wetzel, 1981; Horne and Goldman, 1994). The contribution of the drainage basin depends on the topography, the vegetal cover, the intensity and/or duration of the rains and the amount of phosphorus in soils (Wetzel, 1981). In semi-arid regions where the precipitations are low but torrential, the vegetal cover is scarce and the erosion of extensive grounds is high, the phosphorus moves easily and is transported in dissolved as well as in solid form adsorbed on soils particles. The Chubut River lower valley is a semi-arid region with these characteristics; for that reason, Pr and Pt found in the fresh waters were relatively high. The Pr/Pt ratio depends on the trophic level and is about 0.1 (Hutchison, 1975) in normal conditions. In this work, an average of 0.3 obtained indicated a high proportion of Pr available for phytoplankton growth.

Nitrogen was determined in low average value, similar to that was found in Russian estuaries (Köhler *et al*., 2003). It was mainly present as nitrate, as can be expected in no polluted and aerated waters like Chubut River.

The analysis of the variance of the nutrients indicated that only temporal differences were significant (Table III) in the same way as A_{250}.

Both PO_4-Pr and NO_3-N varied in the same way (Figure 4), showing their consumption in September and March and their production in June and December. This temporary variation was opposite in relation to pigments. Like Pr and Pt, NO_3-N increased slightly towards Rw sampling site.

The ratio N/P was always lower than 15, which indicated that N was the limiting nutrient for the phytoplankton growth. That is proper to this aquatic system placed in a semi-arid region. Horne and Goldman (1994) emphasized that the N below 100 micrograms. L^{-1} can limit the algal growth and this frequently occurs in dry climates whereas in wet climates it is common to find limitation by P.

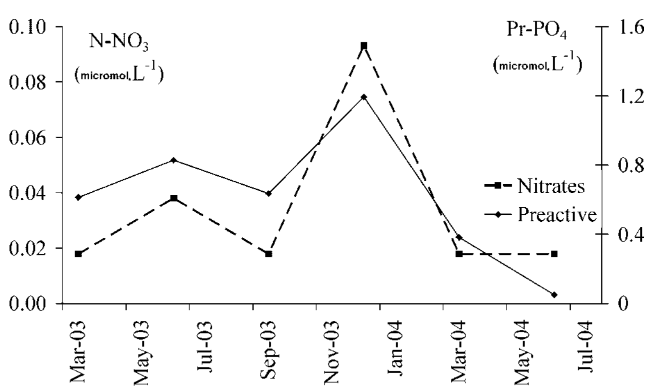

Figure 4. Temporal distribution of reactive phosphorus and nitrate

Silica is another important nutrient because it is essential for the diatoms growth and the dominance of these species in many aquatic environments (Horne and Goldman, 1994). We found that silica was relatively high with low variation in relation to other nutrients. This indicated that, although its consumption by phytoplankton, it was not a limiting nutrient. The Analysis of the Variance showed that there were no significant differences along the time and for the different sampling sites. The concentration in the fluvial section was affected by the clays contribution, since it increased slightly towards Rw, whereas in the reservoir the concentration was related to diatoms growth.

The chlorophyll showed low values and high variation and was always lower than pheopigments. This fact indicates a possible enhancement of chlorophyll degradation promoted by photo-oxidation mainly in periods of strong irradiation that has influence in the upper layers of the euphotic zone (Lorenzen, 1967; Loez, 1995) and by temperature (Wetzel, 1981) especially in these oxygenated waters. Temporal variation of pigments was inverse to that of the nutrients and this variation was higher than the spatial one (Table III).

Conductivity is related to total dissolved solids (TDSs) by a factor. Taking under consideration the sum of major anions (HCO_3^-, SO_4^- and Cl^-) and cations (Ca^{++}, Mg^{++}, Na^+ and K^+) concentrations to express TDS, the factor was equal to 0.81 ($s = 0.06$, $n = 32$). Then, multiplying the average conductivity (20°C) with this factor, a result of 211 mg L^{-1} was obtained that has the mean of a moderate saline water body. Although conductivity presented little variability in all the system, reflecting a poor contribution of soils, ANOVA showed spatial and temporal significant differences (Table III). Minimum and maximum values for all sampling sites were observed at warm and cold seasons, respectively, suggesting that the evaporation, during high temperature periods, did not concentrate salts and the rain, occurring during low temperature periods, did not influence the transport and dragging of salts. All the processes involved did not change the saline matrix of the system (CV is only 15%) and only a higher salinity in the Tw–Rw was detected that may be related to a more dragging effect of minerals in this zone.

The values of *Secchi disk* in the reservoir presented temporal variations that were in accordance to the seasonal change of phytoplankton biomass. These values were higher than the values obtained in the river that showed a relatively temporal stability. This fact may be associated to the mineral origin of the SS, where the decrease registered downstream may be caused by soil erosion. Other related parameters such as SS and turbidity agreed with these results.

DOI: 10.1002/rra

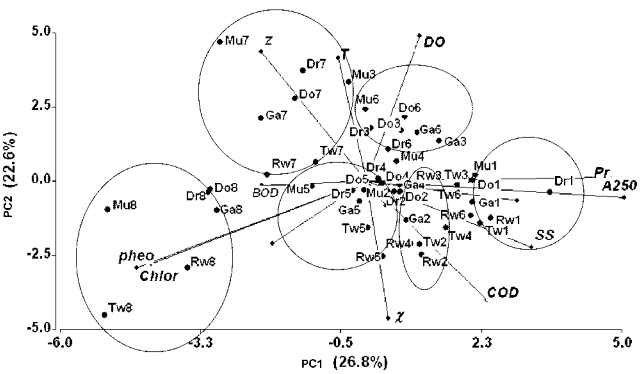

Figure 5. PCA: combinations of sampling sites (letters) and dates (subscript number) and environmental variables on main plane

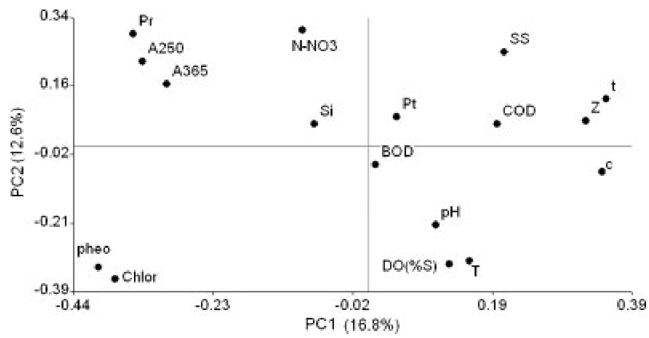

Figure 6. Principal coordinates analysis

The low values of BOD indicated a high water quality, which were in correspondence to the high percentage of saturation in dissolved oxygen observed (91%).

All the sites had similar values of COD except Tw and Rw. In the latter, a slight increase was observed in accord with the values determined for A_{250}, suggesting a higher anthropogenic activity in this section.

Relationships between HSs and limnological parameters: multivariate analysis

Principal Components Analysis (PCA) including only the variables (14) that did not present lack of data was made from matrix of standardized data. The first Principal Component (PC1) accounts for 26.8% of the data variation, and the second (PC2) accounts for 22.6% of the data variation. Thus, the two main PCs explain only 49.4% of the total variance (Figure 5). This shows the influence of diverse factors and the complexity of the system under study, as is common in environments of this nature.

In PCA, A_{250} and Pr were the variables of greatest correlation with the factorial axes, followed by pheopigments, DO, chlorophyll, conductivity, Secchi disk transparency and Temperature. A_{250} and Pr have weight in the first axis in the positive direction, and pigments (pheopigments and chlorophyll) in the negative direction, indicating positive correlation of A_{250} with Pr and negative correlation of these variables with pigments. The four variables show only small weight in the PC2. The variables DO, T and transparency have weight in PC2 in positive direction and conductivity and COD in the negative direction, indicating positive correlation among the former and negative correlation of these variables with the latter ones. Only T has weight in the PC3. The groups of Figure 6 (subjective circles) describe the system. They are performing by dates and not by sampling sites, corroborating the strong temporary influence previously mentioned.

In order to analyse the relations among the variables, including those without complete data, a Principal Coordinates Analysis from the matrix of correlation of the total of the variables (17) was made. Figure 6 show that the main plane explains only 29.4% of the total variance. In general, the correlation coefficients were low. It is important no notice that A_{250} was the variable correlated with greatest number of parameters and with highest correlation index. Those which should be emphasize are the index with Pr (0.83) and inverse with chlorophyll (−0.52) and pheopigments (−0.59) and in smaller degree with nitrate (0.50). Nitrates are correlated with Pr (0.76). It is also important to see that BOD and COD are not correlated to each other, showing that the contribution of anthropogenic organic matter is only slightly significant. Conductivity has positive correlation with turbidity (0.63) and negative with temperature (−0.34), suggesting an increase of TDS at lower temperatures, which support the above discussion.

CONCLUSIONS

The above-described results showed that the processes developed in fresh waters of the lower section of Chubut River, whose complexity was demonstrated by the multivariate analyses, are dominated by those produced in the Florentino Ameghino Reservoir. The HSs are fundamentally generated in the reservoir. These are in very low concentrations typical of their aquagenic origin, exhibit spatial homogeneity and temporal variations follow a temporal distribution similar to the nitrates and reactive phosphorus. The ratio A_{250}/A_{365} showed a predominance of fulvic acids over humic acids. These conclusions corroborate the results of previous works about their mixed origin with superiority of aquagenic HSs.

ACKNOWLEDGEMENTS

The authors thank CONICET for financial support.

DOI: 10.1002/rra

REFERENCES

APHA- AWWA-WEF. 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th edn, American Public Health Association: Washington.

Bloom PR, Leenheer JA. 1989. Vibrational, electronic, and high-energy spectroscopic methods for characterizing humic substances. In *Humic Substances II. In Search of Structure*, Hayes MHB, Mc Carthy P, Malcom RL, Swift RS (eds). John Wiley & Sons Ltd: Chichester; 409–446.

Conzonno VH. 1995. Métodos para análisis químicos. In *Ecosistemas de aguas continentales. Metodologías para su estudio. Tomo I*, Lopretto E, Tell G (eds). Ediciones Sur, La Plata: Argentina; 231–250.

Conzonno VH, Fernández Cirelli A. 1987. Soluble humic substances from the affluents of Chascomús Pond (Argentina). *Archiv. für Hydrobiologie* **109**: 305–314.

Conzonno VH, Fernández Cirelli A. 1987/1988. Aplicabilidad de las determinaciones de absorción en el UV-Visible para la caracterización de las sustancias húmicas en ambientes acuáticos. *Ecosur* **14/15**(25/26): 1–6.

Conzonno VH, Fernández Cirelli A. 1988. Soluble humic substances from Chascomús Pond (Argentina). Factors influencing distribution and dynamics. *Archiv für Hydrobiologie* **111**: 467–473.

Conzonno VH, Fernández Cirelli A. 1996. Humic substances and phytoplankton primary production in Chascomus Pond (Argentina). Facts and Speculations. *Revista de la Asociación de Ciencias Naturales del Litoral* **21**(1): 35–42.

Fooken U, Liebezeit G. 2000. Distinction of marine and terrestrial origin of humic acids in North Sea surface sediments by absorption spectroscopy. *Marine Geology* **164**: 173–181.

Frimmel FH, Abbt-Braun G, Mueller M, Brinkmann T, Lankes U, Saravia F. 2004. Humic matter in water treatment, learning from nature. In *Humic Substances and Soil and Water Environment. Proceedings of XII International Meeting of International Humic Substances Society.* Martin Neto L, Marcondes Bastos Pereira Milori D, Lopes da Silva WT (eds). Embrapa Instrumentação Agropecuária, ISBN 85-86463-12-4: São Pedro, São Pablo, Brazil; 45–47.

Golterman HL, Clymo RS, Ohnstad MAM. 1978. *Methods for Phisical an Chemical Analysis of Fresh Waters*, IBP Handbook No. 8. Blackwell Scientific Publications: Oxford.

Helbling EW, Barbieri E, Marcoval A, Goncalves R, Villafañe V. 2005. Impact of Solar Ultraviolet Radiation on Marine Phytoplankton of Patagonia, Argentina. *Photochemistry and Photobiology* **81**: 807–818.

Hertkorn N, Claus H, Schmitt Kopplin PH, Perdue EM, Filip Z. 2002. Utilization and transformation of aquatic humic substances by autochthonous microorganisms. *Environmental Science and Technology* **36**: 4334–4345.

Horne AJ, Goldman ChR. 1994. *Limnology.* McGraw Hill, Inc.: New York.

Hutchison EG. 1975. *A Treatise on Limnology*, Vol. I, Part 2, Wiley Interscience: New York.

InfoStat. 2008. InfoStat versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Köhler H, Meon B, Gordeev V, Spitzy A, Amon R. 2003. Dissolved organic matter (DOM) in the estuaries of Ob and Yenisei and the adjacent Kara Sea, Russia. *Proceedings in Marine Science* **6**: 281–309.

Loez C. 1995. Determinación de clorofila a. In *Ecosistemas de aguas continentales. Metodologías para su estudio. Tomo I*, Lopretto E, Tell G (eds). Ediciones Sur, La Plata: Argentina; 263–269.

Lorenzen CJ. 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnology and Oceanography* **12**: 343–346.

Martin-Mousset B, Crou JP, Lefebvre E, Legube B. 1997. Distribution et Caractérisation de la Matière Organique Dissoute D'Eaux Naturelles de Surface. *Water Research* **31**: 541–553.

Miserendino ML. 2006. Seasonal and spatial distribution of stoneflies in the Chubut River (Patagonia, Argentina). *Hydrobiologia* **568**: 263–274.

Parks SJ, Baker LA. 1997. Sources and transport of organic carbon in an Arizona river-reservoir system. *Water Research* **31**(7): 1751–1759.

Pasquini AI, Depetris PJ, Gaiero DM, Probst JL. 2005. Material sources, chemical weathering, and physical denudation in the Chubut River Basin (Patagonia, Argentina): implications for Andean Rivers. *Journal of Geology* **113**: 451–469.

Santinelli N, Esteves JL. 1993. Características químicas y fitoplanctónicas del estuario del Río Chubut (Patagonia Argentina). *Naturalia Patagónica. Ciencias Biológicas* **1**: 22–34.

Scapini MC, Conzonno VH, Balzaretti VT, Fernández Cirelli A. 2004. Structural aspects of aquatic humic substances in a Patagonic River. In Humic Substances and Soil and Water Environment. Proceedings of XII International Meeting of International Humic Substances Society. Martin Neto L, Marcondes Bastos Pereira Milori D, Lopes da Silva WT (eds). Embrapa Instrumentação Agropecuária, ISBN 85-86463-12-4: São Pedro, São Pablo, Brazil; 365–367.

Treguer P, Le Corre P. 1975. *Manuel d́analyse des sels nutritifs dans léau de mer (Utilisation de ĺ Autoanalyzer II Technickon ^{R})*, 2eme edn. Université de Bretagne Occidentale: Brest.

Wetzel RG. 1981. *Limnología*. Ediciones Omega, S.A.: Barcelona.

DOI: 10.1002/rra