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Distribution and environmental preferences of diatoms along the Negro River, Patagonia, Argentina

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ABSTRACT

Distribution and environmental preferences of diatoms along the Negro River, Patagonia, Argentina

Surface sediment samples from the Negro River, northern Patagonia, were examined to identify major environmental variables that influence the diatom distribution. Assemblages were highly diverse and heterogeneous and its community structure responds to a salinity gradient. Cluster analysis identified two major diatom zones: the former grouped sites with marine influence and the latter freshwater ones. Partial RDAs analysis indicates that salinity was the main factor affecting diatom communities. Riverine sites assemblages were dominated by tychoplanktonic fragilarioids, while estuarine sites were characterized by marine-brackish taxa. The occurrence of marine taxa in the freshwater estuary shows the maximum intrusion of marine biogenic particles at a more landward position. Results showed that the highest nitrate concentration is found at the lower valley of the river. Ecological preferences of dominant and less represented taxa also suggest the existence of anthropogenic processes that would be affecting the water quality of the river.

Key words: fluvial system, mesotidal estuaries, multivariate analysis, surface sediments, Argentina, Patagonia

RESUMEN

Distribución y preferencias ambientales de los ensambles de diatomeas del Río Negro, Patagonia, Argentina

Muestras de sedimento superficial del Río Negro, Patagonia norte, fueron analizadas para identificar las variables ambientales que en gran medida influyen en la distribución de las diatomeas. En general, los ensambles presentaron una alta diversidad y heterogeneidad, en tanto que la estructura de las comunidades respondió a variaciones en el gradiente de salinidad. El análisis de agrupamiento identificó dos zonas diatomológicas principales: la primera, agrupó los ensambles con influencia marina, mientras que en la segunda se incluyeron los ensambles dulceacuícolas. Los análisis parciales de redundancia indicaron que la salinidad afectó significativamente la distribución de las comunidades de diatomeas. Mientras los ensambles dulceacuícolas estuvieron dominados por pequeñas fragilarioides ticoplanctónicas, los sitios estuarinos estuvieron caracterizados por la abundancia de taxones marino-salobres. La abundancia de taxones marinos en los sitios dulceacuícolas permitió detectar la máxima capacidad de penetración de partículas biogénicas marinas en el tramo superior del estuario. Los sitios ubicados en el valle inferior del río muestran las concentraciones más altas de nitratos en el agua. Las preferencias ecológicas de las especies dominantes y de los taxones menos representativos, también sugieren la existencia de procesos antrópicos que potencialmente estarían afectando la calidad del agua en el río.

Palabras clave: *sistema fluvial, estuarios mesomareales, análisis multivariado, sedimentos superficiales, Argentina, Patagonia*

INTRODUCTION

Composition and distribution of diatom assemblages in freshwater (e.g. rivers and streams) and coastal ecosystems (e.g. estuaries, coastal lagoons, deltas), are determined by the intricate and non-linear relationship between prevailing environmental conditions and biological interactions (Margalef, 1983). Although it is difficult to accurately estimates these attributes due to the biological dynamics of the communities, different methods are available for a valuable approximation of the structure and functioning of the assemblages (Guisan & Zimmermann, 2000).

Biological communities and their major associated environmental variables show complex numerical data, containing an important amount of incomplete and inconsistent information that hinders their ecological interpretation (Birks, 2010). Standard multivariate analysis of ecological observations allows the structuring and systematization of this information, providing insight about some valuable elements for a suitable biological interpretation (Legendre & Legendre, 2012). In the broadest sense, these analyses are essential not only to quantify the autoecology of the species, but also to estimate and prove statistically how an assemblage of species respond simultaneously to natural and anthropogenic stressors (Juggins & Birks, 2012).

Diatoms show rapid changes in their growth rates, abundances and specific composition in response to biotic and abiotic fluctuations (Round *et al.*, 1990). This sensitivity to physical, chemical and biological changes makes them one of the most reliable bioindicators of recent and past environmental changes. Concerning palaeolimnological studies, the species-environment relationship is very important for developing of meaningful and useful quantitative reconstructions of past environmental variables from both lacustrine and marine systems (Birks *et al.*, 2010; Stevenson *et al.*, 2010). The interaction between diatom sediment thanatocoenoses (which integrates the seasonal and spatial variation of communities in the river) and modern environmental conditions, has become the most widely used approach in the last decades to elucidate such ecological information, which may be used as analogues to interpret the fossil record, particularly in Holocene researches (Juggins & Birks, 2012). A better spatial perspective of

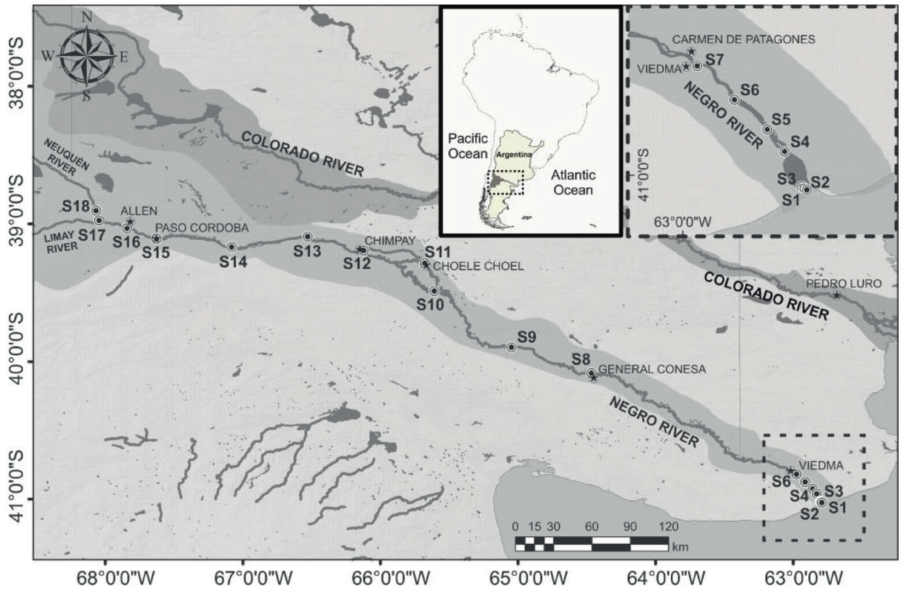

Figure 1. Location map showing sampling sites in the Negro, Limay and Neuquén rivers, north Patagonia, Argentina. *Localización de los sitios de muestreo en los ríos Negro, Limay y Neuquén, Patagonia norte, Argentina.*

diatom autoecology, would provide exhaustive information about biological and environmental reference conditions of a particular aquatic system (Soininen, 2007).

There are several studies related to the taxonomy and ecology of diatoms in Argentina in contrast to other countries in South America. However, this information is still scattered, incomplete and heterogeneous, mainly because these studies are conducted in Buenos Aires and Córdoba provinces (Vouilloud, 2003). Most of the diatomological studies carried out in northern Patagonia have focused on the publication of lists and taxonomic descriptions, or have been part of phytoplankton studies (Santinelli *et al.*, 1990; Sastre *et al.*, 1994; Vouilloud & Leonardi, 2001; Guerrero & Echenique, 2002; Echenique & Guerrero, 2003; Sar *et al.*, 2003; Garibotti *et al.*, 2011). Recently, the composition and distribution of diatom communities, and their relationship with some environmental variables have been analyzed in the middle and lower valley of the Colorado, Negro and Chubut rivers (Espinosa & Isla, 2015; Vélez-Agudelo *et al.*, 2017).

Ecological requirements of diatoms that inhabit freshwater and coastal environments in north Patagonia have not been thoroughly addressed, and further investigations are needed. This study focuses on diatom composition and the major environmental variables that determine their distribution in surface sediments from Negro River using specific numerical procedures. The aim of the present study is twofold: (1) to describe the primary environmental gradient and the corresponding diatom communities, and (2) to determine the environmental requirements of common species that provide information concerning water quality. The increase of autoecological information will allow more reliable ecological inferences and the future development of more accurate calibration models for the north of Patagonia.

STUDY AREA

The Negro River basin covers an area of about 140 000 km^2 and is the largest drainage watershed of Patagonia. It extends from the Andes to the Atlantic Ocean, flowing across the plateau in an NW-SE direction. Two well-defined geomorphological units are recognized in the basin: Andean Patagonia to the west and the extra-Andean plateau lands at the east (Depetris *et al.*, 2005). The upper basin is drained by the Neuquén and Limay rivers; these rivers converge and originate the Negro River, the most important hydrographic system of Patagonia according to hydrological and economical aspects (Fig. 1) (Piccolo & Perillo, 1999). Along its 635 km length, the riverbed varies in width and receives no other tributary. At the middle valley the main course is dominated by meandering channel systems that are activated seasonally (Depetris *et al.*, 2005). The river has a pluvio-nival regime with a mean annual discharge of about 900 m^3/s. Two freshwater inflow peaks are related to winter rainstorms and spring snow melting. The middle and lower basins have an arid to semi-arid climate, with rainfall of no more than 250 mm per year (Coronato *et al.*, 2008). The Negro River estuary has semidiurnal mesotidal conditions, and the mean tidal amplitude may reach up to about 3.3 m. The outlet is characterized by sand-banks and islets with muddy marshes. The ebb-delta has a main channel with depths ranging from 6 to 8 m (Vergara Dal Pont *et al.*, 2017).

Of the eight main rivers draining Argentinian Patagonia, Negro River has the largest drainage basin, the highest total mean annual discharge (49 %) and the highest population density (7.1 inh/km) (Gaiero *et al.*, 2003). Several dams have been constructed at the Neuquén and Limay rivers since the end of the 1960s, modifying the natural hydrological and sedimentological dynamics of the river (Isla *et al.*, 2010). The upper valley of the river is considered one of the most important agricultural areas in the country. Because of these intensive farming practices, different Persistent Organic Pollutants (POPs) have been detected along the valley, and could be a significant source of water pollution in riverine and coastal areas (Miglioranza *et al.*, 2013; Ondarza *et al.*, 2014). Other pollution sources in the basin are also related to occurrence of hydroelectric plants, sewage effluents and petrochemical activities (Migueles, 2019).

MATERIALS AND METHODS

Field and laboratory procedure

Eighteen sampling sites along the Negro River and its two tributaries (Limay and Neuquén rivers) were selected to collect sediment samples (uppermost layer) and measure the environmental variables (Fig. 1). The selected sites include a wide range of fluvial typologies, geomorphological features and land uses. Physical and chemical variables of surface water were measured in winter (July 2014) and summer (February 2015), while surface sediment samples for diatom and sedimentological analyses were collected in winter (July 2014).

Salinity, conductivity, water temperature and pH were measured *in situ* using a Horiba U-10 analyzer. In addition, water samples were taken in polypropylene bottles to measure silica (SiO_2), total hardness and major ions content (HCO_3^-, Cl^-, SO_4^-, NO_3^-, Na^+, K^+, Ca^{2+} and Mg^{2+}). Water samples were acidified (HCl 10 %) and refrigerated for nitrate analysis. All samples were transported in dark to the laboratory for processing following standard methods (APHA, 1998).

Diatom samples were collected from surface sediments of the littoral zone with plastic tubes (20 mm x 100 mm). For each site, three samples were taken and then the uppermost 1 cm of sediment from each one was carefully extracted and combined for cleaning and analysis. Inlet site samples were collected from the foreshore at low tide. The surface sediments make up an integrate sample that can represent all the habitats in the aquatic systems, providing faithful information on physical and chemical water conditions (Smol, 2008). In alluvial floodplains of the rivers, where the slow water flow favours the accumulation of the finest fraction of sediments, the epipelic component constitutes a reliable tool to investigate changes in the fluvial diatoms assemblages (Gómez & Licursi, 2001).

Surface sediment layer (ca. 500 g) was scraped from the littoral zone using a spoon, and placed into airtight plastic bags for sedimentological analysis. In the laboratory, a subsample (ca. 100 g) was dried to quantify the proportion of gravel (> 2 mm in diameter), coarse sand (2 mm-500 µm), medium sand (250-499 µm), fine sand (125-249 µm), very fine sand (62-124 µm) and mud (< 62 µm) using the dry-sieving technique (Folk, 1980). Total Organic Carbon (TOC) content of surface sediments was estimated according to the Walkley & Black (1934) method.

Diatom analysis

Diatom samples were processed by hot digestion using hydrogen peroxide (H_2O_2 30 %) and hydrochloric acid (HCl 10 %) to remove organic matter and carbonates, respectively. Subsequent washings with distilled water were used to eliminate the excess of chemical substances until reaching neutral pH. Then, samples were dried on cover glass and permanent diatom slides were mounted with *Zrax®*. For each sample at least 500 valves were identified and counted in random transects under oil immersion using a *Zeiss Axiostar plus* light microscope. Scanning electron microscope (SEM; Jeol JSM-6460 LV) operated at 15 kV was used to confirm taxonomic classification. Each specimen was identified to the lowest taxonomic level according to the standard literature.

Statistical analysis

Standard ecological multivariate analyses were carried out to identify environmental factors controlling diatom distribution along the river. Principal Component Analysis (PCA), based on a correlation matrix was used in order to reduce data set dimensionality and to examine major patterns of variation in environmental data between all sampling sites. Prior to this ordination, winter and summer data of physical and chemical variables were averaged. PCA was performed for the complete data set (18 sampling sites) and the reduced data set (15 sampling sites), in which the estuarine sites were excluded (S1, S2 and S3).

Diatom counts were expressed as percentages, and for further statistical analysis only those taxa with abundances higher than 2 % in at least one sample were included. Diatom data were square-root transformed, and environmental variables were log (x+1) transformed prior to ordination analysis. Diatom zones were defined

Table 1. Physical and chemical variables of water from 18 sampling sites in the Negro, Limay and Neuquén rivers. Cond: conductivity (µS/cm). T: water temperature (ºC). Sal: salinity (‰). Hard: Total Hardness (mg/L of $CaCO_3$). Concentrations of ions are expressed in mg/L. *Variables físicas y químicas del agua en 18 sitios de muestreo de los ríos Negro, Limay y Neuquén. Cond: conductividad (µ S/cm). T: temperatura del agua (ºC). Sal: salinidad (‰). Hard: Dureza total (mg/L de $CaCO_3$). Las concentraciones de iones son expresadas en mg/L.*

Sample site	Location	Season	pH	Cond	T	Sal	Hard	HCO_3^-	Cl^-	SO_4^{2-}	NO_3^-	Ca^{2+}	Mg^{2+}	Na^+	K^+	SiO_2
S1	41°01'27.3"S ; 62°47'35.8"W	Summer	9.48	6420	22.40	3.60	629	181	1755	148	1.40	140	67	1100	15	35.70
		Winter	8.80	28 300	8.50	17.10	6513	75.70	9406	1440	5.70	400	1323.10	4400	220	0.50
		Mean	**9.14**	**17 360**	**15.45**	**10.35**	**3571**	**128.35**	**5580.50**	**794**	**3.55**	**270**	**695.05**	**2750**	**117.50**	**18.35**
S2	41°01'19.9"S ; 62°47'50.1"W	Summer	9.54	10 200	22.70	5.60	1256	104	2384	224	3.60	208	176	1000	55	20
		Winter	8.75	21 350	8.35	12.40	1553	75.70	1566	1770	0.70	574	28.30	1300	120	4.14
		Mean	**9.14**	**15 780**	**15.53**	**9.00**	**1404.50**	**89.85**	**1975**	**997**	**2.15**	**391**	**102.15**	**1150**	**87.50**	**12.07**
S3	41°01'06.6"S ; 62°48'08.7"W	Summer	9.55	7120	22.60	3.80	864	83.50	2471	126	2.20	178	100.50	1200	30	7.90
		Winter	8.43	13 850	6.50	7.70	1072.60	212.10	5070	480	3.80	272	94.20	2800	7	4
		Mean	**8.99**	**10 480**	**14.55**	**5.75**	**968.30**	**147.80**	**3770.50**	**303**	**3.00**	**225**	**97.35**	**2000**	**18.50**	**5.95**
S4	40°57'38.3"S ; 62°49'49.9"W	Summer	7.51	410	22.00	0.10	93.50	63	78.70	60	10.90	11	16.80	70	1	40.70
		Winter	8.39	290	7.70	0.10	285.40	75.70	73.40	140	6.20	23	54.60	29	1.30	2
		Mean	**7.95**	**350**	**14.85**	**0.10**	**189.45**	**69.35**	**76.05**	**100**	**8.55**	**17**	**35.70**	**49.50**	**1.15**	**21.35**
S5	40°55'25.3"S ; 62°51'34.1"W	Summer	8.75	200	22.10	0	188	174	62.	39	3.70	10	39.10	30	1	5
		Winter	7.72	160	7.90	0	282	60.60	56	150	2.80	21	55	18	0.90	4.40
		Mean	**8.24**	**180**	**15.00**	**0**	**235**	**117.30**	**59**	**94.50**	**3.25**	**15.50**	**47.05**	**24**	**0.95**	**4.70**
S6	40°52'27.4"S ; 62°54'51.1"W	Summer	8.69	200	21.80	0	110	153	58.50	37	4.60	11	20	51	1.40	15.40
		Winter	7.86	160	9.35	0	311	75.70	45.40	170	14.10	40	50.60	15	0.60	6.90
		Mean	**8.28**	**180**	**15.58**	**0**	**210.50**	**114.35**	**51.95**	**103.50**	**9.35**	**25.50**	**35.30**	**33**	**1**	**11.15**
S7	40°49'03.8"S ; 62°58'34.4"W	Summer	8.79	200	21.90	0	125.60	90.40	29.20	29.	2.30	2	28.90	16	0.70	14.80
		Winter	7.82	160	8.35	0	360	128.70	45.40	180	2.50	48	57.60	17	0.70	3.80
		Mean	**8.31**	**180**	**15.13**	**0**	**242.80**	**109.55**	**37.30**	**104.50**	**2.40**	**25**	**43.25**	**16.50**	**0.70**	**9.30**
S8	40°04'58.3"S ; 64°28'05.1"W	Summer	8.69	170	25.00	0	205	180	22	29	0.30	22	36	11	1.40	12
		Winter	7.73	140	8.75	0	182	98.50	21	75	10.00	10	37.60	15	0.80	3.30
		Mean	**8.21**	**160**	**16.88**	**0**	**193.50**	**139.25**	**21.50**	**52**	**5.15**	**16**	**36.80**	**13**	**1.10**	**7.65**
S9	39°53'46.0"S ; 65°02'54.7"W	Summer	8.77	170	22.20	0	415	69.60	475	34	2.70	11	93	170	17	11
		Winter	7.65	140	8.95	0	136	61	17.20	60	10.00	20	21	10	3	2.60
		Mean	**8.21**	**150**	**15.58**	**0**	**275.50**	**65.30**	**246.10**	**47**	**6.35**	**15.50**	**57**	**90**	**10**	**6.80**
S10	39°29'16.2"S ; 65°36'33.2"W	Summer	8.60	200	22.50	0	173	104	52	28	1.40	2	40.30	17	0.60	7.50
		Winter	8.11	180	8.40	0	115	91	31	28	6.20	20	15.60	13	1.20	3.06
		Mean	**8.35**	**190**	**15.45**	**0**	**144**	**97.50**	**41.50**	**28**	**3.80**	**11**	**27.95**	**15**	**0.90**	**5.28**
S11	39°17'07.0"S ; 65°40'42.4"W	Summer	8.64	170	21.30	0	126	160	18.20	31	0.80	2	29.04	20	1.10	8.10
		Winter	7.62	150	8.80	0	123.60	76	38	29	10.00	18	19	10	5.40	3.40
		Mean	**8.13**	**160**	**15.05**	**0**	**124.80**	**118**	**28.10**	**30**	**5.40**	**10**	**24.02**	**15**	**3.25**	**5.75**
S12	39°11'37.4"S ; 66°07'17.5"W	Summer	8.65	150	21.20	0	150	104	36.50	24	2.90	10	30	12	0.80	8.10
		Winter	7.64	130	8.80	0	165	106	42	20	1.00	8	34.80	10	0.90	5.56
		Mean	**8.14**	**140**	**15.00**	**0**	**157.50**	**105**	**39.25**	**22**	**1.95**	**9**	**32.40**	**11**	**0.85**	**6.83**
S13	39°05'30.9"S ; 66°31'45.4"W	Summer	8.82	150	22.00	0	200	55.60	182	24	0.50	6	44	47	0.60	9.90
		Winter	7.81	120	8.70	0	132	106	44.70	19	1.70	18	21	10	2	3.30
		Mean	**8.31**	**140**	**15.35**	**0**	**166**	**80.80**	**113.35**	**21.50**	**1.10**	**12**	**32.50**	**28.50**	**1.30**	**6.60**
S14	39°10'04"S ; 67°04'55.4"W	Summer	8.54	390	20.90	0	261	248.50	20.50	57	0.60	6	59.60	28	2	25.40
		Winter	7.53	120	9.10	0	140	91	27.50	22	12.30	10	27.60	7	0.90	2.55
		Mean	**8.03**	**250**	**15.00**	**0**	**200.50**	**169.75**	**24**	**39.50**	**6.45**	**8**	**43.60**	**17.50**	**1.45**	**13.98**
S15	39°06'30.3"S ; 67°37'38.9"W	Summer	8.83	130	20.90	0	157	118	29.20	19	3.00	3	35.80	7	0.60	4.80
		Winter	7.74	110	9.30	0	99	136	38	17	6.30	9	18.30	50	1.30	2.44
		Mean	**8.28**	**120**	**15.10**	**0**	**128**	**127**	**33.60**	**18**	**4.65**	**6**	**27.05**	**28.50**	**0.95**	**3.62**
S16	39°01'56.0"S ; 67°50'31.1"W	Summer	8.98	160	21.10	0	153	111.00	33.00	30	2.70	18	26	10	2.10	7.30
		Winter	7.82	130	9.10	0	123	76.00	27.50	26	0.50	10	23.50	8	0.80	2.50
		Mean	**8.40**	**140**	**15.10**	**0**	**138**	**93.50**	**30.25**	**28**	**1.60**	**14**	**24.75**	**9**	**1.45**	**4.90**
S17	38°58'30.7"S ; 68°02'42.1"W	Summer	8.89	70	21.80	0	128.70	69.60	47.50	13	2.10	4	28.40	2	0.30	12.40
		Winter	7.90	130	9.10	0	140	106.00	38.00	22	6.50	6	30	10	0.20	3.25
		Mean	**8.40**	**100**	**15.45**	**0**	**134.35**	**87.80**	**42.75**	**17.50**	**4.30**	**5**	**29.20**	**6**	**0.25**	**7.83**
S18	38°54'14.4"S ; 68°04'02.0"W	Summer	8.88	250	21.30	0	182	150.00	22.00	38	3.90	4	41.20	15	1.40	8.50
		Winter	7.87	250	9.05	0	163	98.40	51.60	40	10	26	23.50	14	1.90	3.73
		Mean	**8.38**	**250**	**15.18**	**0**	**172.50**	**124.20**	**36.80**	**39**	**6.95**	**15**	**32.35**	**14.50**	**1.65**	**6.12**

using constrained hierarchical clustering based on Bray-Curtis dissimilarity, and the statistical significance to detect the ecological zones was assessed through the broken stick model. Nonmetric multidimensional scaling (NMDS), using the Bray-Curtis dissimilarity index, combined with a surface fitting function to visualize the distribution of the most abundant species was applied. This function fits a smooth surface using thin-plate splines according to values of the relative percentages. Redundancy Correspondence Analysis (RDA) was conducted to explore the relationship between the transformed diatom abundance data and selected environmental variables. Variance partitioning of physical and chemical variables and sedimentological variables was performed to assess whether each group explains unique aspects of species composition. Significant and independent contribution of the selected variables was estimated by using a series of partial RDAs. The statistical significance of the RDA and partial RDAs were assessed by ANOVA permutation tests. In addition, diatom species richness (S) and Shannon index (H') (log base = *e*) were calculated (Potapova & Charles, 2003).

All analyses were conducted with the statistical software R version 3.2.2 (R Development Core Team, 2015), using the packages "rioja" version 0.9-5 (Juggins, 2015) and "vegan" version 2.3-0 (Oksanen *et al.*, 2015).

RESULTS

Environmental variables

Physical, chemical and sedimentological data are listed in Tables 1 and 2. According to pH values, the surface water of Negro River is slightly basic to basic. Sites closest to the mouth (S1, S2 and S3) had the highest values for this variable both in

Table 2. Sedimentological variables from 18 sampling sites in the Negro, Limay and Neuquén rivers. Total Organic Carbon (TOC) and categories of grain size are expressed in percentage (%). *Variables sedimentológicas de 18 sitios de muestreo de los ríos Negro, Limay y Neuquén. El Carbono Orgánico Total (TOC) y el tamaño de grano son expresados en porcentajes (%).*

Sample sites	TOC	Gravel (>2mm)	Coarse Sand (2mm-500µm)	Medium Sand (250-499µm)	Fine Sand (125-249µm)	Very Fine Sand (62-124µm)	Mud (<62µm)
S1	0.18	4.07	38.07	20.68	31.61	5.48	0.10
S2	0.25	6.07	15.60	12.34	45.53	16.95	3.50
S3	1.20	0	2.19	7.32	28.47	22.92	39.10
S4	1.65	0	1.82	4.80	12.45	30.53	50.40
S5	1.63	0.10	4.42	16.99	22.05	29.97	26.47
S6	1.57	0	0.96	2.49	10.04	33.43	53.08
S7	0.91	0	3.63	3.18	15.58	33.07	44.54
S8	0.72	0	0.21	0.46	31.51	40.84	26.97
S9	0.53	0.41	1.10	4.29	34.50	37.12	22.57
S10	0.82	23.09	1.19	2.04	15.94	25.51	32.22
S11	0.82	8.68	1.02	8.03	50.44	20.35	11.47
S12	0.41	60.61	5.05	8.61	13.14	6.91	5.69
S13	1.13	0.57	0.67	4.64	26.45	37.06	30.61
S14	0.70	0	0.13	1.55	36.61	35.93	25.78
S15	0.18	0.26	0.43	12.94	64.78	16.83	4.77
S16	0.57	46.06	20.53	17.45	10.02	3.16	2.78
S17	1.95	19.29	15.79	20.82	22.25	12.06	9.79
S18	3.10	37.96	7.72	9.67	13.28	13.03	18.34

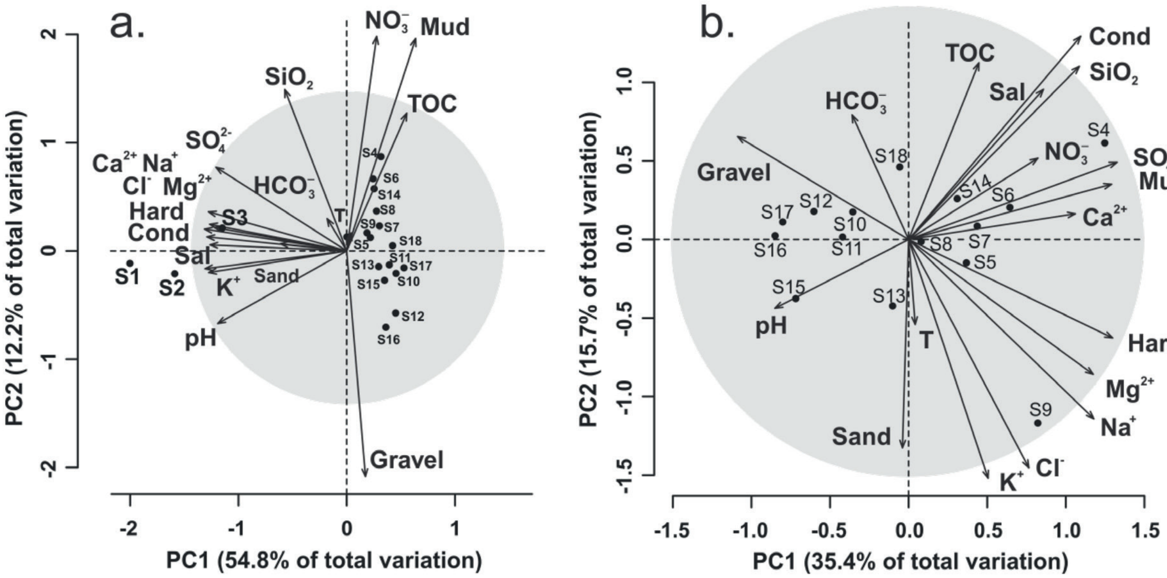

Figure 2. Principal component analysis (PCA) diagram of the environmental data from the Negro River with all sampling sites (a) and only freshwater sites (b). Environmental variables with vectors whose lengths exceed the radius of the equilibrium contribution circle (shadowing in the plot) could be interpreted with greatest confidence. *Gráfico del análisis de componentes principales de las variables ambientales medidas en el Río Negro con todos los sitios de muestreo (a) y únicamente con los sitios dulceacuícolas (b). Las variables ambientales cuya longitud del vector excede el radio del círculo de contribución al equilibrio (sombreado en el gráfico) contribuyen de manera significativa en la varianza total de los datos.*

summer and winter. A seasonal trend occurred with SiO_2 average concentration: 14.1 ± 10.2 mg/L in summer and 3.4 ± 1.4 mg/L in winter. Surface water temperature oscillated between 6.5 and 9.3 ºC in winter and between 20.9 and 25 ºC in summer. It should be noted that in thirteen sampling sites, higher nitrate values were observed in winter. At the others five sampling sites (S2, S4, S5, S12 and S16), the highest values were recorded in summer.

The first two principal components (PCA) based on the total set of environmental variables explained more than 67 % of the total variation. The first axis explained 54.8 % of the total variation, showing a strongly increase of the ionic gradient toward the estuarine sites (from right to left, Fig. 2a). In this component, the group formed by sites S1-S3 exhibited the highest values of pH, conductivity, salinity, hardness, Cl^-, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Na^+, K^+ and sand. The second axis accounted for 12.2 % of the variance, showing positive correlation between SiO_2, NO_3^- and mud, but negative correlation between these variables and gravel percentages.

When the sampling sites near to the mouth (S1, S2 and S3) were excluded from the PCA analysis (Fig. 2b), the first two components explained 51.2 % of the total variation. The first axis accounted for the 35.4 % of the total variation attributed to conductivity, salinity, hardness, SO_4^{2-}, NO_3^-, Ca^{2+}, Mg^{2+}, Na^+, SiO_2 and muds. The group formed by sites S4-S7 and S9 showed the highest values for these variables, describing an ion gradient toward the inland waters closest to the mouth. The sites located at the upper basin (S10-12 and S15-17) had the highest gravel percentages, which in turn showed a negative correlation with the first axis. The second axis explained 20.1 % of the variance, showing a negative correlation with Cl^-, K^+ and sand.

Diatom assemblages: species composition and distribution patterns

Diatom flora identified in the Negro River was taxonomically diverse and included a total of 320

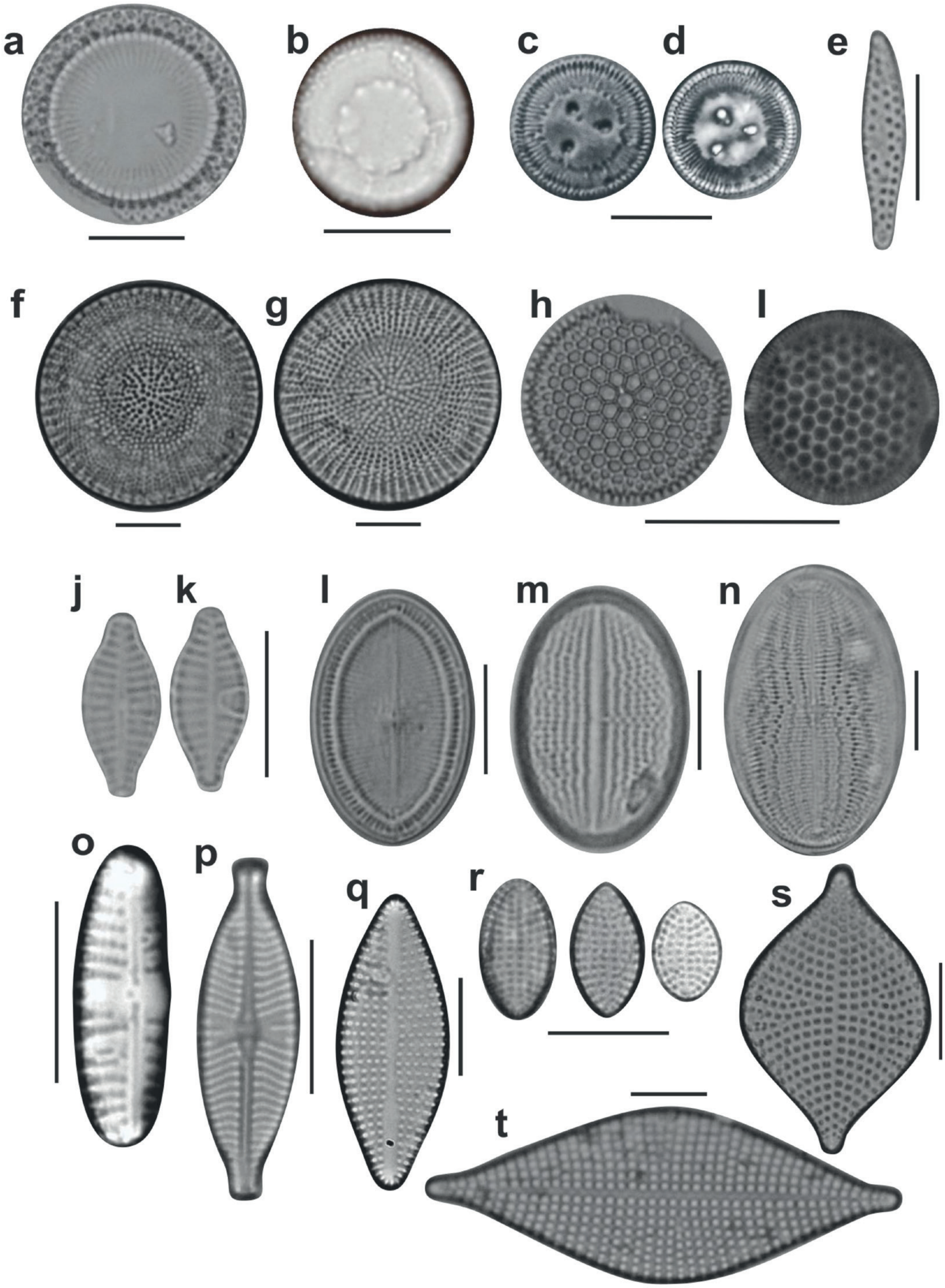

Figure 3. Predominant diatoms in fluvial and estuarine sediments from the Negro River under LM: (a) *Paralia sulcata*; (b) *Paralia sulcata* var. *coronata*; (c-d) *Cyclotella ocellata*; (e) *Cymatosira belgica*; (f-g) *Stephanodiscus agassizensis*; (h-i) *Shionodiscus oestrupii*; (j) *Planothidium rostratum* (raphe valve); (k) *Planothidium rostratum* (sternum valve); (l) *Cocconeis placentula* (raphe valve); (m) *C. placentula* (sternum valve); (n) *Cocconeis pediculus*; (o) *Reimeria uniseriata*; (p) *Geissleria decussis*; (q) *Delphineis surirella*; (r) *Delphineis minutissima*; (s-t) *Rhaphoneis amphiceros*. *Imágenes en microscopio óptico (ML) de las diatomeas dominantes en sedimentos fluviales y estuariales del Río Negro.*

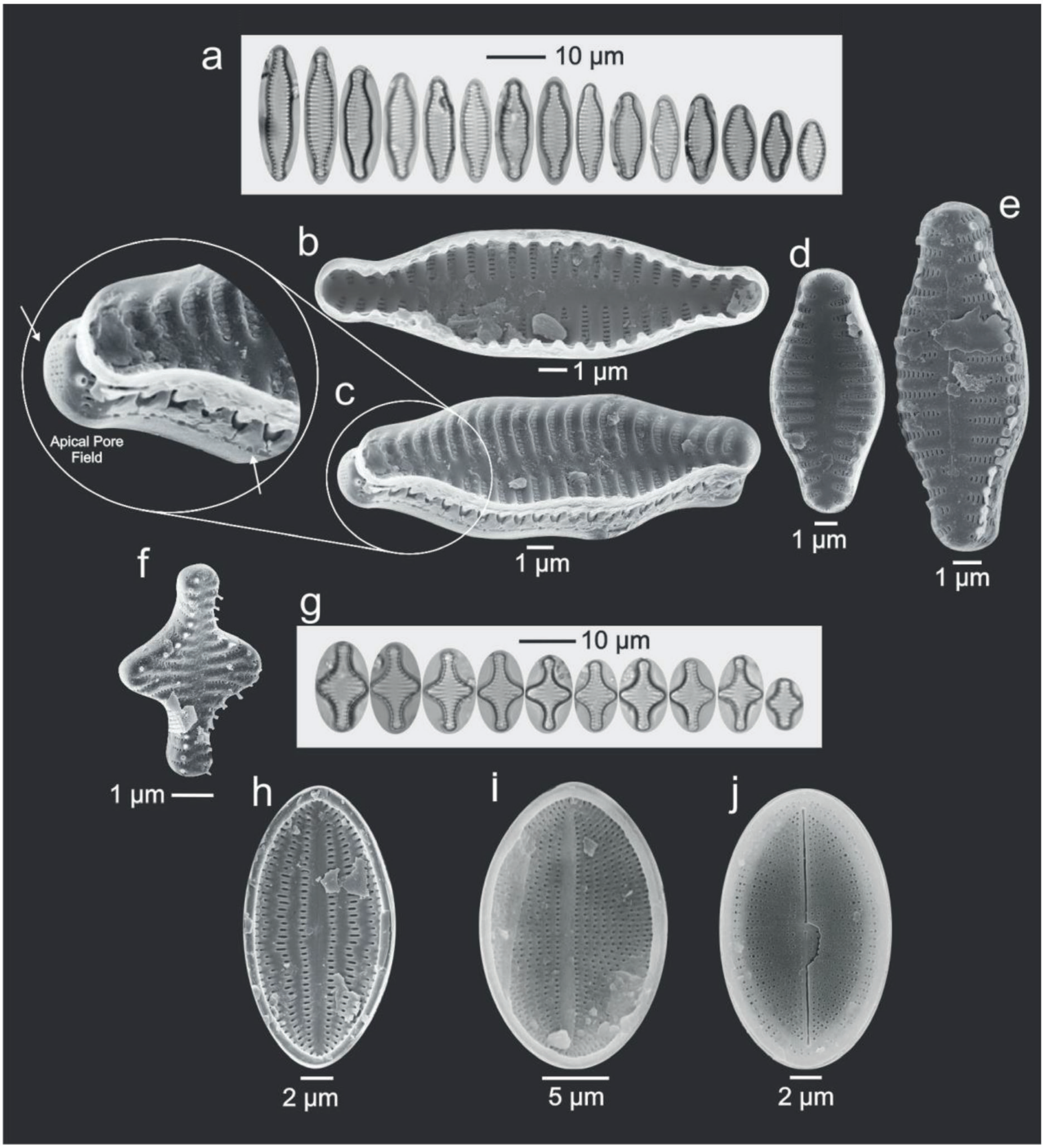

Figure 4. Predominant diatoms in fluvial and estuarine sediments from the Negro River: (a) morphological variability of *Staurosira binodis* in LM; (b-d) SEM internal view of *S. binodis*, arrows shows the apical pore field and the spatulate spines; (e) SEM external view of *S. binodis*, note the spines located between striae; (f) SEM external of *Staurosira construens*; (g) morphological variability of *S. construens* in LM; (h) sternum valve of *Cocconeis euglypta* (SEM internal view); (i) sternum valve of *Cocconeis placentula* (SEM internal view); (j) raphe valve of *C. placentula* (SEM external view). *Diatomeas dominantes en sedimentos fluviales y estuariales del Río Negro: (a) variabilidad morfológica de* Staurosira binodis *en ML; (b-d) vista interna de* S. binodis *con SEM, las flechas indican el campo de poro apical y las espinas en forma de espátula; (e) vista externa de* S. binodis *con SEM, note las espinas en las interestrías; (f) vista externa de* Staurosira construens *con SEM; (g) variabilidad morfológica de* S. construens *con ML; (h) valva sin rafe de* Cocconeis euglypta *(vista interna con SEM); (i) valva sin rafe de* Cocconeis placentula *(vista interna con SEM); (j) valva con rafe de* C. placentula *(vista externa con SEM).*

taxa (species, forms and varieties) representing 74 genera. Only 30 taxa showed relative abundances higher than 2 % in at least one sample (Figs. 3 and 4); that makes up about 9.3 % of the total species registered. The remaining 91.7 % of less represented taxa were uncommon or rare species. Genera with higher number of species were *Nitzschia* Hassal (51), *Navicula* Bory (36), *Gomphonema* Ehrenberg (13) and *Thalassiosira* Cleve (12).

Species richness (S) among the sites varied between 44 and 84 taxa, and the Shannon diversity index (H') fluctuated between 2.66 and 3.43. S18 site situated at the Neuquén River was the most diverse (S = 84 and H' = 3.43), while the S9 site located at the middle basin of the Negro River was the less diverse (S = 44 and H' = 2.66). Overall, a trend towards higher diversity was observed at the upper basin of the river (S13-S18 sites). Fifteen sampling sites showed a number of taxa higher than 60.

Constrained cluster analysis indicated four main diatom zones based on a broken stick model criterion (Fig. 5). A highly difference in the structure of the diatom assemblages between the estuarine sites (zone I) and typically freshwater ones was observed (zone II, III and IV). Diatom composition of the estuarine sites (S1, S2 and S3) in zone I, was characterized by the dominance of marine-brackish tychoplankton as *Cymatosira belgica* Grunow, *Rhaphoneis amphiceros* (Ehrenberg) Ehrenberg, *Delphineis minutissima* (Hustedt) Simonsen, *Delphineis surirella* (Ehrenberg) Andrews, *Paralia sulcata* (Ehrenberg) Cleve, *Paralia sulcata* var. *coronata* (Ehrenberg) Andrews and *Shionodiscus oestrupii* (Ostenfeld) Alverson, Kang & Theriot. Their abundances decreased in freshwater sites of the same diatom zone I (S4, S5, and S6) where populations of small, 'chain-forming' freshwater tychoplanktonic diatoms (henceforth fragilarioids) dominated, such as *Staurosira binodis* Lange-Bertalot, *Staurosira construens* Ehrenberg, *Staurosira venter* (Ehrenberg) Cleve & Möller, *Staurosirella* sp. 1 Williams & Round, *Pseudostaurosira brevistriata* (Grunow) Williams & Round and *Punctastriata lancettula* (Schumann) Hamilton & Siver. In this zone a significant abundance of *Cocconeis euglypta* Ehrenberg was also recorded.

A distinctive feature of the middle and upper basin of the Negro River, as well as the Limay and Neuquén rivers sampling sites (zones II, III and IV), is the overwhelming dominance of small fragilarioids similar to those observed at sites S4, S5 and S6 of zone I (Fig. 5 and 6). Excluding estuarine sampling sites, this group jointly makes up between 32 and 60 % of the samples. The middle basin of the river was dominated by *S. construens* (16 to 25 %) and *S. venter* (8 to 18 %), while the upper basin was dominated by *S. binodis* (16 to 38 %). Other important species in these zones were: *C. euglypta* and *C. placentula* Ehrenberg, *Planothidium rostratum* (Østrup) Lange-Bertalot and *Amphora pediculus* (Kützing) Grunow ex Schmidt. Centric forms were represented by *Stephanodiscus agassizensis* Håkansson & Kling, *Aulacoseira granulata* (Ehrenberg) Simonsen, *Aulacoseira ambigua* (Grunow) Simonsen and *Cyclotella ocellata* Pantocsek. It is noteworthy that in zone IV, which is represented by a single sample in the lower basin of Neuquén River, *S. binodis* and *A. ambigua* were not recorded. By contrast, these species showed high abundances at the Limay River.

Diatom assemblages and environmental variables

Variance partitioning analysis displayed that physical and chemical variables independently explained 37.1 % of the community diatoms turnover, while the individual effect of the sedimentological variables accounted for 12.1 %. Thus, this last group of variables was excluded from the RDA analysis, due to the low contribution in the variation of the community structure. The first two axes of the complete data set represented 40.9 % of the accumulated variance in the biological data (Table 3). The first constrained canonical axis accounted for 84.3% of the variance explained by the relationship between diatoms and the environmental variables, while the second axis explained an additional variance of 8.7 %. ANOVA permutation showed that only the first canonical axis was highly statistically significant ($p = 0.005$).

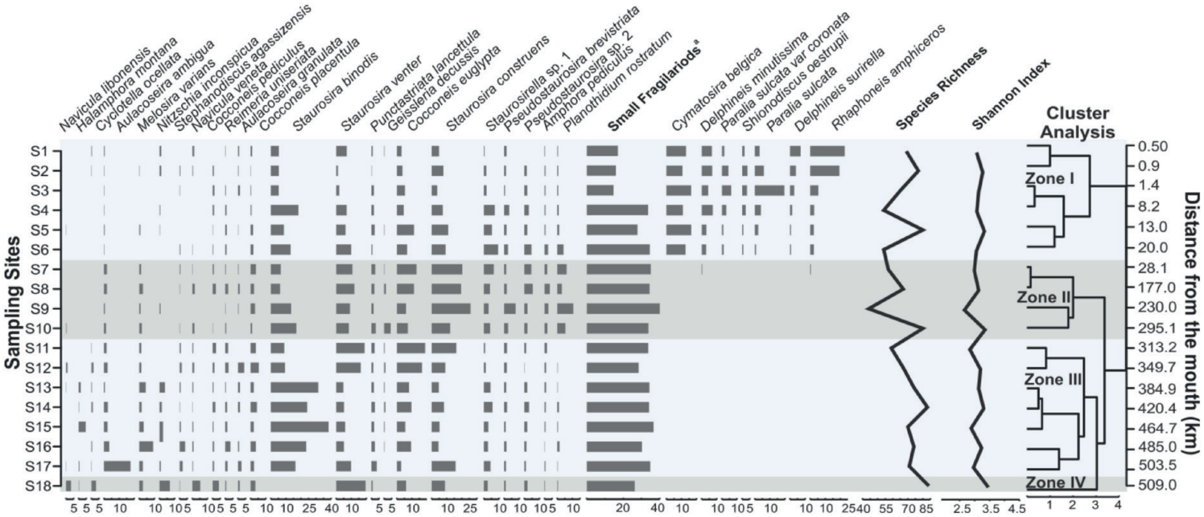

Figure 5. Relative abundance of the most common (> 2 %) diatom taxa in surface sediment samples. Species richness, Shannon index and cluster analysis from the Negro River are also included. [a] Abundance percentage of small fragilarioids includes 17 taxa belonging to *Pseudostaurosira*, *Punctastriata*, *Staurosira* and *Staurosirella*. *Abundancia relativa de los taxones más comunes (> 2 %) en muestras de sedimento superficial. En el gráfico también se incluyen la riqueza de especies, el índice de Shannon y el análisis de agrupamiento. [a] El porcentaje de abundancia de pequeñas fragilarioides incluye 17 taxones pertenecientes a los géneros* Pseudostaurosira*,* Punctastriata*,* Staurosira *and* Staurosirella.

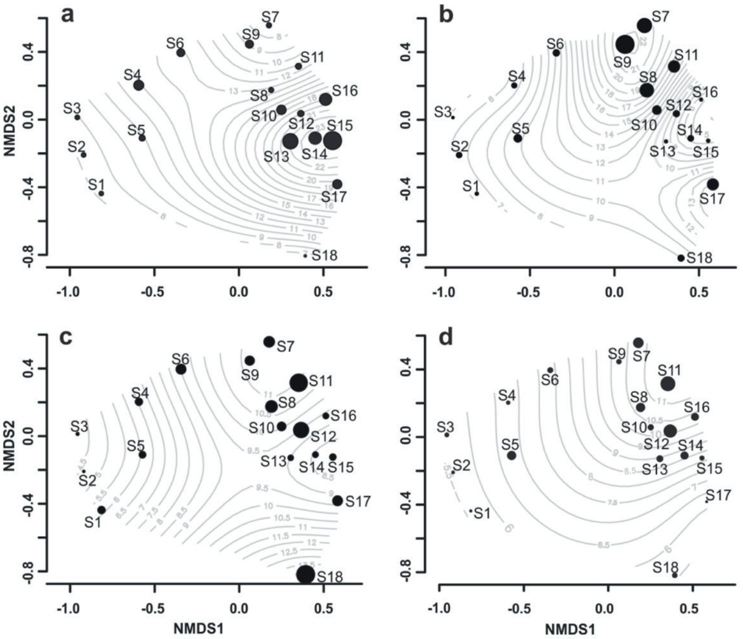

Figure 6. Non-metric multidimensional scaling plot based on the diatom composition from the Negro River. Contour lines show a smoothed surface of the abundance relative of *S. binodis* (a), *S. construens* (b), *S. venter* (c) and *C. euglypta* (d). Filled black circles represent the estimate abundance relative in each sampling site. *Gráfico de escalamiento multidimensional no métrico basado en la composición de diatomeas del Río Negro. Las líneas de contorno muestran la superficie suavizada de la abundancia relativa de* S. binodis *(a),* S. construens *(b),* S. venter *(c) y* C. euglypta *(d). Los círculos negros representan la abundancia relativa estimada en cada sitio de muestreo.*

Table 3. Redundancy analysis (RDA) results between common diatoms taxa and selected environmental variables. *Resultados del análisis de redundancia (RDA) entre los taxones de diatomeas comunes y las variables ambientales seleccionadas.*

Eigenvalues of the correlation matrix				
	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalue	0.096	0.009	0.005	0.002
Species-environment correlations	0.86	0.73	0.46	0.68
Cumulative Percentage Variance:				
- Species data	37.1	40.9	42.8	44
- Species-environment relationship	84.3	93	97.4	100
Intra set correlations of environmental variables				
Salinity	0.94	-0.31	-0.07	0.07
Nitrate (NO_3^-)	0.02	0.94	-0.09	-0.32
Silicon dioxide (SiO_2)	0.60	0.25	0.52	-0.53
Bicarbonates (HCO_3^-)	0.07	-0.20	-0.73	-0.64

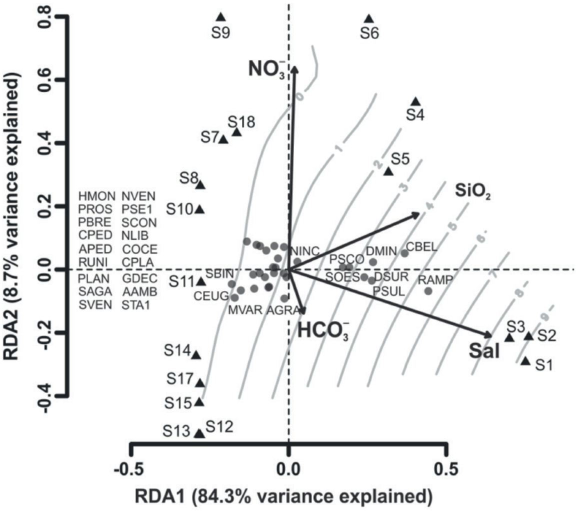

Figure 7. RDA triplot of the Hellinger-transformed diatom abundance data constrained by selected environmental variables. Contour lines display a smooth surface related with salinity gradient. Filled black triangles represent sampling sites and filled grey circles the diatom taxa. For acronyms of the species names, see Table 4. *Representación gráfica del RDA que relaciona los datos de abundancia transformados (Hellinger) y las variables ambientales seleccionadas. Las líneas de contorno indican una superficie suavizada relacionada con el gradiente de salinidad. Los triángulos negros representan los sitios de muestreo y los círculos grises los taxones. El nombre científico de las especies y los códigos se encuentra en la Tabla 4.*

The first canonical axis displayed a strong positive correlation with salinity (r = 0.94) indicating an important effect of this variable on the distribution pattern of the diatom assemblages. Partial RDAs also indicated that this variable accounted for 21.1 % of the individual effect on the diatom composition. Nitrate showed a strong positive correlation with the second axis (r = 0.94), however the canonical axis was not significant (p = 0.940). Silicon dioxide (p = 0.993) and bicarbonates (p = 0.992), also showed no significance in the model.

As shown in the RDA triplot, most of taxa were located in the negative side of the RDA1 axis (salinity gradient) and they are associated with freshwater sampling sites (Fig 7). The species in the positive side are related to those sites closest to the mouth of the river. Taxa associated with high salinity values were *C. belgica*, *R. amphiceros*, *D. minutissima*, *D. surirella*, *P. sulcata*, *P. sulcata* var. *coronata* and *Shionodiscus oestrupii*. On the other hand, the species associated with typical freshwater sampling sites were *S. binodis*, *S. construens*, *S. venter*, *Staurosirella* sp 1, *P. brevistriata*, *P. lancettula*, *C. euglypta*, *C. placentula*, *P. rostratum* and *A. pediculus*.

Table 4. Codes and list of diatom species with relative abundances higher than 2 % of the total sample. *Códigos y lista de las especies de diatomeas con abundancias relativas superiores al 2 % del total de la muestra.*

Acronym	Diatom taxa
APED	*Amphora pediculus*
AAMB	*Aulacoseira ambigua*
AGRA	*Aulacoseira granulata*
CEUG	*Cocconeis euglypta*
CPED	*Cocconeis pediculus*
CPLA	*Cocconeis placentula*
COCE	*Cyclotella ocellata*
CBEL	*Cymatosira belgica*
DMIN	*Delphineis minutissima*
DSUR	*Delphineis surirella*
GDEC	*Geissleria decussis*
HMON	*Halamphora montana*
MVAR	*Melosira varians*
NLIB	*Navicula libonensis*
NVEN	*Navicula veneta*
NINC	*Nitzschia inconspicua*
PSUL	*Paralia sulcata*
PSCO	*Paralia sulcata* var. *coronata*
PROS	*Planothidium rostratum*
PBRE	*Pseudostaurosira brevistriata*
PSE1	*Pseudostaurosira* sp. 2
PLAN	*Punctastriata lancettula*
RUNI	*Reimeria uniseriata*
RAMP	*Rhaphoneis amphiceros*
SOES	*Shionodiscus oestrupii*
SCON	*Staurosira construens*
SBIN	*Staurosira binodis*
SVEN	*Staurosira venter*
STA1	*Staurosirella* sp. 1
SAGA	*Stephanodiscus agassizensis*

DISCUSSION

Diversity indexes and gradient analyses indicate that diatom assemblages of surface sediments from Negro River are highly diverse and heterogeneous, and their community structure responds to an ionic gradient that increases with the proximity of the estuary, being salinity, conductivity, pH, cations and anions the main environmental predictors.

Grain size and TOC reflected local sedimentological characteristics in some areas of the river. The retention of the clay-silt fraction in the dam systems located upstream of the Limay and Neuquén rivers, as well as the morphology of the channels and riparian vegetation, would explain the high percentage of gravel at the upper valley of the Negro River (Isla *et al.*, 2010). Large proportions of the sand fraction are recorded in the sites located at the Miguel bank (S1 and S2), one of the two banks that are develop parallel to the ebb and flow current of the mouth. The accretion of this sandy littoral banks and the inlet migration from south to north is a consequence of the littoral drift (del Río *et al.*, 1991; Isla & Bertola, 2003).

Although S3 site is located at the mouth of the river and showed a diatom composition similar to S1 and S2, it is part of an intertidal sector that constitutes a muddy marsh characterized by the presence of halophytic vegetation such as *Sporobolus* and *Sarcocornia*. Muddy sediments in freshwater sites (S4-S7) of the lower valley seem to be a function of the progressive increase in water turbidity in the last few years ago. This recent trend in the increase of the suspended particles in the river, is related to extreme rainfall conditions, soil erosion, intense agriculture pressure, urbanization, steep slopes and desertification (Marizza *et al.*, 2010; Abrameto *et al.*, 2017)

Some features as grain size, freshwater ionic concentration and abundance of marine, brackish and freshwater diatoms indicate that sampling sites S4, S5 and S6 correspond to the middle/upper estuary transition. Total hardness, sulphate concentration and the isohaline of 0.1 ‰ recorded at site S4 (approximately 8.2 km to the mouth) set this boundary and reflect the maximum estimated saline intrusion in the river (Perillo, 1995).

Although there is no mixture with the sea water, tidal waves generates flood and ebb currents that transport organic and inorganic material toward the lower limit of the upper estuary (Wolanski *et al.*, 2009). In this sense, diatom analysis of surface sediments from the Negro River reflects that marine biogenic particles transported by tides may enter into the river up to 20 km from the inlet (site S6), near to Viedma city (S7). It should be also considered that the physical effects of tides are transferred upstream up to 70 km from the inlet (D'Onofrio *et al.*, 2010).

This high tidal intrusion is favoured because the final riverbed stretch is a wide fluvial channel highly influenced by the energy of the tides. These geomorphological conditions contrast with other coastal systems from northern Patagonia as the Colorado and Chubut estuaries (Vergara Dal Pont *et al.*, 2017). Colorado delta has an extensive network of channels, while Chubut river is narrow and meandering and the tidal influence is reduced to few kilometers from the inlet (Perillo *et al.*, 1989; Piccolo & Perillo, 1997).

Diatom assemblages on surface sediments occurring along transects of transitional zones between marine, brackish and freshwater habitats, have been used as modern analogues in order to estimate the continental input and marine intrusion in other micro and mesotidal coastal ecosystems of Argentina and Uruguay (Hassan *et al.*, 2009; Licursi *et al.*, 2010; Espinosa & Isla, 2015; Perez *et al.*, 2018). These diatom spatial discontinuities can therefore be important in late Quaternary to elucidate the response of coastal environments to natural and anthropogenic forces that shape coastlines through previous time periods (Cooper *et al.*, 2010).

Taking into account the previous work by Espinosa & Isla (2015), a turnover of epiphytes and planktonic taxa by small tychoplanktonic fragilarioids was observed in the middle and upper river basin. These results might provide indirect evidence of limnological condition changes between 2006/2007 (samples of the former study) and 2014/2015 (samples of present study). Fragilarioids taxa as *Staurosira* (Ehrenberg), *Staurosirella* (Williams et Round) and *Pseudostaurosira* (Williams et Round) are benthic/tychoplanktonic diatoms with a wide range of ecological preferences, capable of growing in shallow aquatic environments and littoral areas of flowing waters and deep lakes (Bennion *et al.*, 2010). This group of small araphid diatoms is considered as *r-strategist*, responding to physical changes (e.g. habitat availability, turbidity) more than to chemical changes in aquatic environments. Overall, if these diatom taxa are abundant, the ecological interpretation is difficult (Gell & Reid, 2014).

In lowland rivers and floodplains ecosystems, these taxa seem to have affinities for disturb, oligo-mesohaline waters, low light conditions and they have also been linked to an increase in both suspended solids and macrophytes (Gell *et al.*, 2002). Using modern and fossil diatom analyses, Vélez-Agudelo *et al.* (2017) and Fayó *et al.* (2018) have also detected an increase of small tychoplanktonic fragilarioids than planktonic taxa (e.g. *Aulacoseira* spp.) in the Colorado River (north Patagonia). Such results are interpreted as salinity, depth and turbidity water changes in recent historical times likely due to river flow regulation, decrease river discharge and increase of industrial and agricultural activities.

Particularly in the Negro River, the dominance of small tychoplanktonic fragilarioids and epiphytic taxa (e.g. *Cocconeis* spp. and *Gomphonema* spp.) would be associated to aquatic macrophytes habitats (emergent, submersed or rooted-floating), which seem to be favored by the recent decrease in sediment discharge and nutrient input (Arribere *et al.*, 2003; Abrameto *et al.*, 2017). Since the construction of the dam system in the early 1970s, the river has undergone significant variations in sediment transport downstream, causing changes in the structure and function ecosystem that strongly affect its biotic assemblages (Vergara Dal Pont *et al.*, 2017). However, further refine studies are needed to improve the understanding of the complex relationship between diatoms and macrophytes communities, and its response to hydrological changes along the river.

Nitrate content in surface waters of the Negro River, higher than 1 mg/L, reflects anthropogenic inputs and nutrient-enrichment. But these values are less than 13 mg/L, which is the maximum level allowed for protection and development of aquatic biological communities (CWQG, 2012). It is noteworthy that in this study the highest mean nitrate values are recorded in sites S4-S7 where low species richness and Shannon diversity index are also observed, except in the S5. This trend is in agreement with Abrameto *et al.* (2017), who observed an increasing eutrophication condition toward the lower valley. They argue that chemical fertilizers and pesticides discharge in agricultural areas, sewage treatment plant and industrial inputs from Guardia Mitre, Zanjon Oyuela, Viedma and Carmen de Patagones, significantly influence the distribution of nitrate in this stretch of the river. These results provide critical information indicating the need to develop a holistic and integrate approach to improve monitoring programs in the river, since nitrate pollution is one the major threats in arid/semi-arid aquatic ecosystem worldwide (Cook *et al.*, 2010).

Ecological preferences of some less abundant taxa also seem to reflect environmental signals of these human impacts in the river. Perhaps the most distinctive feature is the persistent abundance of *C. euglypta*, epiphyte taxa that thrives in wide environmental conditions, but that is usually more abundant in mesotrophic, alkaline lakes and rivers with medium to high concentrations of major ions. Although it shares ecological similarities with *C. placentula* and *C. lineata*, some studies suggest that *C. euglypta* tolerates moderate levels of organic pollution (Romero & Jahn, 2013; Delgado & Pardo, 2015; Espinosa & Isla, 2015). Otherwise, *C. placentula* and *C. pediculus* have been found in environments with high phosphorus concentrations and high turbidity (Rott *et al.*, 1998). Other less represented taxa as *A. ambigua*, *A. granulata*, *S. agassizensis*, *A. pediculus*, *Navicula veneta* Kützing and *Nitzschia inconspicua* Grunow, also suggest possible underlying turbid and nutrients problems occurring throughout the river (Reavie & Smol, 1998; Martínez de Fabricius *et al.*, 2003; Kiss *et al.*, 2012; Delgado & Pardo, 2015).

CONCLUSIONS

1. The gradient analysis describes a significant effect of ionic strength on diatom assemblages along the Negro River.
2. The transition between the estuary and the freshwater environment is interpreted as a function of the grain size, dissolved ionic concentration and the abundance of marine, brackish and freshwater diatoms.
3. The mean nitrate values support the idea that the lower valley exhibits a clear pollution gradient.
4. Ecological preferences of dominant and accompanying taxa also display a possible deterioration along the river caused by the industrial and land use activities.
5. The numerical approach provided the basis to understand major chemical gradients underlying diatom distribution. This is a key issue as data is scarce in fluvial and coastal environments from northern Patagonia. These tools combined with other datasets from the region, will allow for the development of valuable and reliable diatom models required to enhance quantitative palaeoenvironmental inferences.

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