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Modern diatom assemblages in surface sediments from meso-macrotidal estuaries of Patagonia, Argentina

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Abstract The composition and distribution of diatom assemblages from surface sediments in the littoral zone of meso and macrotidal estuaries from Patagonia (Argentina) were studied with the aim to recover modern data on diatom distribution applicable to future paleoenvironmental reconstructions in coastal areas of Southern South America. Twenty-seven sites were selected to cover the maximum spatial distribution along the Negro, Chubut, Santa Cruz valleys and San Julián Bay. Salinity, water temperature, pH, sediment grain size and organic matter content were measured. Multivariate methods (cluster, CCA and NMDS analysis) were explored to analyse the relation between biotic and abiotic data. The results showed that the spatial distribution and species composition of diatom assemblages are strongly related to salinity and temperature. The diatom assemblages of riverine conditions (without salt-wedge influence) were characterized by high abundances of the freshwater diatom species *Cocconeis placentula*, *C. euglypta* and *Aulacoseira granulata,* meanwhile high abundances of marine and marine-brackish species such as *Paralia sulcata*, *Rhaphoneis amphiceros*, *Delphineis surirella* and *Diploneis interrupta* were characteristic of estuarine conditions (i.e. under salt-wedge influence). As temperature is directly related to latitude there are differences between northern and southern Patagonian communities. Different proportions or combinations of the taxa produce the distinction between assemblages. In southern estuaries with lower temperatures, the diatom assemblages are less diverse.

Key words: environmental *proxies*, salinity changes, multivariate analysis, sediments, southern South America

Resumen: Ensambles de diatomeas actuales en sedimentos superficiales de estuarios meso-macromareales de Patagonia, Argentina. Se estudió la composición y distribución de ensambles de diatomeas en la zona litoral de estuarios meso-macromareales de Patagonia (Argentina) con el objetivo de recabar información aplicable a futuras reconstrucciones paleoambientales en áreas costeras del sur de Sudamérica. Se seleccionaron veintisiete sitios que cubren una máxima distribución espacial a lo largo de los valles de los ríos Negro, Chubut, Santa Cruz y Bahía San Julián. Se midieron la salinidad, temperatura del agua, pH, sedimentos y contenido de materia orgánica. A través de métodos multivariados (análisis de agrupamiento, CCA y NMDS) se analizaron datos bióticos y abióticos. Los resultados mostraron que la distribución espacial y la composición de especies de diatomeas están fuertemente relacionadas con la salinidad y la temperatura. Los ensambles de diatomeas de ambientes fluviales (sin influencia marina) se caracterizaron por altas abundancias de *Cocconeis placentula*, *C. euglypta* y *Aulacoseira granulata,* mientras que los ambientes estuariales (con influencia marina) estuvieron caracterizados por *Paralia sulcata*, *Rhaphoneis amphiceros*, *Delphineis surirella* y *Diploneis interrupta.* Dada la relación de la temperatura con la latitud, se hallaron diferencias entre las comunidades del norte y sur de Patagonia. La distinción entre ensambles está dada por las diferentes proporciones de los taxones, en los estuarios del sur con bajas temperaturas, fueron menos diversos.

Palabras clave: indicadores ambientales, cambios de salinidad, análisis multivariado, sedimentos, sur de Sudamérica

Introduction

Estuaries are transitional states, suffering more acutely the outcome of climate variability. However, variability and unsteadiness are intrinsic properties of estuarine ecosystems; their biological communities are well adapted to several temporal variability scales and to spatial gradients of key factors, such as salinity or temperature (Gameiro et al. 2007).

Diatoms are considered the most important component of benthic microalgal assemblages in estuarine and shallow coastal environments. They are organisms that are very sensitive to environmental changes (Vos & De Wolf 1988). In that sense, they are chosen to estimate these variations from the past, assuming that their ecological preferences did not change over the period considered (Espinosa et al. 2003).

In estuaries, the salinity is variable depending on the waves, tidal influence and freshwater river discharge (0 to 36.5 ‰). Diatomological studies from continental aquatic ecosystems have been much more common than marine or coastal studies (Mackay et al. 2003). Regarding with South America diatom ecological studies on estuarine and shallow coastal waters are very scarce compared to freshwater and open ocean habitats ones. In this sense, some work has been done on the northern coast of Argentina in relation to assessing the distributions of diatom assemblages from three microtidal estuaries (tides < 2 m) of Buenos Aires province, in order to achieve ecological information useful as modern analogues in diatom-based paleoecological reconstructions in the region (Hassan et al. 2006, Espinosa et al. 2006, Hassan et al. 2007).

In coastal sediments of South America, diatoms have been used to record environmental changes in salinity related to Holocene sea-level changes (Espinosa et al. 2003, Garcia-Rodríguez et al. 2004). However, the reconstruction of coastal history based on diatom biostratigraphical techniques (e.g. Espinosa 2001) relies on the classifications of taxa according to freshwater, brackish or marine indicative conditions. More recently, a quantitative approach was achieved based on salinity transfer function (Hassan et al. 2009), producing a new generation of high-resolution procedures (Espinosa et al. 2012). The application of these techniques depends on a complete knowledge of modern diatom communities and their relationship with their environment with regard to physical and chemical proxies. These kinds of studies in meso-macrotidal estuaries (tides > 2 m) are very scarce in Argentina, although there are some contributions about the Negro and Chubut Rivers (Sastre et al. 1990, Villafañe et al. 1991, Sastre et al. 1994, Pucci et al. 1996). Particularly in north Patagonia, estuarine diatoms have been used to infer paleoenvironments in the Negro River and Chubut River inlets (Escandell et al. 2009, Escandell & Espinosa 2012) based only on autoecological data extrapolated from other regions of the world.

Patagonian rivers flow across a rocky desert to discharge in the South Atlantic Ocean. Their watersheds are cutting volcanic plateaus crowned by Patagonian pebbles (Isla & Cortizo 2013). Quaternary marine terraces indicate the fluctuation of the sea level and tectonic effects (Rutter et al. 1989, Pedoja et al. 2011) while fluvial terraces are also showing significant changes in their watersheds related to piedmont glaciers at the foot of the Andes Cordillera. Distances from amphidromic point (no tides) and the width of the continental shelf cause tidal ranges of over 4 m along the Patagonian coast (Piccolo & Perillo 1999, Isla & Bujalesky 2008). Due to these large tidal ranges within flat country, Patagonian estuaries are thus associated with extensive marshes (Bortolus et al. 2009, Isla et al. 2010a).

The scarcity of regional information about diatoms autoecology has prevented the development of quantitative models for Southern South America. Thus, in this paper we present information about the diatom composition and distribution along four fluvial/estuarine systems located in Patagonia region (South Argentina). The main goals are: a) to document the regional distribution of modern diatom assemblages from surface sediments, and b) to define the relationship between these assemblages and the environmental parameters recorded. Considering environmental differences between northern and southern Patagonia is expected to: i) the composition of diatom assemblages will be different in the estuaries of northern Patagonia impacted by human settlements and agricultural activity than in the estuaries of southern Patagonia corresponding to pristine areas with little population, we would expect high species diversity and increased abundance in impacted sites due to nutrients supply, ii) the temperature will be an environmental forcing that influences the distribution of diatom communities from Northern and Southern Patagonia, iii) furthermore, we will expect to find a spatial variability on the distribution and composition of diatom communities along each estuary, caused by salinity changes in tidal environments.

Materials and Methods

Study area: Patagonia is located between the subtropical high-pressure belt and the subpolar low-pressure zone. Mean annual temperature diminishes from 40°S to 55°S with median temperatures ranging from 16°C at Northern Patagonia to 5°C in Tierra del Fuego, Southern Patagonia (Coronato et al. 2008). The Patagonian Andes are placed perpendicular to the westerly wind belt that carries humidity from the Pacific to the Atlantic Ocean. In this sense, Eastern Patagonia is almost a desert with precipitations below 200 mm/year. However, higher discharges were delivered from allochthonous rivers flowing eastwards (Negro, Chubut and Santa Cruz Rivers). Regarding with the vegetation, shrubs dominate Patagonia while forests are limited to the piedmonts of the Andes or around glacial lakes. Significant variations of the Patagonian estuaries were also caused by the Holocene fluctuation of the sea level (Isla & Bujalesky 2008; Schellmann & Radtke 2010; Isla and Cortizo 2013).

Negro River: The Negro River originates at the junction of the Neuquén and the Limay Rivers (Fig. 1), and crosses the Northern Patagonia to discharge more than 1000 m^3/s to the South Atlantic Ocean. Several dams were constructed along its watershed to control water availability for fruit production in the floodplains. The Middle valley is characterised by meanders (Choele Choel islands) that sieves the concentration of pollutants (Isla et al. 2010b). At the lower valley, an extended area has been subject to a Holocene sea-level fluctuation responsible for much of the sedimentation that enclosed it (Auer 1974). The irrigation practices failed in those areas. The inlet of the river migrates to the north in response to the long shore drift (Del Río et al. 1991). Mesotidal conditions dominate at the inlet (2.95 m at Punta Redonda) and are also affecting the lower valley where the cities of Viedma and Carmen de Patagones are located 20 km from the inlet. Salinity declines sharply in the last 2 km of the valley (Isla et al. 2010b).

Chubut River: The Chubut River also originates at the Andes Cordillera and crosses the Patagonian plateau (Fig. 1). This watershed diminished significantly since the Maximum Glaciation due to fluvial capture at the headlands, close to the Cordillera (Isla & Cortizo 2013). A large dam (Florentino Ameghino Dam) was constructed half way along this watershed, and consequently the discharge of this river (very low by nature) drops significantly after it.

San Julián Bay: San Julián bay operates as a coastal lagoon dominated by a macrotidal regime. The bay is divided into two ovoid embayments, separated by a salient where San Julián harbour is situated. For the purpose of this paper, it was considered as an estuarine environment although the freshwater input is minimum (Fig. 1).

Santa Cruz Estuary: Two rivers, Chico de Santa Cruz River and Santa Cruz River flow into a macrotidal inlet forming the Santa Cruz estuary (Fig. 1). Two harbours dominate this estuary: Puerto Santa Cruz and Punta Quilla, the latter with higher activity in the last years. Santa Cruz River has a discharge of the same order of magnitude as the Negro River. The estuary is morphologically asymmetric with cliffs at the southern margin and a coastal plain to the north (Schellmann 1998). Freshwater inflow provided by the Chico de Santa Cruz River increases significantly the discharge volumes. This cause a segregation of flows that remains by long distances within the estuary: some areas concentrate marine salinities while other areas receive freshwater seeps from the cliffs. As these rivers diminished their discharges during the Holocene, they are characterized by small flux areas in relation to their wide and deep alluvial valleys. While the Negro and Santa Cruz Rivers have annual discharges over 600 m^3/s, the Chubut River outflows with less than 50 m^3/s.

Field and laboratory methods: Diatom samples and abiotic data were obtained along the valleys of the Patagonian Rivers during summer (February 2006, 2007 and January 2010). 27 sites were selected to represent the maximum heterogeneity along the aquatic environments (rivers and bays, Fig. 1), spanning wide variations in salinity, morphology, tidal action, river flow and substrate. The basins of the Negro and Chubut Rivers, Santa Cruz River inlet

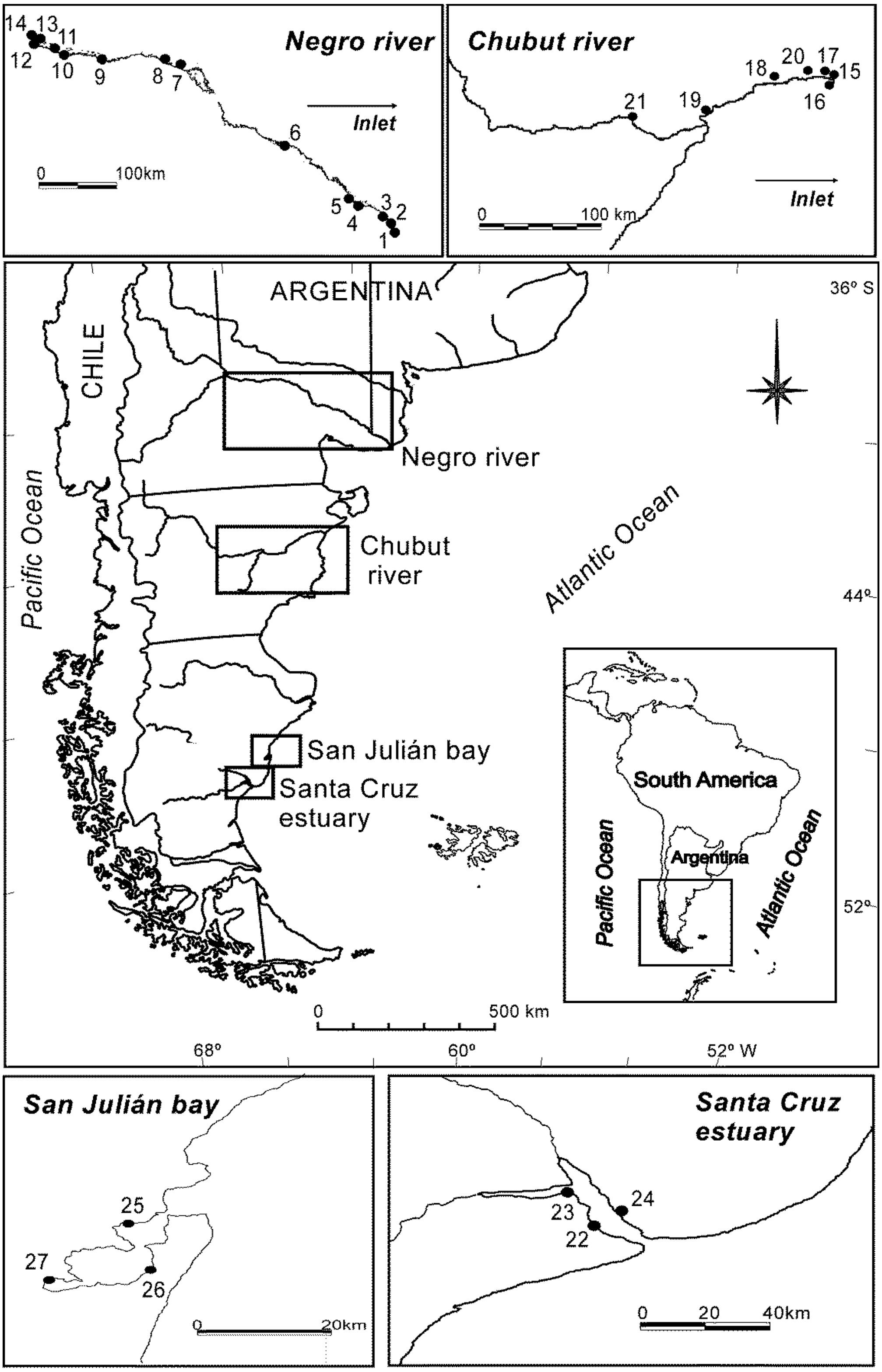

Figure 1.- Location of the study areas: Negro River, Chubut River, San Julián Bay and Santa Cruz Estuary.

and San Julián Bay were included. At each site, salinity, water temperature and pH were measured from the subsurface water column (no more than 1 m depth) using a Horiba U-10 water quality meter. All the measurements were taken during low tide and at Negro River inlet salinity was measured during low and high tides to check the limit of marine influence in the estuary. In addition, a surface sediment sample (0.5 kg) was taken for grain size analysis and organic matter content. At laboratory, sand was sieved while mud (silt and clay) was tested in relation to the settling velocity of the particles (pipette method, Folk 1974). Sand (>0.063 mm) is assumed to be transported in the bed-load fraction while mud (silt 0.004–0.063 mm and clay <0.004 mm) are assumed to be transported in the suspended fraction. Organic matter was measured according to the Walkey & Black (1934) method.

Surface sediments samples were taken in triplicate with a 20 mm diameter x 100 mm length plastic tube from the littoral zone of each site. The top 1 cm of sediment was scraped with a spatula and placed into airtight plastic bags. Diatom assemblages from surface sediments are assumed to contain a time-integrated flora, a mixture of both living epipelic diatoms and the remains of diatoms that died at the sampling site or were transported from adjacent environments.

5 g of dry sediment were oxidized with hydrogen peroxide (30%) and hydrochloric acid (10%) to remove organic matter and carbonates, washed 4 or 5 times with distilled water, and diluted to a total volume of 100 ml. After complete homogenization, a subsample of 20 µl was transferred to a coverslip and air-dried. Permanent slides were mounted with Naphrax®. On each slide at least 300 diatom valves were counted in random transects, using an optic microscope at 1,000x magnification. The identification of species was based on the local and standard diatom taxonomic literature. Nomenclature and taxonomic authorities follow Algaebase (Guiry & Guiry 2009). Ecological groups of De Wolf (1982), Denys (1991/1992) and Vos & De Wolf (1988, 1993) were used to classify diatoms taxa according to their salinity and habitat preferences in order to relate them to the monitored environmental conditions.

Data analysis: Percentages of each taxon are based on a sum of total diatoms (relative abundances). Multivariate methods were used to analyse the percentage data and objectively assess trends in the data using the software package PAST version 2.17 (Hammer 2001). A hierarchical cluster analysis was performed on the surface sediment samples using Simpson as similarity measure and a single-linkage algorithm (correlation coefficient: 0.8422). Differences among samples considering diatom composition were also assessed by means of NMDS (non-metric multidimensional scaling), a robust ordination technique for community analysis (Minchin 1987) used to create similarity matrixes using Euclidean distance. A numerical measure of the closeness between the similarities in the lower dimensional space is called stress. Stress values range from 0 to 1, with 0 indicating perfect fit and 1 indicating the worst possible fit. Solutions were obtained for one, two and three dimensions. The best solution –in terms of trade-off between complexity associated with increased dimensionality and reduction of stress– was chosen for interpretation. Canonical correspondence analyses (CCA) relate species composition to measured environmental factors. CCA ordinations utilize log transformed environmental variables. An unrestricted Monte Carlo permutation procedure with 999 permutations tested the significance of the first two ordination axes. Only those taxa with an occurrence higher than 2% were considered in the analyses.

Results

Environmental data: As shown in tables I and II, the sampled estuaries and associated fluvial systems were characterized by neutral to alkaline (pH 6.8 - 8.69) water. Summer temperatures (January and February) decreased from north to south, ranging from 23.7 °C to 13.9 °C. In general, sediments were mostly dominated by sands (mean percentage = 58.73%) with the exception of some sites with estuary influence as Criadero (Negro estuary), Iberpesca and Recreo (Chubut estuary) and Isla Cormorán (San Julián Bay) where the dominant fractions were silt (mean percentage = 30%) and clay (mean percentage = 25%).

Diatom assemblages : The diatom analysis of the 27 surface sediments samples (Fig. 1) reveals generally well preserved frustules. The assemblages represent both autochthonous and allochthonous diatoms; no attempt was made to separate the two groups. A total of 162 species were identified (see Appendix 1). The taxa that exhibited low relatively percentages (i.e. less than 2%) contributed very little to the statistical analysis, as their occurrence in a sample may have resulted from contamination by allochthonous inputs (Whiting & McIntire 1985).

Negro River: A total of 107 diatom species were identified in sediments from the Negro River, only 35 were represented on the diagram (>2%). Fourteen sample sites were ordered within the diagram according to the distance (km) from the mouth: Banco Miguel (0), Villarino (1.5), Criadero (15), Colonia La Luisa (145), Conesa (158), Colonia La Josefa (273), Chimpay (366), Chelforó (401), Villa Regina (451), Paso Córdova (496), Allen (517), Neuquén break (542), Neuquén main (542) and Limay (545), (Fig. 2).

Table I. Environmental parameters from Negro River (sites 1 to 14), Chubut River (sites 15 to 21), Santa Cruz Estuary (22 to 24) and San Julián Bay (sites 25 to 27). LT: low tide, HT: high tide.

Sample sites	Location	pH	Temperature (°C)	Salinity (‰)	Sand (%)	Mud (%)	Clay (%)	Organic matter (%)
1. Banco Miguel	41°01´24.8"S/62°47´41.9"W	7,6	21,85	22.75 (LT)/26.9 (HT)	100	0	0	0
2. Villarino	41°01´7.5"S/62°48´8.5"W	7,68	20,7	9.15 (LT)/27.1 (HT)	48,65	38,86	12,48	0,7
3. Criadero	40°55´26.6"S/62°51´33.5"W	7,3	23	0 (LT)/0.1 (HT)	30,02	41,13	28,86	1,9
4. Colonia La Luisa	40°11´53.44"S/64°13´2.89"W	7,4	22	0	44,5	22,5	33	1,05
5. Conesa	40°07´59.15"S/64°30´26.86"W	7,78	21,5	0	75,5	13	11,5	0,6
6. Colonia La Josefa	39°35´37.1"S/65°28´12.6"W	7,5	21,9	0	46,15	31,09	22,76	1,2
7. Chimpay	39°09´40.76"S/66°08´40.4"W	7,55	20,8	0	62,5	22,49	15	0,7
8. Chelforó	39°07´15"S/66°31´22.92"W	7,25	22	0	57,08	26,89	16,04	0,34
9. Villa Regina	39°10´0.1"S/67°05´35.5"W	7,61	22,3	0	79	12	9	0,4
10. Paso Córdova	39°06´30.2"S/67°38´8.0"W	6,8	21,8	0	60	31,01	9	0,9
11. Allen	38°56´58.01"S/67°51´8.48"W	7,2	23	0	67	20,49	12,5	0,94
12. Limay	38°58´37.4"S/68°02´40.7"W	8,1	19,8	0	83,64	4,29	12,06	1
13. Neuquén break	38°58´55.6"S/68°01´16.48"W	7,48	21	0	88,89	1,1	10	0,8
14. Neuquén main	38°54´40.76"S/67°58´10.54"W	7,7	20,5	0	87,66	0,96	11,18	0,4
15. Iberpesca	43°19´50.4"S/65°03´45.1"W	7,575	20,55	2,1	14,55	43,05	42,4	2,8
16. Recreo	43°19´35.9"S/65°03´39.0"W	7,64	20,5	0,2	16	44	40	2,95
17. Rawson	43°18´25.2"S/65°06´0.4"W	8,22	21,3	0	45	35	20	1
18. Gaiman	43°17´58.8"S/65°29´21.5"W	8,69	20,3	0	40	32,5	27,5	4,6
19. F. Ameghino dam	43°41´54.0"S/66°28´1.2"W	8,28	16,4	0	85	1,25	13,75	0,6
20. Trelew lagoon	43°14´50.3"S/65°13´53.2"W	7,91	22,1	0,2	52,5	25	22,5	7,9
21. Alto Las Plumas	43°43´12.4"S/67°17´19.3"W	8,37	23,7	0	50	30	20	2,6
22. Casilla Pescadores	50°05´12.0"S/68°29´20.5"W	7,72	14	23,75	45	24	31	2,6
23. Cañadón Misioneros	49°58´17.7"S/68°34´49.98"W	8,4	13,9	2,3	85	2,5	12,5	0,5
24. Baliza Ojos	50°04´11.7"S/68°24´5.2"W	7,87	15,1	13,4	68,6	11	20,4	0,8
25. Cormorán island	49°16´17.1"S/67°43´50.4"W	7,71	15,8	36,3	25,2	45,8	29	1,09
26. El Rincón	49°21´35.0"S/67°41´41.6"W	7,72	18,55	36,5	62,83	18,55	18,62	0,64
27. Chacra Ezeiza	49°19´32.5"S/67°47´48.4"W	7,7	19	36,5	65,4	22,8	11,8	0,45

Table II. Descriptive statistics of environmental parameters measurements at sampling stations

Variable	N	Mean	Stand. Dev.	Min.	Max.
pH	27	7.73	0.41	6.8	8.69
Temperature	27	20.12	2.75	13.9	23.7
Salinity	27	6.78	12.55	0	36.5
Sand	27	58.73	22.72	14.55	100
Mud	27	22.27	14.53	0	45.8
Clay	27	18.99	10.13	0	42.4
Organic matter	27	1.46	1.65	0	7.9

Diatom diagram shows the dominance of marine/brackish species at the three sites closest to the inlet (15 km; Fig. 2). This inlet assemblage was composed of tychoplanktonic taxa: *Cymatosira belgica* Grunow, *Delphineis surirella* (Ehrenberg) G.W. Andrews, *Raphoneis amphiceros* (Ehrenberg) Ehrenberg and *Paralia sulcata* (Ehrenberg) Cleve. Furthermore, the Villarino marsh was also dominated by the aerophilous species *Diploneis interrupta* (Kützing) Cleve. From Colonia La Luisa site (145 km from the inlet) to the Limay site (545 km from de inlet), the brackish/freshwater taxa *Cocconeis placentula* Ehrenberg, *Cocconeis euglypta* Ehrenberg, *Aulacoseira granulata* (Ehrenberg) Simonsen and *Stephanodiscus hantzschii* Grunow dominated. Thus, they are indicating that freshwater conditions prevail at the middle and upper valley. Besides, *Cocconeis euglypta* was dominant in the Upper valley and Conesa site and *C. placentula* was dominant in the Middle valley.

Chubut River: A total of 78 diatom species were identified in sediments along the Chubut River, 24 of them reached relative abundances higher than 2% in at least one sample (Fig. 3). Seven samples sites were ordered according to the distance (km) from the mouth: Iberpesca (0.22), Recreo (0.35), Rawson (6), Trelew lagoon (18), Gaiman (36), Florentino Ameghino dam (130) and Alto Las Plumas (185).

The freshwater, planktonic diatom *Stephanodiscus hantzschii* was dominant from the inlet to the headlands (Iberpesca, Recreo, Rawson, Gaiman and F. Ameghino dam), with the exception of the polluted lagoon close to Trelew city (Trelew lagoon site) and Alto Las Plumas site. In Trelew lagoon, the dominant species was *Hantzschia amphioxys* (Ehrenberg) Grunow (aerophilous and brackish/freshwater), accompanied by *Hippodonta hungarica* (Grunow) Lange-Bertalot, Metzeltin & Witkowski (epipelic and brackish/freshwater). Alto Las Plumas site was characterized by the assemblage constituted by *Cyclotella meneghiniana* Kützing (planktonic and brackish/freshwater) and *Epithemia sorex* Kützing and *Gomphonema parvulum* (Kützing) Kützing (both freshwater epiphytes). Marine/brackish species such as *Opephora pacifica* and *Tryblionella hungarica* were present only at the inlet (Iberpesca site) in very low percentages. Thus, freshwater/brackish and freshwater diatoms were the dominant groups along the Chubut River.

San Julián Bay: 35 diatom taxa were indentified in the three stations sampled at the San Julian Bay; 21 were selected for the diagram following the statistical significance criteria (Fig. 4a). Marine and marine/brackish assemblages were dominant. The marine/brackish and tychoplanktonic *Paralia sulcata* (60%) accompanied by marine plankton, *Actinoptychus senarius* (Ehrenberg) Ehrenberg and *Coscinodiscus marginatus* Ehrenberg were dominant at Chacra Ezeiza (18 km from the coast). Moreover, *Navicula salinicola* Hustedt (marine/brackish epipelon) was the dominant taxa at El Rincón site (98%). Besides, Isla Cormorán was characterized by a mixture assemblage: marine brackish taxa as *Raphoneis amphiceros*, *P. sulcata* and *Tryblionella compressa* (J.W. Bailey) Poulin and freshwater taxa as *Cocconeis neodiminuta* Krammer and *Frustulia rhomboides* (Ehrenberg) De Toni.

Santa Cruz estuary: 42 diatom taxa were identified although only 22 were presented in the diagram (>2%; Fig. 4b). Marine brackish and brackish benthos epipelon assemblages were dominant in the estuary. *Caloneis westii* (W.Smith) Hendey in Cañadón Misionero (24 km from the inlet), *Navicula cincta* (Ehrenberg) Ralfs in Casilla Pescadores (6 km from the inlet) and *Tryblionella levidensis* W. Smith in Baliza Ojos (9 km from the inlet) were the most important species.

Statistical analysis: The cluster analysis for the 27 surface sediment samples (Fig. 5) shows two clear groups. Cluster 1 was characterised by the north Patagonian study sites (i.e. Negro and Chubut Rivers) while cluster 2 was characterised by south Patagonian sites (i.e. San Julián bay and Santa Cruz estuary). Cluster 1 shows low similarity (0, 40) of Trelew lagoon sample (Chubut River) with respect to the others samples, this is associated with high percentages of the aerophilous taxa *Hantzschia amphyoxis*. The samples corresponding to sites situated near the inlet of north Patagonian Rivers (Recreo and Iberpesca in Chubut River and Villarino, Banco Miguel and Criadero in Negro River) were separate from the others. The six samples of cluster 2 are grouped with high similarity and they all belonging to sites with high salinities and low temperatures with the exception of Cañadón Misionero site (2.3‰) that is located in the inner part of the estuary and receive an important freshwater input.

The variation within –and overlap between– groups obtained by cluster analysis were best explored using the ordination method NMDS (Fig. 6a). The two methods jointly provided a complete analysis of the diatom data. NMDS arranged diatom samples in a specified dimensional space according to the rank order of their ecological similarities (stress value of 0.1706). This value indicates that the data indeed remained in the two dimensional space. The NMDS ordination plot revealed differences in the structure of the assemblages. Samples showed a distributional pattern clearly related to type of environment. NMDS diagram shows that samples of cluster 2 of figure 5 belonging to estuaries from south Patagonia, remained together in the negative axis 1 with the samples from the Negro River inlet (see the number corresponding to each sampling site in table I). All of them are characterized by the dominance of marine/brackish plankton, tychoplankton and benthos diatoms typical of estuarine environments. The samples of no tidal influence sites were ordered at positive section of axis 1. Axis 2 separate freshwater samples of the Negro River (negative correlation) where dominate epiphyte and brackish/freshwater taxa and Chubut River (positive correlation) with the dominance of freshwater plankton.

Partial CCA (Fig. 6b) showed that temperature, salinity and organic matter were the strongest environmental variables influencing diatom distribution. The rest of the environmental variables did not explain significant portions of the diatom variances and thus were excluded. Axis 1 and 2 explained 50.52% of the variance of this dataset (Table III). The first axis (26.33% of the explained variance) showed a positive correlation with salinity and negative correlation with temperature and organic matter. Sites were ordered along this axis following mainly salinity with coastal/marine sites located to the right side, and brackish/freshwater sites towards the left side. The second axis (24.19% of the explained variance) was positively correlated with organic matter and salinity, and negatively with temperature. Consequently, sites were ordered along axis 2 following organic matter content and temperature gradient, with cold estuaries environments towards the top and mild continental environments towards the bottom of the diagram.

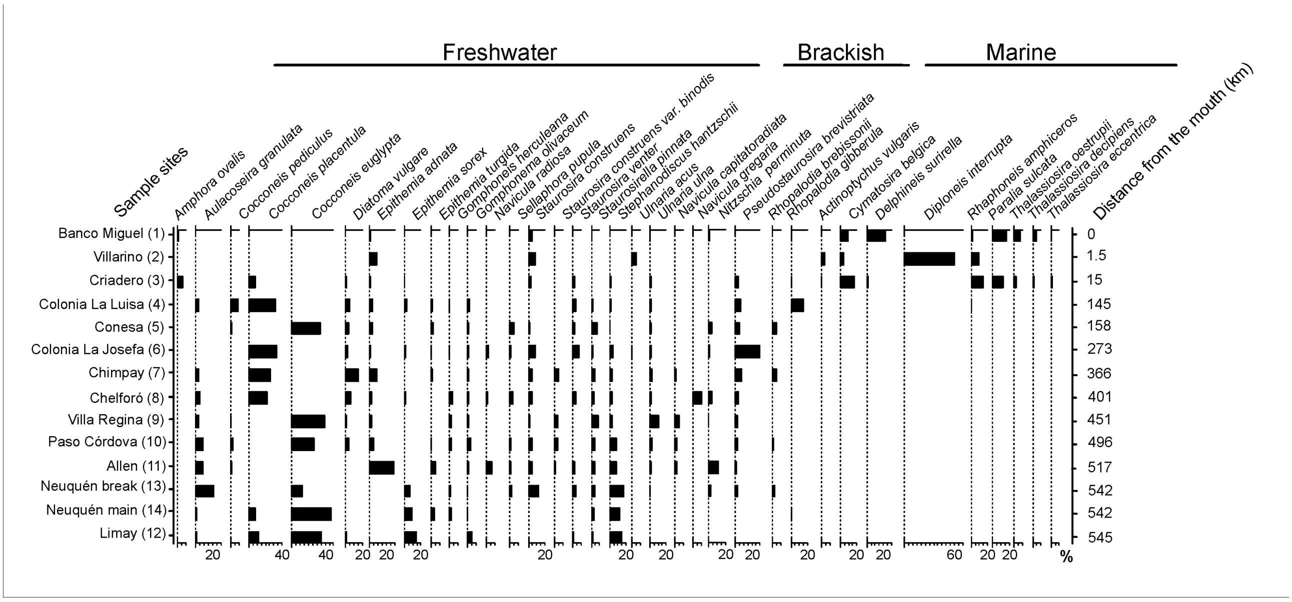

Figure 2.- Relative abundances of the most common (>2%) diatom taxa from Negro River.

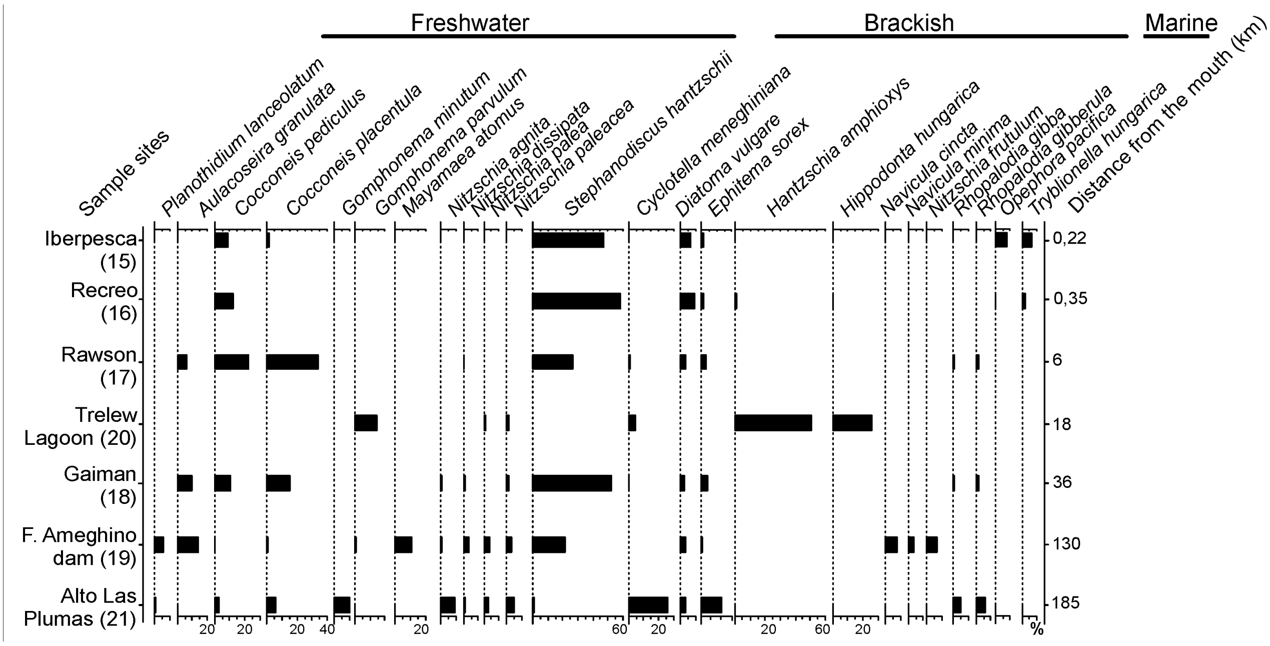

Figure 3.- Relative abundances of the most common (>2%) diatom taxa from Chubut River

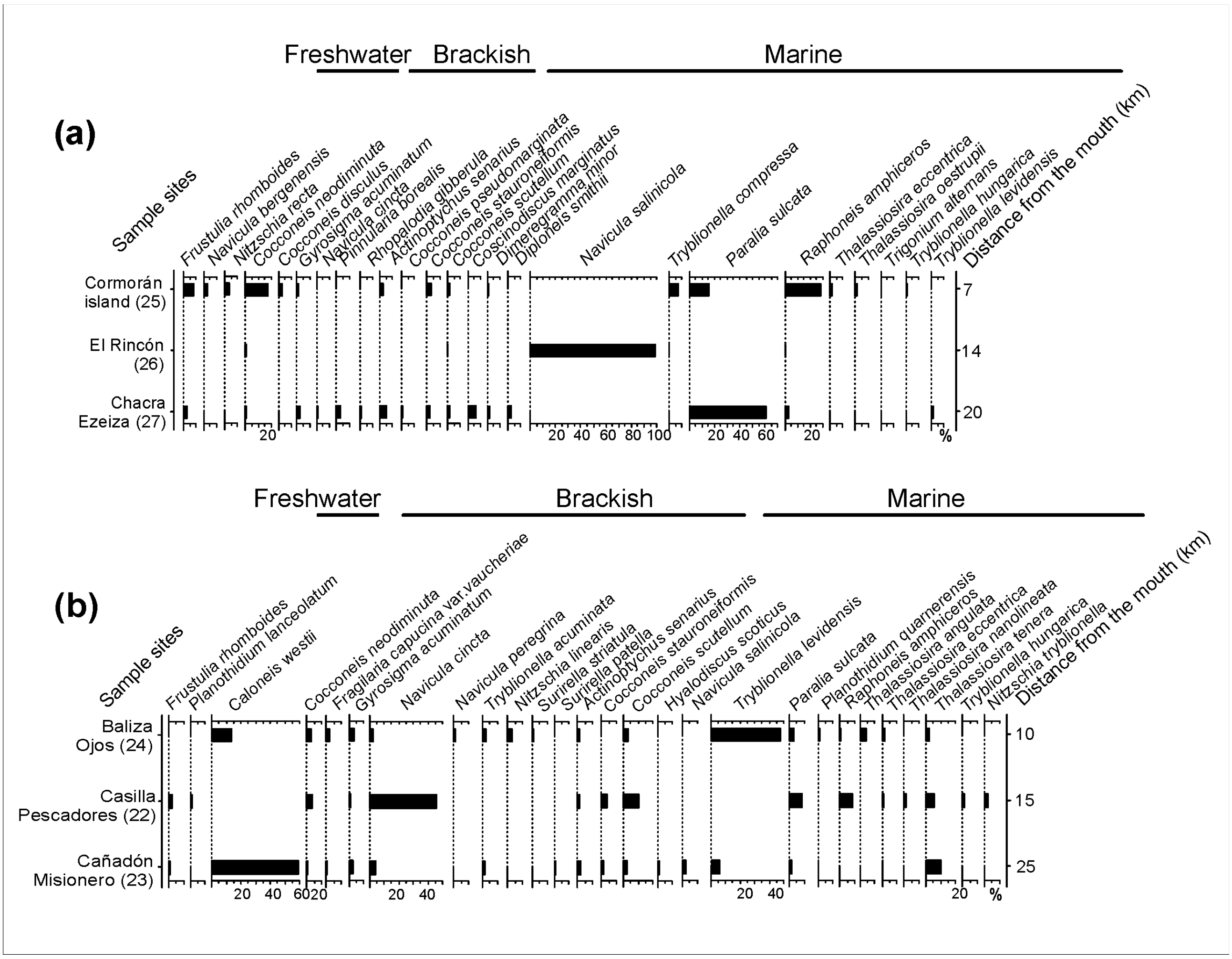

Figure 4.- Relative abundances of the most common (>2%) diatom taxa from (a) San Julián bay and (b) Santa Cruz estuary-

Discussion

The hydrological dynamics of the Patagonian estuaries affected benthic diatom community both at spatial and temporal scale. As a result of the complex dynamics between the rivers and the sea, the marine influence appeared as one of the main factors affecting spatial diatom composition and distribution. Some previous studies in estuaries and similar systems (McIntire 1978, Admiraal 1984, Underwood 1994, Hassan et al. 2006) pointed out that salinity has a key role in the diatom community composition and distribution.

At Patagonia region, paleoenvironmental reconstructions (salinity and depth changes) extending back to the Late Holocene were possible using ecological data gathered from European authors as De Wolf (1982), Vos & De Wolf (1993) and Denys (1991/1992), and the fragmentary information about the ecology and distribution of modern diatoms in estuarine and coastal systems of Southern South America (Escandell et al. 2009, Escandell & Espinosa 2012).

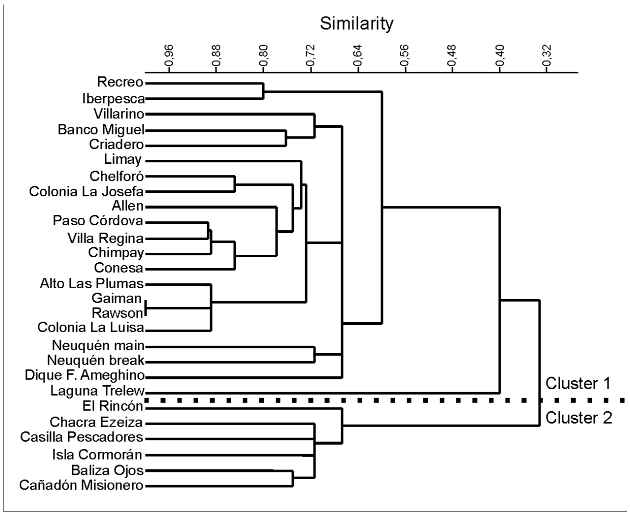

Figure 5.- Cluster analysis of the twenty-seven surface sediment samples from Patagonia.

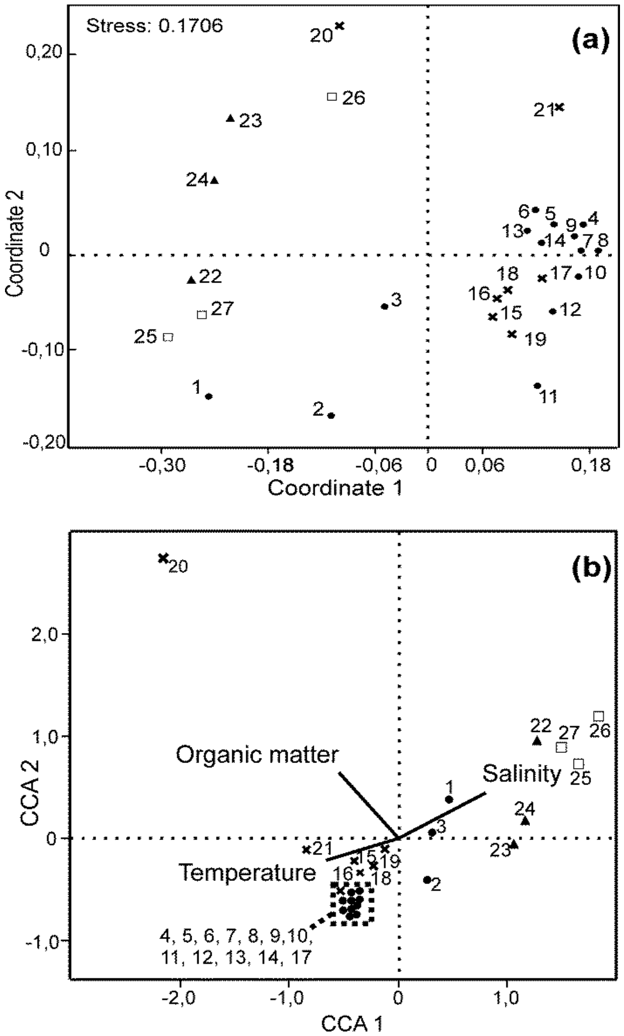

Figure 6.- (a) NMDS ordination plot from Negro River (black circles), Chubut River (gray crosses), San Julián bay (gray squares) and Santa Cruz estuary (black triangles) and (b) CCA ordinaton plot showing the relationship between environmental variables and sampling sites.

Table III.- Canonical Correspondence Analysis (CCA) results

Axis	Eigenvalues	% variance explained	Permutation test (p)
1	0.83288	26.33	0.03774
2	0.76533	24.19	0.00111
3	0.65681	20.76	0.00111
4	0.36645	11.58	0.0222
5	0.33283	10.52	0.00111
6	0.20903	6.608	0.02664
7	2.8281^{-11}	8.94^{-10}	0.6437

Trace: 3.163
Trace p: 0.00111

The present study showed that salinity solely cannot be considered as a driving force determining diatom species composition and distribution in these systems, although temperature must be considered a very important environmental factor as shown in the Canonical Correspondence Analysis (CCA).

As shown in table 1, water in inner areas had different salinity characteristics compared to the coastal water but the summer temperatures were very similar throughout the course of Negro and Chubut Rivers (north Patagonia) from the headwaters to the inlets. Different temperatures values were observed according to the latitude, the northern estuaries (Negro and Chubut Rivers, 40° and 43° S respectively) had higher temperatures than southern estuaries (San Julián Bay and Santa Cruz River, 49° and 50° S respectively). Salinities were variable between the four estuarine environments due to the effect of tides on meso and macrotidal regimes.

CCA showed that the effect of salinity and temperature on the species distributions is significant, indicating that environmental processes control the distribution of sedimentary diatoms along Patagonian estuaries in predictable ways. Salinity and temperature were strongly correlated with CCA Axis 1, positive and negative, respectively. Organic matter is associated to CCA Axis 2. Southern sites are positive associated to salinity within the CCA because no samples were taken in the associated fluvial systems (freshwater).

The results indicate that, although mixing of allochthonous and autochthonous diatoms could occur, estuarine diatom assemblages reflects major environmental gradients in Patagonian estuaries and associated fluvial systems and thus can be effectively used for quantitative reconstructions of former environmental conditions.

Hierarchical Cluster Analysis segregated diatom communities into two assemblages depending on the temperature and the NMDS segregated diatom community in to two assemblages according to the salt-wedge. River flow magnitude and fluctuations imply strong physicochemical variability. So, changes in salinity play a significant effect on the marine/brackish assemblages of the estuaries that are subjected to the freshwater input from rivers. Where the tidal effects decrease a short distance (200 m) from the mouth freshwater diatoms are dominant; this is the case of the Chubut estuary characterized by diatom assemblages of riverine conditions with high abundances of *Stephanodiscus hantzschii*, *Cocconeis placentula*, *C. pediculus* and *Aulacoseira granulata*. On the other hand, in the Negro River estuary where the tidal influence reaches 15 km from the mouth, dominant diatom assemblages are characteristic of tidal channels: *Cymatosira belgica*, *Delphineis surirella* and

Paralia sulcata, and salt-marshes: *Diploneis interrupta* (Vos & De Wolf 1993b). In macrotidal estuaries marine diatoms such as *Paralia sulcata* and *Rhaphoneis amphiceros* colonize tidal banks and marshes as occur in San Julián Bay and brackish assemblages represented by *Caloneis westii*, *Navicula cincta* and *Tryblionella levidensis* are dominant at Santa Cruz estuary where freshwater influence is significant; the two rivers converging at the estuary reflect these fluvial effects.

The presence of some euryhaline taxa (such as *Paralia sulcata*) in estuarine systems from Patagonia is according with changing salinity regimes, where the taxa are more selected according to their ability to adapt to changing salinity rather than to their salinity optima (Snoeijs 1999). Dominance of euryhaline/brackish taxa has been observed in estuaries of Buenos Aires province (Hassan et al. 2006, 2007) and Brazilian coastal lagoons (Sylvestre et al. 2001). The responses of diatoms to salinity can vary widely among species and within species. There is evidence that a diatom species can display different salinity optima in different water bodies, although tolerance ranges generally overlap (Cumming & Smol 1993). Rovira et al. (2012) reported that the most abundant and frequent diatom species in the Ebro Estuary (Spain) can tolerate a wide range of conductivity levels that are directly related to the salinity; especially in estuarine conditions where there is high physicochemical variability. Although our study reinforces these assumptions, the marine/brackish dominant species *Cymatosira belgica*, *Delphineis surirella, Diploneis interrupta* and *Paralia sulcata* do not appear in the riverine area and consequently we can characterize this as a typical estuarine assemblage. In contrast, most abundant freshwater diatoms (such as *Aulacoseira granulata*) inhabit in both environments with different proportions or combinations to produce the distinction between assemblages. This broad tolerance of diatom communities to environmental changes in estuarine systems have been previously reported (Underwood 1994, Hassan et al. 2007, Bate & Smailes 2008). At Yaquina estuary (Oregon, USA), the distribution of diatoms was regulated primarily by mean salinity and characteristics of the sediment (Amspoker & McIntire 1978). Prominent taxa in the river above Yaquina Bay exhibited overlapping distributions along the salinity gradient to a location in brackish water where the mean salinity was approximately 5‰ and sharp changes of assemblages composition were detected.

Negro River: Negro River is dominated by freshwater diatoms along its middle and lower floodplain (Fig. 2). Pucci et al. (1996), analyzing microalgal composition from water samples and the environmental parameters in only three sites along the Negro River, concluded that the composition of these assemblages was homogeneous between sites during low tide (S‰=0.052 to 0.32). For samples taken during high tide salinity rose up to 26‰ at the inlet decreasing to values under 0.19‰ in the other two stations. Accordingly, diatom assemblages were more diverse, and dominated by brackish-freshwater forms in the two inner stations; and by coastal-marine taxa in the outer station. At the present study, diatom analysis from the fourteen sediment samples along Negro River allowed to recognize marine-brackish assemblages (*Cymatosira belgica*, *Paralia sulcata*, *Raphoneis amphiceros* and *Diploneis interrupta*) in the three nearest sites of the river inlet extending to 15 km although salinity values were low (0.1 ‰ at Criadero site during high tide, see table 1).

The impact of organochlorine pollutants (OCPs) is low at the estuary of Negro River (Isla et al. 2010b). They diminish from the headlands to the inlet with a slight increment at the beginning of the Lower Valley. Higher concentrations of OCPs in the Upper Valley are directly related to fruit and agricultural activities, and the intensive use of pesticides. Probably the presence of riparian vegetation along the Middle and Lower valleys in addition to the dynamics of the estuary has led to a dilution effect of these compounds at the lower valley (Isla et al. 2010b). Diatom assemblages of Upper Valley sites are dominated by *Cocconeis euglypta* while in the Middle Valley the dominant taxon is *Cocconeis placentula*. C. *euglypta* is assumed to be more tolerant to organic pollution than *C. placentula* (Kelly et al. 2005). According to Monnier et al. (2007) *C. euglypta* is ubiquitous, abundant in mesotrophic waters and supports different pollution levels and tolerant to high conductivity water (Romero & Jahn 2013).

Chubut River: Diatom assemblages from the Chubut River indicate that freshwater conditions dominates along the valley with assemblages composed mainly by the planktonic *Stephanodiscus hantzschii* accompanied by the epiphytes *Cocconeis pediculus* Ehrenberg and *Cocconeis placentula* living at salinities of 0 to 2‰ (Fig. 3). The absence of *Cocconeis euglypta* along the Chubut River may be explained by a much lower concentration of pesticides. The sites Recreo and Iberpesca are close to the coast but the marine influence is very scarce, even during high tides. Some marine/brackish diatoms as *Opephora pacifica* (Grunow) Petit and *Tryblionella hungarica* (Grunow) Frenguelli were only be sampled at the inlet. The Chubut River has a wave-dominated and shallow inlet, and therefore, is dominated by freshwater diatoms. The availability of freshwater at the mouth is at least 40 to 60% per day for low and high river discharges respectively (Helbling et al. 1992). These results were in coincidence with the reports of Santinelli et al. (1990). At Trelew lagoon site brackish aerophilous *Hantzschia amphyoxis* accompanied by *Hippodonta hungarica* dominate. This assemblage is different to the others along the river because this site is a very shallow and brackish environment with salinity of 0.2‰ and higher percentage of organic matter; it is supplied by water discharging agriculture channels and diatom assemblages are favored by very high nutrient concentrations. This is an environment with anthropogenic impact. Vacht et al. (2014) found that the diatom community composition of riparian soils could potentially indicate anthropogenic disturbance levels, especially through the abundance, absence, or presence of specific species as *Hantzschia amphioxys*. NMDS and cluster analysis reflect the different diatom composition of Trelew Lagoon site in relation to the others.

San Julián Bay: Very little is known about the diatom distribution along macrotidal estuaries. In this sense, San Julián Bay operates as a coastal lagoon dominated by a macrotidal regime. The water characteristics were mainly controlled by the effect of the tidal inflow of coastal water. Conditions in San Julián Bay were not homogeneous despite the large tidal exchange of water. The lagoon is hypersaline (36.3 to 36.5 ‰) in summer, temperatures are variable according to sampling sites. The near shore sites have lower temperatures than those in the interior. Fluctuations in temperature are influenced by water inputs from the ocean. The higher temperature site (Chacra Ezeiza) is the farthest from the coast. Marine diatom assemblages characterized this estuary. Cormorán Island and Chacra Ezeiza sites presented typical tidal flats assemblages (Vos & De Wolf 1993b) with marine plankton and tychoplankton (*Paralia sulcata*, *Rhaphoneis amphiceros* and *Actinoptychus senarius*). El Rincón site, on the other hand, showed a monospecific assemblage of *Navicula salinicola*, a small epipelic species that is favoured by high concentrations of nitrogen compounds. This monospecific pattern may be attributed to shallow trends within the site in contrast that occur in Lagoa de Araruam (Brazil) where the presence of this species was attributed to anthropic activities (Sylvestre et al. 2001). *Navicula salinicola* was also reported dominating at a tidal flat at North Sea in Germany during autumm (Scholz & Liebezeit 2012).

Santa Cruz Estuary: Diatom assemblages of Santa Cruz Estuary were different from the other estuaries. At this latitude (50° S), summer temperatures were the lowest recorded in the study (between 13.9 and 15.1°C). There are also differences between the three sample sites of Santa Cruz Estuary and they are directly related to the salinity. At Cañadón Misionero and Baliza Ojos (salinities of 2.3 and 13.4‰ respectively), *Caloneis westii* and *Tryblionella levidensis* were the dominant taxa. In Casilla Pescadores (23.75‰), *Navicula cincta* and *Cocconeis scutellum* Ehrenberg were the most representative taxa. The high discharge of the Santa Cruz River provoked a mixing with the high-salinity tidal effect (Isla & Cortizo 2013). The future construction of two dams along this river: Condor Cliff and La Barrancosa projects (Capdevila et al. 2008) will alter the freshwater input to the lower valley and therefore changes in the diatom distribution are expected to occur.

The need to understand present day – in order to predict future-, climate changes greatly enhances the interest to understand past environmental dynamics (Bradley 2000). To obtain environmental records that extend beyond instrumental measurements, quantitative reconstruction techniques are needed (Birks 1998), and diatoms are excellent bioindicators. This paper increases the knowledge of ecological requirements of Southern South American estuarine diatom communities and provides useful data for environmental reconstructions in coastal settings of the region.

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