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### An Introduced Polychaete in South America – Ecologic Affinities of *Manayunkia speciosa* (Polychaeta, Sabellidae) and the Oligochaetes of Uruguay River, Argentina

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#### 1. Introduction

#### 1.1 The Uruguay River and its alluvial valley

Rivers are very dynamic and complex ecosystems. The elements forming their structure are the riverbed, the bank area and the alluvial valley. They can change in size and complexity according to their geological features and the climate of the region they cross. This variation in the horizontal axis, the flow regime, the physicochemical characteristics of the water and the type and density of the vegetation determine, in fluvial systems, a spatial heterogeneity, in agreement with the temporal dynamism (Sabater et al., 2009).

South America is characterized by the vast extension of its river basins, among which the Amazon, Orinoco and Del Plata can be cited. Due to the geologic structure of the continent, the rivers of these basins, generally long and with an abundant flow drain into the Atlantic Ocean. Among the main rivers forming the Del Plata Basin are the Paraguay, Paraná and Uruguay (Bonetto & Hurtado, 1998).

Uruguay River flows along 1800 km, starting in Brazil, and passing through Argentina and Uruguay before draining in the estuarial system of the Río de la Plata. The Uruguay River basin comprises some 365,000 km<sup>2</sup> with a complex geology, forming a mosaic of volcanic (basaltic flows) and sedimentary rocks and quaternary alluvial sediments (Bonetto & Hurtado, 1998; López Laborde, 1998). These Holocene formations occurred during a long period of some 5000 years under a tropical/subtropical humid climate relatively homogeneous in the whole basin, giving rise to partially erosioned soils covered by young sediments, mainly eolian (Iriondo & Kröhling, 2004). According to its hydrographic features it can be divided into three sections: upper, middle and lower. The Upper Uruguay, fast and hardly navigable, starts in Brazil at the confluence of Pelotas and Canoas rivers extending to

the drainage of the Piratiní River, along some 800 km with a slope of approximately 43 km; the middle section is ca. 600 km long reaching the locality of Concordia (Entre Ríos Province, Argentina) where the Salto Grande hydroelectric dam is found; the lower section is 350 km long and presents a slope of only 3 km.

The average discharge of Uruguay River is 4500m<sup>3</sup>/s with extremes varying from 800 to 14,300 m3/s and although its discharge level is lower than the Paraná River's, its variability is higher (from 11 to 17 %). Its waters are transparent with much less solids in suspension than in the Paraná River (López Laborde, 1998; Nagy et al., 1998). The swelling of Uruguay River takes place in winter, with a secondary maximum in spring and a minimum flow in summer-autumn. An increase in the discharge has been observed since 1950 and the modifications in its flow have been due to the Salto Grande hydroelectric dam (Nagy et al., op. cit.). Both Uruguay and Paraná rivers show a strong oscillation in phase with the ENSO events (El Niño/La Niña Southern Oscillation), with remarkable swellings during the mild events (or El Niño) and normal to low discharges during the cold events (or La Niña) (Pasquini & Depetris, 2006). Its alluvial valley is formed by ecosystems with wetlands of relative extension and importance where the gallery forest connected to the drainage system stands out. The basin is found in the phytogeographic territory corresponding to the Amazonic ("Paranaense" province) and Chaqueño ("del Espinal" and Pampean provinces) domains. It presents numerous edaphic communities and marginal forests formed by species from the Paranaense province, although impoverished in a northern-southern direction (Cabrera & Willink, 1973). These marginal or gallery forests are narrow strips, densely populated by fairly tall species like "ibirapitá" (Peltophorum dubium (Spreng.), "lapacho rosado" (Tabebuia impetiginosa ((Mart. ex DC.) Standl.), "azota caballo" (Luehea divaricada Mart.), and "pitanga" (Eugenia uniflora L.). Towards the Lower Uruguay in the riparian zone of Argentina and in the islands there is an almost absolute predominance of "sarandí blanco" (Phyllanthus sellowianus Müll.) (Bonetto & Hurtado, 1998; Cabrera, 1971). This abundant riverside vegetation plays an important role in the intake of coarse particulate allochthonous material which is then used by the benthic organisms associated to the riverside, determining a fauna characteristic of these environments.

With respect to the uses of the river, navigation is concentrated mainly in the lower section towards the mouth of the Río de la Plata River, in the middle section it is navigated by ships of reduced draft while upstream, in Brazilian territory, navigation is in small boats. The main use of its waters is connected with the generation of hydroelectric energy (e. g. the Salto Grande hydroelectric dam, already mentioned, and two other dams in its upper section).

The recreational use of the river includes fishing, sports practice and bathing resorts in riverside Argentinian cities such as Gualeguaychú and Colón. The impact on the river is due to an inadequate management of the resources, such as agricultural, industrial and transport activities, the production of energy, and the habitat fragmentation (Bonetto & Hurtado, 1998).

#### 1.2 Benthic invertebrates

Invertebrates are among the most adapted organisms in fluvial ecosystems, playing a fundamental role in the transference of energy from the inferior levels (macrophytes,

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algae and detritus) to the superior consumers (aquatic vertebrates and birds). As they have evolved in an unpredictable environment, many of them have developed opportunistic ways of life and generalistic diets. Their communities often show a great plasticity with respect to their vital features and reproductive strategies which they optimize according to the environmental characteristics (Barbour et al., 1999; Rodrigues Capítulo et al., 2009).

The community of organisms living at the bottom of a water body constitutes the benthos. The characteristics of the physical habitat, the water quality and the availability and quality of the food determine, to a great extent, the presence and abundance of benthic organisms. In fluvial environments the current and type of substratum also determine the benthic structure. The current produces a homogenizing effect of the environment, diminishing diversity, removing particles and favoring the gaseous exchange. The type of substratum can be formed by sediments of different granulometry, such as gravel, sand, silt and clay, and by riparian vegetation. Spatial and temporal variations of the fluvial systems determine that the communities of organisms inhabiting the upstreams usually differ from those inhabiting the middle and downstreams. Besides, it is possible to distinguish a littoral benthos, with habitat heterogeneity, greater diversity, and coarse particulate organic matter, and a profoundal benthos with less available habitats, moderate diversity and thin particulate organic matter (Margalef, 1983).

The study of the benthic community of Uruguay River has been carried out by Ezcurra de Drago & Bonetto (1969) and Di Persia & Olazarri (1986) concerning fouling and arborescent sponges; bivalves and gastropod mollusks; and caddisflies, mayflies and true flies insects (Bonetto & Hurtado, 1998). Nevertheless, and differently from other great South American rivers as the Paraná, Paraguay and Río de la Plata, where the oligochaetes have been extensively studied (Armendáriz et al., 2011; Ezcurra de Drago et al., 2004, 2007; Marchese & Ezcurra de Drago, 1992; Marchese et al., 2005; Montanholi-Martins & Takeda, 1999; Takeda, 1999), the Uruguay annelid fauna has received, so far, scarce attention.

#### **1.3 The introduced species**

The introduction of species is one of the processes which produce major alterations in the biodiversity of ecosystems. Even though it is not a problem caused only by human activity, the number of species involved and the frequency of their relocation has grown enormously as a result of the expansion of transportation and trade (Glasby & Timm, 2008; Penchaszadeh, 2005). The continental aquatic environments are highly susceptible to the accidental or deliberate introduction of exotic species. Besides, the natural connection of the basins and the dispersion capacity of the aquatic organisms together with the human activity determine the possibility of invasion of new species (Ciutti & Cappelletti, 2009). In these aquatic environments, the transfer of exotic species occurs through a wide variety of ways and means, for example, in maritime transportation, in the ballast water of ships (El Hadad et al., 2007).

In South America, most of the coastal ecosystems between the Río de la Plata Estuary and central Patagonia have been modified due to the introduction of exotic organisms. From 31 identified cases, 6 have caused a considerable ecological impact (Orensanz et al., 2002). In this region, the introduction of crustaceans such as *Balanus glandula* Darwin, 1854 and

*B. amphitrite* Darwin, 1854; mollusks, such as *Crassostrea gigas* (Thumberg, 1793), *Corbicula fluminea* (Müller, 1774), *C. largillierti* (Philippi, 1844), *Limnoperna fortunei* (Dunker, 1857), and *Rapana venosa* (Valenciennes, 1846), and polychaetes such as *Ficopomatus enigmaticus* (Fauvel, 1923) have been recorded in the last 40 years (Penchaszadeh, 2005). Recently, the presence of *Manayunkia speciosa* Leidy, 1858 (Armendáriz et al., 2011) has been registered in Uruguay River.

*M. speciosa* is a small polychaete strictly from freshwater, first recorded in Schuylkill River (USA) in 1858. Some decades later its distribution extended through the whole Nearctic Region, being recorded in the eastern and western lotic systems of North America (Brehm, 1978; Hazel, 1966; Holmquist, 1967; Krecker, 1939; Leidy, 1883; Mackie & Qadri, 1971; Meehean, 1929; Spencer, 1976). A hundred and fifty years after its first record, it is found for the first time in the Neotropical Region, enlarging its limit distribution from the United States of America to Argentina. Although it could not be determined whether its introduction had been recently, it can be assumed that *M. speciosa* arrived at the Uruguay River through the ballast water of commercial ships (Armendáriz et al., 2011).

In recent years, the studies on *M. speciosa* have been focused in some aspects of its population dynamics and in its role as intermediate host of Myxozoa parasites of Salmonidae (Bartholomew et al., 1997; Bartholomew et al., 2006; Stocking & Bartholomew, 2007). Due to the lack of information on the ecological requirements of *M. speciosa* together with the scarce knowledge on the annelid fauna of the Lower Uruguay River, we propose, in this chapter, to analyze the main ecological variables which could have determined the establishment of *M. speciosa* with the oligochaete annelids, forming the benthic assemblage of the Lower Uruguay River.

#### 2. Methodology

#### 2.1 Study area

The study was carried out in the Lower Uruguay River (Figure 1). Eight sampling sites were selected between  $33^{\circ}$  05′ S 58° 12′W,  $33^{\circ}$  5.94′ S 58° 25′ W. Sites S1 to S5 were established on the main river basin, site S6 at the centre of Bellaco Bay, site S7 inside Ñandubaysal Bay, and site S8 in Inés Lagoon. Sites S1 to S3 were located upstream of a pulp mill industry on the Uruguayan coast. Site S4 is an area of sediment accumulation, located downstream of the mentioned industry. Site S5 is located at the centre of the main riverbed. It is characterized by a high current velocity and a pronounced erosion effect. Hence, it constitutes a clean water zone with a limited offer of refuge and food for the benthic fauna. Site S6, located in a zone with calm waters, with bulrush (*Schoenoplectus californicus* (C. A. Mey) Palla) and riverside vegetation is influenced by Gualeguaychú River and Gualeguaychú city. Site S7 is also located in a zone with a low current velocity, Ñandubaysal Bay, with low waters and abundant littoral vegetation. Depending on the seasonal hydrological regime of the river, this site is connected with site S8 that shows lentic characteristics.

The sediments of the study area were formed by plastic silty clay with small proportions of scarcely sorted quartz sand (Iriondo & Kröhling, 2004).

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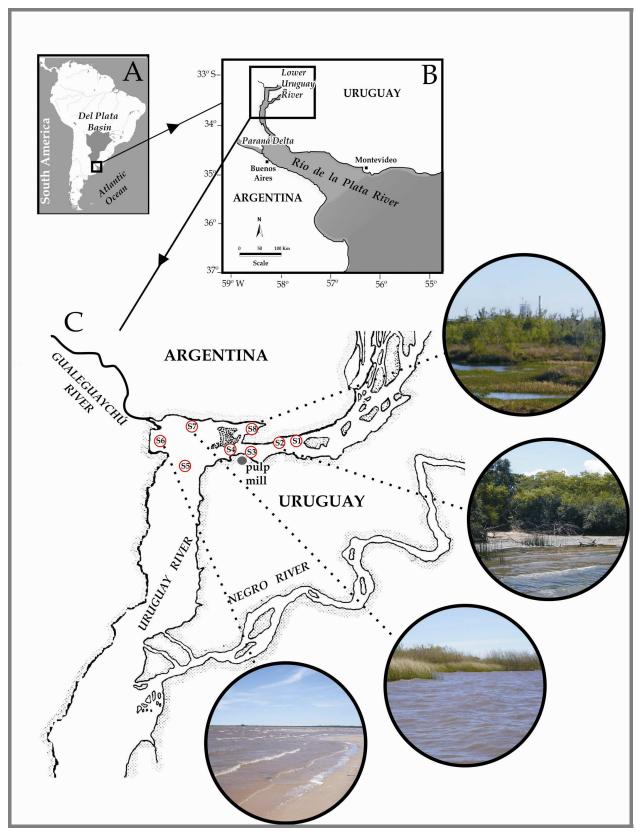


Fig. 1. A) Map of Del Plata Basin in South America. B) Study area in the Lower Uruguay River. C) Sampling sites: sites S1 through S5 at the main course of the Uruguay River, S6 at Bellaco Bay, S7 at Ñandubaysal Bay, and S8 at Inés Lagoon. Photos: A. Rodrigues Capítulo.

#### 2.2 Sampling of the annelid assemblage

The work methodology included the taking of samples with a van Veen dredge (470 cm<sup>2</sup>) from a boat (Figure 2). Seasonal samples, from November 2007 through March 2009, were extracted in the 8 mentioned sites, distinguishing, when possible, the main riverbed and the bulrushes from the coast (Fig. 1). Site S8 was not sampled during autumn and winter due to the scarce depth of the river and the abundant aquatic vegetation which made navigation impossible. Six replicates in each site and season were collected. The samples were fixed *in situ* with formaldehyde 5%. The following physicochemical variables were measured: Temperature in °C and pH (Hanna HI 8633), dissolved oxygen in mg/l (Ysi 52), conductivity in  $\mu$ S/cm (Lutron CD-4303) and turbidity in UTN (Turbidity meter 800-ESD). Due to difficulties with the equipment, depth could only be measured in spring, summer and autumn. In March 2009, water samples for the analysis of nutrients (nitrates, NO<sub>3</sub><sup>-</sup>; nitrites, NO<sub>2</sub><sup>-</sup>; ammonium, NH<sub>4</sub><sup>+</sup>; and soluble reactive phosphorus PO<sub>4</sub> <sup>3-</sup>) were also taken.



Fig. 2. Van Veen dredge used for collecting the benthic samples in Lower Uruguay River, Argentina.

In the laboratory, the samples were washed with a 500  $\mu$ m mesh sieve, dyed with erythrosine B, and the organisms were separated manually from the sediment under stereoscopic microscope. Annelids were identified under optical microscope according to keys and specific bibliography (Brinkhurst & Marchese, 1992; Glasby & Timm, 2008;

Opinion 2167, ICZN, 2007; Pettibone, 1953; Struck & Purschke, 2005). Final preservation of the organisms was made in alcohol 70%.

Indirect measurements of the content of organic matter (OM) in sediment of the collected samples were measured using the loss organic ignition method (LOI: loss organic ignition) at 500°C during 4 h, with a previous drying of 48 h at 60°C (APHA, 1998).

#### 2.3 Statistical analysis

The annelid assemblage was evaluated through the application of ecological indexes: diversity (H') (Shannon-Wiener, in log<sub>e</sub>), evenness (E') and specific richness (R: number of species or taxa). The frequency of occurrence of each species was calculated as FO (%) = 100 m<sub>i</sub>/M, where m<sub>i</sub>: is the number of samples containing species i, and M: the total number of samples. According to this, the species were classified in: Constant (C): with a FO  $\geq$  75 %; Frequent (F): with 75% < FO  $\leq$  50%; Accesorial (A): 50% < FO  $\leq$  25%; and Incidental (I): FO < 25%.

A Pearson correlation was performed in order to analyze the relationship between: 1) percentage of the organic matter sediment vs. individual abundance (N) of *M. speciosa* and 2) percentage of the organic matter sediment vs. total abundance of oligochaetes.

The relationship between the sampled sites and the environmental variables recorded was explored by a Principal Components Analysis (PCA). A Detrended Correspondence Analysis (DCA) was applied to the biotic data in order to determine if the species responded linearly to gradients or passed through some environmental optimum. Due to the fact that the maximum length of gradients in standard deviation units in this analysis was 3.328, a model of unimodal response for the species has been assumed. It was decided to apply a Canonical Correspondence Analysis (CCA) (Ter Braak & Smilauer, 2002) to explore the relationships between the annelid abundance and the environmental variables recorded in the sampling sites. The abundances of the species were  $\log_e (x+1)$  transformed, and all the species presenting a frequency of occurrence higher than 3 % were included (Table 1). The physicochemical variables were standardized. Their inflation factors were smaller than 3. Depth was excluded from the analysis due to the lack of records corresponding to winter 2008. Significance of all canonical axes was evaluated through the Monte Carlo test (499 permutations under the reduced model, P < 0.05). The two first axes of the ordination were selected for the graphical representation.

#### 3. Results

#### 3.1 Physicochemical characteristics of the water

The physicochemical characteristics of the water in the section of the Uruguay River studied varied according to the following values: temperature fluctuated from 9.8 (S6, June 08) to 30.6 °C (S6, December 08), conductivity from 42 (S8, March 09) to 177  $\mu$ S/cm (S6, February 09), pH from 6.57 (S1, March 09) to 9.22 (S7, February 08), turbidity from 10 (S5, February 08) to 193 UTN (S1, December 08), depth from 0.5 (S1, June 08) to 18 m (S3, March 09), dissolved oxygen from 4 (S6, March 09) to 12.8 mg/l (S7, Sep 08), and organic matter from 0.083 (S3, in the main stream of the river, February 08) to 23.67 % (S7, in closed systems with vegetation, March 09). Nutrients were measured in only one sampling and varied according to the following values: nitrates, 0.082 (S8) to 0.564 mg/l (S5); nitrites, 0.001 (S3) to 0.027 mg/l (S7); ammonium, 0.004 (S8) to 0.486 mg/l (S4); and phosphates, 0.035 (S8) to 0.766 mg/l (S6).

#### 3.2 The annelid assemblage and its ecological affinities

The annelid assemblage studied in the Uruguay River during the period comprised a total richness R=40 taxa, most of which belonged to the oligochaete Naididae, being significant the quantity of species of Naidinae and Pristininae (Table 1). According to their frequency of occurrence and taking into account all the sampling sites, no species was constant. Only two taxa, *Aulodrilus pigueti* and Megadrili, were frequent; 11 taxa, among them *Limnodrilus hoffmeisteri*, *Bothrioneurum americanum*, *Nais variabilis*, *Pristina longidentata*, Enchytraeidae, *Narapa bonetoi*, and *Manayunkia speciosa* were accessories, while the rest of the taxa were incidental. Nevertheless, considering sites S3 and S7, *M. speciosa* was constant during the studied period.

ТАХА				FO
Oligochaeta				
Naididae	Tubificinae	Aulodrilus pigueti Kowalewski, 1914	Ар	F
		Limnodrilus hoffmeisteri Claparède, 1862	Lh	А
		Limnodrilus udekemianus Claparède, 1862	Lu	Ι
	Rhyacodrilinae	Bothrioneurum americanum Beddard, 1894	Ba	А
	-	Branchiura sowerbyi Beddard, 1892	Bs	Ι
	Naidinae	Stylaria fossularis Leidy, 1852	Sf	Ι
		Dero (Dero) pectinata Aiyer, 1930	Dp	Ι
		Dero (Dero) digitata (Müller, 1773)	Dd	Ι
		Dero (Dero) righii Varela, 1990	Dr	Ι
		Dero (Aulophorus) furcata (Müller, 1773)	Df	Ι
		Dero (Aulophorus) costatus	Dc	Ι
		(Marcus, 1944) emm. Harman, 1974	DC	1
		Allonais lairdi Naidu, 1965	Al	Ι
		Nais variabilis Piguet, 1906	Nv	А
		Nais communis Piguet, 1906	Nc	Ι
		Nais pardalis Piguet, 1906	Np	А
		Slavina appendiculata (d'Udekem, 1855)	Sa	Ι
		Slavina isochaeta Cernosvitov, 1939	Si	Ι
		Slavina evelinae (Marcus, 1942)	*	Ι
		Stephensoniana trivandrana (Aiyer, 1926)	St	Ι
		Bratislavia unidentata (Harman, 1973)	Bu	Ι
		Chaetogaster diastrophus (Gruithuisen, 1828)	Cdt	Ι
		Chaetogaster diaphanus (Gruithuisen, 1828)	Cdp	Ι
	Pristininae	Pristina osborni (Walton, 1906)	Ро	А
		Pristina proboscidea Beddard, 1896	Рр	Ι
		<i>Pristina jenkinae</i> (Stephenson, 1931)	Рj	А
		Pristina acuminata Liang, 1958	Ра	Ι
		Pristina americana Cernosvitov, 1937	Pam	А
		Pristina leidyi Smith, 1896	*	Ι
		Pristina longidentata Harman, 1965	Plo	А
		Pristina aequiseta Bourne, 1891	Pae	Ι
		Pristina sima (Marcus, 1944)	$\mathbf{Ps}$	Ι
		Pristina longisoma Harman, 1977	*	Ι
		Pristina macrochaeta Stephenson, 1931	Pm	Ι

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ТАХА			FO
Opistocystidae	Trieminentia corderoi (Harman, 1970)	Tc	Ι
	Crustipellis tribranchiata (Harman, 1970)	Ct	Ι
Enchytraeidae	•	EN	А
Narapidae	Narapa bonettoi Righi y Varela, 1983	Nb	А
Megadrili	, 0,	ME	F
Polychaeta			
Aeolosomatidae	Aeolosoma sp.	*	Ι
Sabellidae	Manayunkia speciosa Leidy, 1858	Ms	Α

Table 1. List of annelid species collected at the sampling sites in the Uruguay River (Argentina), with the abbreviation of each species showed in CCA biplot and the frequency of occurrence category: F, frequent (75 % < FO  $\leq$  50 %), A, accessory (50 % < FO  $\leq$  25 %); I, incidental (FO < 25 %).\* Taxa not included in the CCA (frequency of occurrence < 3%).

The ecological indexes applied showed certain fluctuations during the year in the sampling sites (Figure 3). Diversity (H') varied between 0.43 and 2.12. Site S5 presented the lowest H' values, while site S6 showed the highest ones. Evenness (E') varied between 0.24 and 0.87, although it was rather regular in all the sites throughout the period of study. Taxonomic richness (R) varied between 2 and 16, and it was very fluctuating in almost all the sampling sites, except in site S7 that showed similar values during the year (Fig 2).

Annelid density (N) varied between 66 (S5, spring) and 3985 ind./m<sup>2</sup> (S8, summer) (Figure 4). *M. speciosa* was present in sites S3 and S7 in all the seasons, while it was never recorded in site S8; in the rest of the sampling sites its presence was variable during the year. Its density varied between 4 (S6, spring) and 517 ind./m<sup>2</sup> (S3, autumn). Two egg-bearing females were found in March 2009.

Figure 5 shows an individual of *M. speciosa* —with part of its tube built by fine sediments—, and another annelids representative of the assemblage found in Lower Uruguay River, such as the naidid oligochaetes Naidinae and Tubificinae.

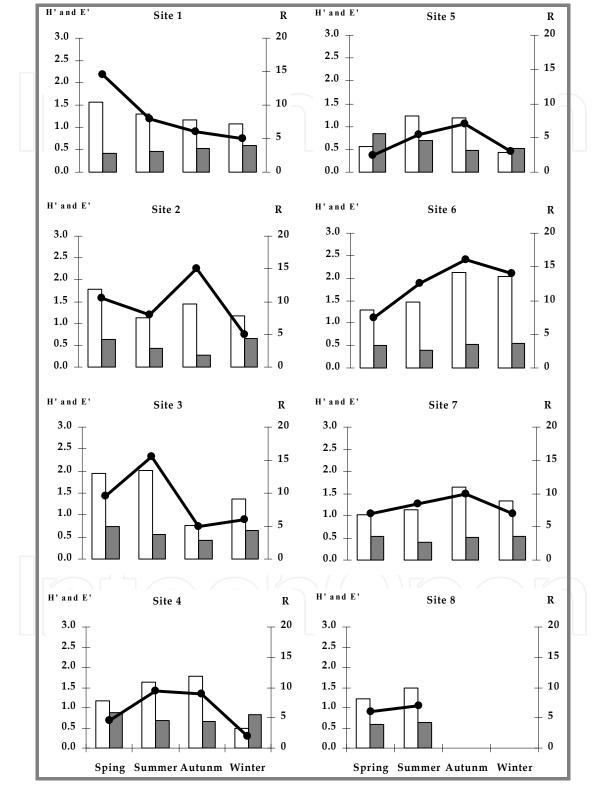
The Pearson correlation coefficient demonstrated a positive relationship between the abundance of *M. speciosa* and the percentage of organic matter in the sediments (r= 0.508; P< 0.001), as well as between the total abundance of oligochaetes in the samples and the percentage of organic matter in the sediments (r= 0.278; P< 0.05).

According to the PCA results, the first two axes explained 57.4 % of the cumulative variance of the data set (Table 2).

	Axis 1	Axis 2	Axis 3	Axis 4	p-value
PCA-eigenvalue	0.337	0.237	0.180	0.134	p vulue
PCA-cumulative % variance	33.7	57.4	75.4	88.9	
	0.592	0.340	0 242	00.9	
CCA-eigenvalue	0.592	0.340	0.242	0.171	
CCA-cumulative % variance explained	38.6	60.7	76.4	87.6	0.004
(species-environment relation)			-		-

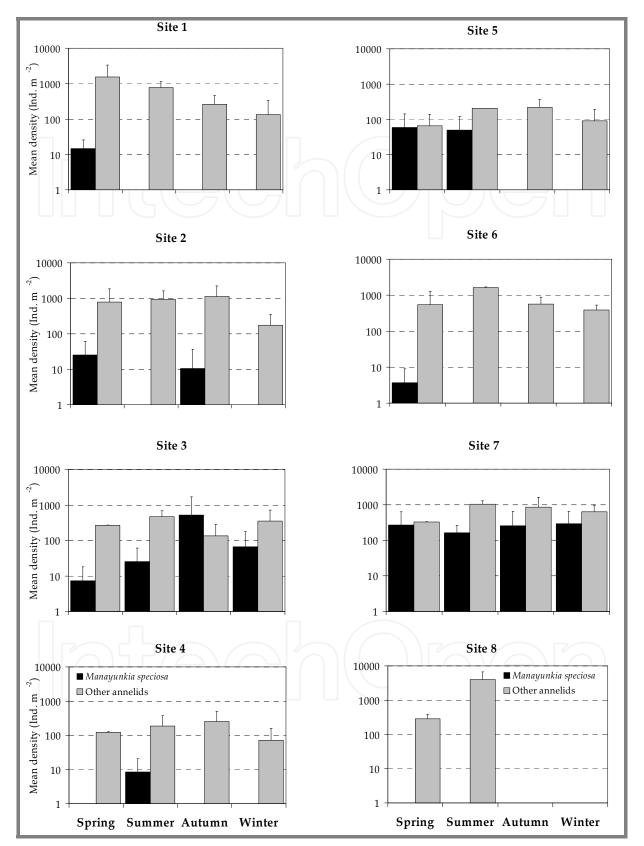
Table 2. Summary results from the PCA and CCA ordination analysis.

Figure 6 represents the ordination of the sampling sites studied determined by the two first axes (axis 1, eigenvalue: 0.337; axis 2, eigenvalue: 0.237). The variables that best correlated



with axis 1 were temperature and dissolved oxygen, while pH and conductivity correlated with axis 2. A marked seasonality in the ordination of the sites was observed.

Fig. 3. Ecological Indexes at the sampling sites in Lower Uruguay River. The Taxonomic Richness (R) is shown in black line, Evenness (E') in gray bars and Shannon-Wiener Diversity (H') in white bars.

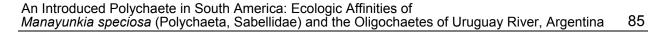


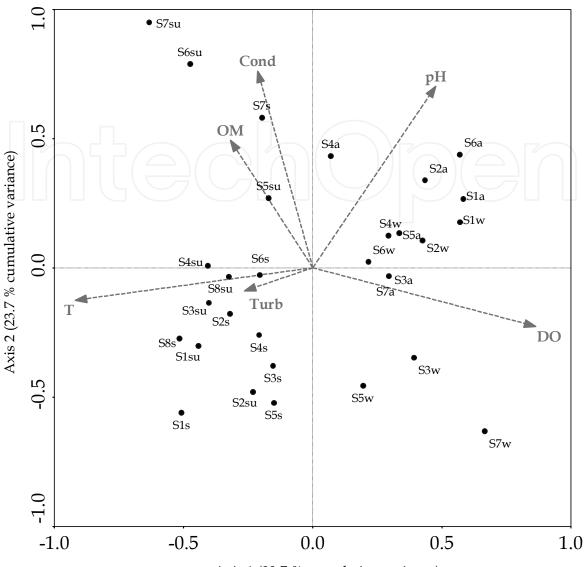
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Fig. 4. Mean abundances of the introduced polychaete *Manayunkia speciosa* and the other annelids (Oligochaeta and Polychaeta Aeolosomatidae) collected during the study at the Lower Uruguay River, Argentina.



Fig. 5. A. *Manayunkia speciosa* specimen collected in the Lower Uruguay River, Argentina. The arrow indicates part of the tube formed by fine sediments agglutinated. B. Oligochaeta Naididae: Naidinae (left) and Tubificinae (right).





Axis 1 (33.7 % cumulative variance)

Fig. 6. Ordination (PCA) diagram of the first two axes showing the variation in the sampling sites (dots) among habitat related variables (arrows). S1- S8: sampling sites; su: summer; a: autumn; w: winter; s: spring.

All the sampling sites corresponding to autumn and winter were grouped in the right (top and bottom) quadrants of the biplot, while the sampling sites corresponding to spring and summer were grouped in the left quadrants.

The first two axes of CCA explained 60.7 % of the cumulative variance from the data set (axis 1, eigenvalue: 0.592; axis 2, eigenvalue: 0.340) (Figure 7). The variables that best correlated with axis 1 were pH (*r*= 0.613) and conductivity (*r*= 0.533), while turbidity (*r*= 0.525) and temperature (*r*= 0.409) were the variables that best correlated with axis 2. The annelid species were distributed along a gradient associated to turbidity, for example *Dero digitata, Stephensoniana trivandrana, Trieminentia corderoi,* and *Slavina appendiculata,* among others. *Bratislavia unidentata, Limnodrilus hoffmeisteri, Branchiura sowerbyi,* and *Pristina osborni* were associated to organic matter. *Stylaria fossularis* markedly separated from the rest of the

species and presented a strong association with conductivity and pH. Otherwise, *M. speciosa*, did not show any particular association with the physicochemical variables and grouped together with other species as *Narapa bonettoi*, *Limnodrilus udekemianus*, *Aulodrilus pigueti*, *Pristina jenkinae* and the Enchytraeidae, close to the intersection of the axes.

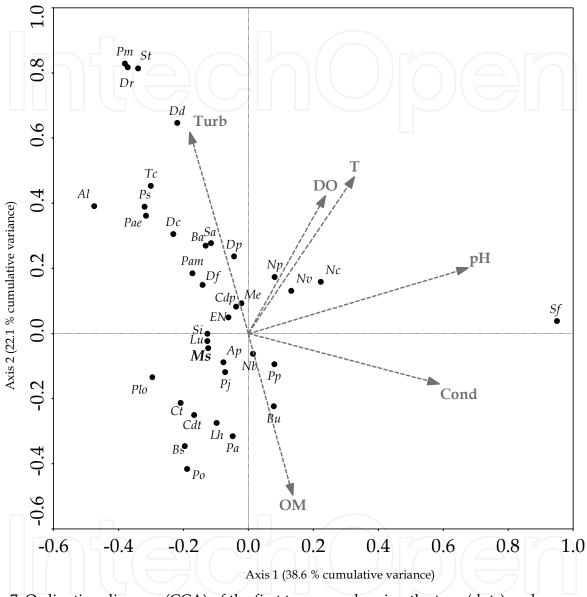


Fig. 7. Ordination diagram (CCA) of the first two axes showing the taxa (dots) and environmental variables (arrows). Taxa abbreviations are as in Table 1.

#### 4. Discussion and conclusions

 Macroinvertebrates are good indicators of the ecological quality of rivers; they integrate the changes occurring over time in the environmental conditions. The species respond to the environmental variables through the gradients and the communities of specific assemblages can be used as entities in order to describe the biological state of rivers under certain environmental conditions. Ordination techniques are used to describe the species-environment associations and to identify the factors which could influence the

habitat preference of each taxon. It is a way of understanding the range of tolerance of a particular species to certain environmental variables (Adriaensses et al., 2007). In this study, ordination techniques were used in order to understand the environmental preferences of each species. Nevertheless, as the ordination only analyzes the correlations between environmental variables and species distribution, and does not reveal causal relationships, it cannot be used as a tool to determine the real factors within the many variables which could be affecting the species distribution (Lin & Yo, 2008).

The Principal Components Analysis (PCA) showed that the tendency in the habitat distribution between the sampling sites was affected, to a great extent, by seasonality. The principal environmental variables acting in the ordination of the sites were temperature, conductivity and dissolved oxygen. The Canonical Correspondence Analysis (CCA) evaluated the relationship between the species populational abundance and the environmental variables. The length of the arrows representing certain variables, such as turbidity, pH and conductivity shows the impact they have had on the annelid assemblage. Some species showed a strong association with turbidity (e.g. Dero digitata and Stephensoniana trivandrana), others to organic matter (e.g. Bratislavia unidentata and Limnodrilus hoffmeisteri), while others, like Manayunkia speciosa, Aulodrilus pigueti, Narapa bonettoi did not show a particular association with the physicochemical variables and grouped together close to the intersection of the axes. L. hoffmeisteri is a species frequently associated to eutrophic environments (Dumnicka, 2002; Krodkiewska & Michalik-Kucharz, 2009; Särkkä, 1987) and together with A. pigueti dominates in the alluvial plain environments of Paraná River (Ezcurra de Drago et al., 2007). N. bonettoi is a species commonly associated to coarse sediment and sand, generally found in the main riverbed of Paraná and Paraguay rivers, where it can reach high densities (Ezcurra de Drago et al., 2007; Marchese et al., 2005; Takeda et al., 2001). Nevertheless, we found it mainly in shallow zones (bays) with abundant aquatic vegetation.

• The oligochaetes are important organisms in the aquatic systems due to their impact on the sediment structure and water-sediment exchanges. Their role in the trophic nets and as intermediate hosts for several myxozoan parasites of fishes of economic importance is significant, as well as their application in pollution monitoring programs and their potential use to reduce sludge volumes in sewage treatment systems (Martin et al., 2008). Current studies on the zoobenthos of Uruguay River only deal with the fauna of its middle and upper sections (Ezcurra de Drago & Bonetto, 1969; Amestoy et al., 1986; Di Persia & Olazarri, 1986; Pintos et al., 1992). The annelid fauna of the lower section has not received much attention so far. In our work, the freshwater polychaete *Manayunkia speciosa* was associated with the oligochaete assemblage formed by cosmopolitan organisms, like Naididae (mainly Tubificinae and Naidinae) and Enchytraeidae (Martin et al., 2008), and an endemic family of the Neotropical region, Narapidae (Ezcurra de Drago et al., 2007).

Such assemblage showed certain similarities with the oligochaete fauna recorded in other large South American rivers as Paraguay and Paraná (Ezcurra de Drago et al., 2007; Marchese et al., 2005; Monthanoli & Takeda, 1999; 2001), although a higher number of species have been recorded in our study in the Lower Uruguay River. Among the especies registered in all these rivers, *A. pigueti, L. hoffmeisteri, L. udekemianus, Bothrioneurum americanum, Pristina americana, P. longidentata, Stephensoniana* 

trivandrana, Trieminentia corderoi and Narapa bonettoi can be mentioned. Ezcurra de Drago et al., (2007) noticed that the benthos of large rivers (with sandy-mobile beds) has a high similarity world-wide, with high abundances, low biomass and low species richness. In the lateral dimension of the alluvial plain, these authors (Ezcurra de Drago, op. cit.) observed a gradient from the main channel to the temporary marginal wetlands, with an increase in the species richness, diversity and biomass, and a decrease in abundance. In the alluvial river-plain transect they found a higher occurrence of oligochaetes in channels and lakes. The spatial heterogeneity, habitat structure, temporal instability and high productivity determine the highest importance of the transversal dimension over the longitudinal in the large rivers with alluvial plain. Vertical dimension also plays an important role in these systems, as it offers refuge for the hatching of eggs and the eclosion of juveniles, providing a habitat for these first stages which represent a faunistic reserve of the system under adverse conditions. Many invertebrate species, the oligochaetes among them, can move to the sediments during the drought phases until favorable conditions are restored. In the Parana River system, the benthic assemblage exhibits more variation in an extended spatial scale, where geophysical processes occur, rather than in smaller scales.

We observed a similar complex as pointed out by Marchese et al., (2005), and Ezcurra de Drago et al., (2007) in Paraná River, in which the highest richness and diversity were recorded in the marginal habitats (or floodplain lakes), that represent the highest spatial and temporal heterogeneity of habitats. Likewise, these authors recorded the highest densities in the main riverbed with values much higher than the ones registered by us. In our study, the highest abundances of individuals have been found in sites with high values of richness and diversity (as bays and lagoon), but not in the main riverbed. In our case, the low values of abundance, diversity and richness observed in the main riverbed (S4), may be due to the influence of the pulp mill industry placed upstream. It is known that the benthic invertebrates are exposed to xenobiotic chemicals in several ways, like the direct contact with sediment through the tegument or the ingestion of contaminated sediment, as the pulp mill effluents and their sediments contain possibly bioavailable and toxic amounts of resin acids,  $\beta$ -sitosterol and chlorophenolics (Merilaïnen & Oikari, 2008).

In the large South American Rivers (e.g. Paraná and Paraguay), the composition of the benthic assemblage and the total density show changes determined by the annual pulse of floods and the degree of connectivity of the alluvial environments, and by the long term phenomena involving ecological successions. For the benthic communities, the low water periods represent a more significant stress factor than the floods (Ezcurra de Drago et al., 2004; 2007; Montanholi & Takeda, 2001). Nowadays, the ENOS (El Niño/ Southern Oscillation) is one of the most widely studied weather phenomena. During the sampling period in the Lower Uruguay River, the ENOS (El Niño/La Niña) events underwent a cold phase or "La Niña" with transition to a neutral phase (information of the Departamento Climatológico, Servicio Meteorológico Nacional, Argentina, www.smn.gov.ar), related with periods of normal to low discharges (Pasquini & Depetris, 2007). Montaholi & Takeda (2001) recorded Aulodrilus pigueti in the Upper Paraná River during the low water phase, where it was dominant. In our study, this species appeared in every site and sampling occasion. Other studies associated A. pigueti with fine sediment and a high content of organic matter (Ezcurra de Drago et al., 2007; Lin & Yo, 2008). Montaholi & Takeda (op. cit.) considered that its highest

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abundances in the alluvial lagoons during the low water phase and its low density in lotic environments is an adaptation to lentic environments and muddy substrate with a high quantity of organic matter and low velocity of current. Besides, its asexual reproductive mode would be intensified during these low water periods.

- Many studies indicate that the anthropogenic pressure in natural ecosystems favors the invasion and persistence of non-native species when the habitat conditions are modified (Gabel et al., 2011). Polluted areas could accumulate a higher quantity of invasive species than the less impacted areas (Crooks et al., 2011). Freshwater ecosystems are particularly affected by biological invasions. The artificial navigation routes, when connecting the freshwater systems previously separated by natural biogeographic boundaries, constituted important ways of invasion for non-native species, especially with maritime ports as entrance gates from overseas. Freshwater species can be transported attached to the hulls of ships (in continental aquatic systems) or in their ballast water (Gabel et al., 2011). The anthropogenic alteration of the abiotic factors (pollution, physic disturbances, artificial navigation routes, etc.) facilitates biological invasions (Crooks et al., 2011; Gabel et al., 2011). Estuaries, coastal lagoons and inner waters generally present more species of introduced invertebrates than the marine environments (Zaiko et al., 2011). This can be due to the fact that they are more vulnerable environments (variety of habitats, salinity gradient, nutrient enrichment, etc.) and with a higher anthropogenic pressure --extensive shipping activities, proximity to invasive corridors, e.g. channels, artificial substrata, degradation of habitat, over-fishing-. Some authors even consider that these ecosystems should be treated as "hot spots" for the introduction of species (Zaiko et al., 2011) and therefore the management strategies and monitoring programs tending to prevent the invasion impacts should be focused on them. The efforts to improve the environmental conditions and to prevent the mechanisms which favor potential invaders should be encouraged.
- The biogeographic aspects of the genus *Manayunkia* arises certain interest because it includes species which have colonized freshwater environments (Rouse, 1996). Benthic and littoral organisms can present a high degree of endemism, being an example *M. speciosa* (Margalef, 1983). Its distribution has been restricted to North America, with a rather disperse and also intriguing distribution pattern. Nevertheless, and as with other polychaete species, it is supposed that its distribution has been widened due to human activities, both intentionally (aquiculture) and inadvertently (basins interconnection and maritime transportation activities) (Glasby & Timm, 2008). As early as in the first decades of the 20<sup>th</sup> century, Meehean (1929) suggests its entrance in Lake Superior through the ballast water of maritime ships.
- *Manayunkia speciosa* is a tube-dwelling organism, with poorly developed parapods and therefore a limited swimming capacity (Croskery, 1978). It builds its tubes with fine particles clustered by mucus secretions (Leidy, 1883). Under certain conditions —when disturbed— it can leave the tube and roam freely in the sediment (Rouse, 1996; Stocking & Bartholomew, 2007). Like other freshwater polychaetes, it has modified its reproductive strategies for protecting their larvae from osmotic stress. Although the knowledge on its reproductive ecology is scarce, it reproduces sexually or asexually inside the tube, forming typically large and yolky eggs. The juveniles develop within

the tubes and when they become young adults, they leave the refuge to build their own ones (Croskery, 1978; Glasby & Timm, 2008). Like other representatives of the Fabricinae subfamily, it is believed that they are suspension feeders, and in a facultative way they are surface deposit feeders, consuming fine organic detritus and microalgae. As they require great quantities of organic matter and in order to optimize their ingesta, these polychaetes are able to select the size of the particles. They either reject or use those bigger particles in the building of their tubes (Stocking & Bartholomew, 2007). Successful non-native invertebrates often present ecological characteristics which result beneficial in disturbed environments, e.g. short generation time, early sexual maturity, high fecundity, a wide food range, euryhalinity and tolerance to pollution and habitat degradation (Gabel et al., 2011). Some of these characteristics would have allowed M. speciosa the settlement in the Lower Uruguay River. As regards its ecological requirements, Brehm (1978) found this polychaete associated to silt-clay sediments, scarce sands, abundant organic matter, salinities up to 11 ‰, and temperatures between 7 to 31 °C. Besides, Margalef (1983) mentioned M. speciosa as a resistant species to polluted water. In the Lower Uruguay River, we have found this species mainly in calm water sites with aquatic vegetation and abundant organic matter. Despite the very low abundance of ovigerous females collected in March 2009, we suggest that the population of *M* speciosa could be well established in the Lower Uruguay River. During biological invasion, "lag times" are frequently observed between the first introduction and the consequent spread of the species, and ussually extend from decades to centuries. Several species have been present for long periods without an apparent major impact before rapidly increasing their populations and becoming highly consequential (Simberloff, 2011). We hypothesize that a similar event might be occurring with *M. speciosa*. Furthermore, the only record of this polychaete on the South American Uruguay River highlights that it goes unnoticed due to its small size, infaunal lifestyle and low abundances. According to Brehm (1978), who reported that most records are based on a few individuals, we found ca. 500 ind./m<sup>2</sup>. Only Hiltunen (1965) recorded more than 45,000 ind./m<sup>2</sup> at the mouth of Detroit River (USA). Nevertheless, this does not imply that introduced species that now have scarce or no repercussion

could eventually have a greater impact on the involved ecosystems in the future.

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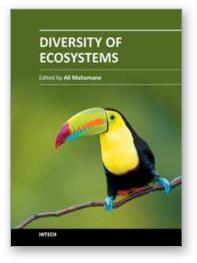
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The ecosystems present a great diversity worldwide and use various functionalities according to ecologic regions. In this new context of variability and climatic changes, these ecosystems undergo notable modifications amplified by domestic uses of which it was subjected to. Indeed the ecosystems render diverse services to humanity from their composition and structure but the tolerable levels are unknown. The preservation of these ecosystemic services needs a clear understanding of their complexity. The role of research is not only to characterise the ecosystems but also to clearly define the tolerable usage levels. Their characterisation proves to be important not only for the local populations that use it but also for the conservation of biodiversity. Hence, the measurement, management and protection of ecosystems need innovative and diverse methods. For all these reasons, the aim of this book is to bring out a general view on the function of ecosystems, modelling, sampling strategies, invading species, the response of organisms to modifications, the carbon dynamics, the mathematical models and theories that can be applied in diverse conditions.

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