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Linking hydrological connectivity with the richness and composition of aquatic invertebrates across the Paraná River floodplain, Argentina

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ABSTRACT

Linking hydrological connectivity with the richness and composition of aquatic invertebrates across the Paraná River floodplain, Argentina.

Lateral hydrological connectivity can affect the community characteristics by promoting dispersal of organisms between the river and its floodplain. We investigated the species richness, specific diversity, and relative abundance of microcrustaceans transported by the flood and the macroinvertebrates retained by *Pontederia crassipes* Mart. roots in five floodplain lakes with different connectivity within the RAMSAR Site Humedales Chaco. Due to differences in the slopes of the floodplain studied, the connectivity was determined by the topographic location of each lake, rather than by the distance from the river. During the hydrological connection, there was a transverse gradient in the physical and chemical characteristics of the water, with an increase in the electrical conductivity and a decrease in dissolved oxygen and suspended solids in the more isolated sites. A total of 62 species of microcrustaceans (48 cladocerans and 14 copepods) and 63 taxa of macroinvertebrates were identified. The microcrustaceans had high species richness in the three most connected lakes, whereas the macroinvertebrates had a peak in taxa richness in the lakes with intermediate connectivity. The low beta diversity (βw) for both microcrustaceans (14.63 %) and macroinvertebrates (16.9 %) indicated that taxa richness varied little between sites with different hydrological connectivity. In both assemblages, cluster analysis based on the similarity of the relative abundance grouped the three sites more connected and separated from the less connected sites. Our results suggest that species richness followed different patterns in different assemblages across the lateral gradient and that the hydrological connectivity was more related to the relative abundance of microcrustaceans and macroinvertebrates than to the classical measures of βw. Species diversity indices did not vary between sites. The high total species richness of invertebrates is mainly a result of the spatial arrangement of the different floodplain lakes.

KEYWORDS: lateral hydrological connectivity, riverine wetlands, β diversity, pulse regime, RAMSAR site Humedales Chaco, South America.

RESUMEN

Vinculando la conectividad hidrológica con la riqueza y composición de los invertebrados acuáticos a través de la llanura aluvial del río Paraná, Argentina.

La conectividad hidrológica lateral puede afectar las características de la comunidad al promover la dispersión de organismos entre el río y su planicie de inundación. Nosotros investigamos la riqueza de especies, la diversidad específica y la abundancia relativa de los microcrustáceos transportados por la inundación y los macroinvertebrados retenidos por las raíces de Pontederia crassipes *Mart. en cinco lagunas con diferente conectividad dentro del Sitio Ramsar Humedales*

Chaco. Debido a las diferencias en las pendientes de la planicie de inundación estudiada, la conectividad estuvo determinada por la ubicación topográfica de cada lago, más que por la distancia al río. Durante la conectividad hidrológica hubo un gradiente en las características físicas y químicas del agua con aumento en la conductividad eléctrica y decrecimiento del oxígeno y de los sólidos suspendidos en los sitios más aislados. Se identificaron 62 especies de microcrustáceos (48 cladóceros y 14 copépodos) y 63 taxones de macroinvertebrados. Los microcrustáceos tuvieron alta riqueza de especies en los tres lagos más conectados, mientras que la riqueza de taxones de macroinvertebrados alcanzó su máximo en la conectividad intermedia. La baja βw tanto para los microcrustáceos (14.63%) como para los macroinvertebrados (16.9%) indicó que la riqueza de taxones varió poco entre sitios con diferente conectividad hidrológica. En ambos ensambles, el análisis de conglomerados basado en la similaridad de la abundancia relativa agrupó los tres sitios más conectados y los separó de los menos conectados. Nuestros resultados sugieren que la riqueza de especies sigue diferentes patrones en distintos ensambles y que la conectividad hidrológica estuvo más relacionada con la abundancia relativa que con las medidas clásicas de βw. Los índices de diversidad de especies no variaron entre sitios. La alta riqueza total de invertebrados se debió a la disposición espacial de diferentes lagos de planicie a través del gradiente de conectividad hidrológica.

PALABRAS CLAVES: *conectividad hidrológica lateral, humedales ribereños, β diversidad, régimen de pulsos, Sitio Ramsar Chaco, Sudamérica.*

INTRODUCTION

In riverine landscapes, connectivity has been extensively used to describe spatial connections (e.g. Tockner et al., 1998, Ward et al., 1999, Amoros & Bornette, 1999, 2002, Robinson et al., 2002). In an ecological context hydrologic connectivity is used to refer to water-mediated transfer of matter, energy, and/or organisms within or between elements of the hydrologic cycle (Pringle, 2001). Many original lotic theories and models adapted from other ecosystems have been proposed to explain patterns of structural and functional biocomplexity across spatio-temporal scales and process in river networks (Thorp et al., 2006). Beta diversity (βw), generally defined as 'the extent of species substitution or biotic change along environmental gradient' (Whittaker, 1972), has been used to test for variations in community structures between habitat patches in floodplain rivers.

Along a connectivity gradient, each of the biotic components reaches peak diversity at different sites. In the Danube floodplain, some groups such as aquatic macrophytes peak at an intermediate level of connectivity, whereas others such as fish species peak at sites with high connectivity to the main channel (Tockner et al., 1998).

Classical measures of beta diversity (βw) only consider species richness, but do not report changes in abundance of each species along the connectivity gradient. This distinction is fundamental, and dramatically different results can be obtained when relative abundance information is included (Anderson et al., 2010). Indeed, comparing analyses of βw that emphasize species identities alone (with a strong role for rare species) with those that emphasize differences in relative abundances (where common and numerically dominant species play a strong role) can yield useful insights into the specific nature of community-level changes.

The connectivity of floodplains with their river channels affects invertebrates and habitat quality, and many organisms move between rivers and floodplains (Batzer et al., 2016). According to Dube et al. (2019), the lateral hydrologic connectivity (measured as the negative distance of a wetland from the river) acts mainly indirectly on zooplankton, aquatic invertebrates and large branchiopod crustaceans, by altering local environmental conditions.

The RAMSAR Site Humedales Chaco includes part of the Paraná and Paraguay River floodplains on their western banks. The frequency, duration, and timing of the connections vary with the river level and with the topographic position of the lakes in relation to the main channel (Neiff, 1990). Floating meadows dominated by *Pontederia crassipes* can cover 30-100 % of the available surface area of the lakes that receive the frequent overflow of the river (Neiff et al., 2014).

During rising water, floating macrophytes are retained in the oxbow lakes by the riparian forest located in the sand bars and the roots act as filters for inorganic sediments supplied by the river (Poi de Neiff et al., 1994). In this section of the Paraná floodplain, the resetting processes mentioned for other rivers (Junk et al., 1989) where floating aquatic and semi-aquatic macrophytes are exported and provide organic matter and nutrients to the main channel communities, do not occur.

Studies carried out in different sections of the Paraná River have evaluated the role of the hydrological connectivity and water levels on the abundance and species richness of communities such as macrophytes (Dos Santos & Thomas, 2007, Neiff et al., 2014), benthos (Marchese & Ezcurra de Drago, 1992), zooplankton (José de Paggi & Paggi, 2008), invertebrates associated with macrophytes (Poi de Neiff & Carignan, 1997) and fish (Agostinho et al., 2004, Neiff et al., 2009). However, to our knowledge, few studies have investigated how the relationship between the transport of organisms by the water during the lateral hydrological fluxes in the floodplain, which is a valuable descriptor of river dynamics (Welcome, 1985), is related to variation in the structure of aquatic communities.

The aim of the present study was to analyze the species richness, specific diversity, and relative abundance of microcrustaceans transported by the flood and the macroinvertebrates retained by *Pontederia crassipes* roots in five floodplain lakes with different connectivity to the main channel within the RAMSAR site Humedales Chaco. Beta diversity was measured to quantified changes in species richness (species turnover) and species composition across the lateral connectivity gradient within the floodplain studied. Both microcrustaceans and macroinvertebrates assemblages play important roles as food for juvenile and adult fish in the floodplain lakes (Agostinho et al., 2004, Neiff et al., 2009, Paggi & José de Paggi, 1990), which serve as breeding areas for migratory fish species.

The following questions are answered:

- *¿Do species richness and βw follow different patterns in different assemblages?*

- *¿Does the relative abundance of macroinvertebrates and microcrustaceans change across the connectivity gradient?*

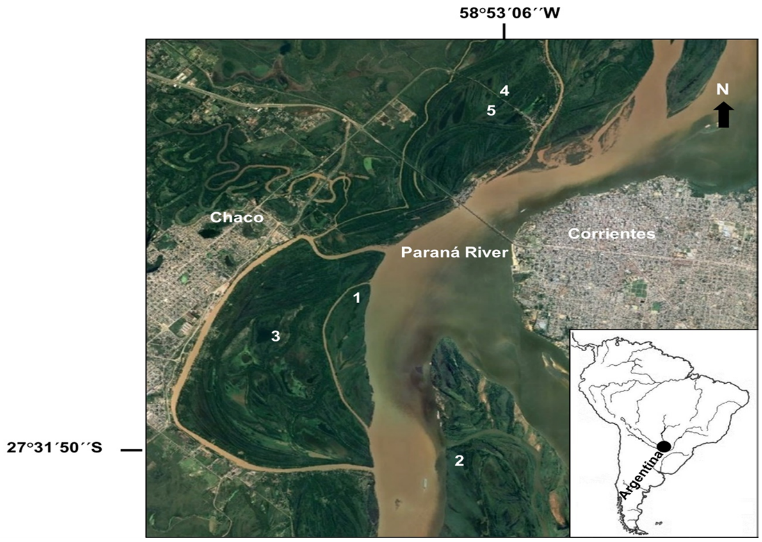

Figure 1. Study area in the Lower Paraná River (Argentina): Site 1. Chouí Island; Site 2: Palomera Island; Site 3: Barranqueras Island; Site 4: El Puente Lake; Site 5: San Nicolás Lake. *Área de estudio en el tramo bajo del río Paraná (Argentina). Sitio 1. Isla Chouí. Sitio 2. Isla Palomera. Sitio 3: Isla Barranqueras. Sitio 4. Laguna El Puente. Sitio 5. Laguna San Nicolás.*

MATERIALS AND METHODS

Study area

The study area is located on the west bank of the Paraná River, 30 km downstream of its confluence with the Paraguay River (between 27° 17′ 40″ S; 58° 32′ 44″ W and 27° 29′ 48″ S; 58° 53′ 36″ W, Fig. 1), within the RAMSAR site Humedales CHACO (Argentina). At this margin, the active intact floodplain is 8 km wide and contains a wide variety of lakes of different origin, size, and morphometry. Some of them were directly connected by a short channel, whereas others were indirectly natural by embankments, swamps, or temporary ponds. The eastern bank of the river is flanked by an 8 to 10 m high terrace. The area has a humid subtropical climate (Bruniard, 1996, 1999), characterized by warm summers and mild winters with low incidence of frost.

The hydrological regime of the Paraná River is irregular, with floods of varying intensity, recurrence and amplitude (Neiff, 1990). The hydrographs of this river reflect irregularities in the distribution of precipitation in long-term data. Between 1996 and 1998, the El Niño Southern Oscillations (ENOS) resulted in unusually wet conditions in lower South America, while the opposite phenomena were recorded between 1999 and 2009.

In the present study, we selected five accessible lakes located in a hydrological connectivity gradient, for which previous information on water conditions and their main biotic assemblages during high and low water was available. The connectivity of the lakes was defined by determining the date of first connection to the river in relation to the water level of the Paraná River in the gauge located near the study sites (Casco et al., 2021). Due to differences in the slopes of the study floodplain, the connectivity of each site was determined by the topographic location of each lake, rather than by the distance from the river.

Sites 1, 2 and 3 are meander scroll ponds located on islands near the west bank of the Paraná River, dominated by aquatic plants such as *Louisiella elephantipes* (Nees ex Trin.) Zuloaga, *Persicaria acuminata* (Kunth) M.Gómez and *Pontederia crassipes* Mart.. These sites are periodically flooded when the river level reaches 3.8 m (site 1), 4.00 m (site 2) and 4.10 m (site 3). During the connection, the water transports organisms and materials from the Paraná River to the floodplain at an average velocity of 0.73 m/s through a narrow channel of five to eight meters wide. During low water, the flow velocity is significantly reduced and in some situations the flow stops, creating temporary lentic characteristics.
Sites 4 and 5 are oxbow lakes that are indirectly connected three or more times a year when the water level of the Paraná River is above 4.20 and 4.85 m at Puerto Corrientes, respectively. These two shallow lakes support a high biomass of the floating macrophyte *P. crassipes*, which covers less than 50% of the lake surface at site 4 and nearly 95% of the lake surface at site 5. During the flow, surface currents of the order of 0.10-0.20 m/s were observed for several days.

Sampling methods

Sampling was carried out at all sites on December 21st, 2015, when the hydrometer in the Corrientes gauge reached 7.41 m and all sites were connected to the Paraná River. PULSE 2.0 software (Neiff & Neiff, 2004) was used to establish the hydrological connectivity in this section of the Paraná River by determining the number of flooded days and isolation of each site between January and December 2015 from the river stage data at the beginning of the connection. The period 2000-2017 was chosen to determine the occurrence of the water level of 7.41 m. For further information, see Casco et al. (2021).

The following environmental variables were measured: electrical conductivity (U-53 Multiparameter), water transparency (Secchi disc), dissolved oxygen (YSI 54A Water Quality), pH (WTW 330/SET-1 digital pH meter). Suspended solids and, total nitrogen and total phosphorus concentrations were determined using methods described in APHA (1995).

The microcrustaceans transported by the water entering the floodplain lakes were collected using a net of 1.3 m long with an open area of 0.08 m^2 and a mesh size of 100 µm. This mesh size was chosen because it was difficult to filter out flood water with nets with smaller pore size (e.g. 50 µm) which would have allowed the capture of smaller organisms such as rotifers. At each site and in the Paraná River, three nets (n=18) were submerged to 0.5 m, removed after 10 min and the organisms retained were fixed in 4% formaldehyde solution. In the laboratory, the total sample was quantified (ind m^{-3} s^{-1}) using a stereoscopic microscope and identified to species level using a microscope (100 x). The water volume of the sample filtered by each net (m^3) was calculated by multiplying the cross-section area of the net (m^2) by the water speed (m/s) by the sampling time (600 seconds). The abundance (ind $m^{-3}s^{-1}$) was calculated by dividing the total number of microcrustaceans per sample by the volume of water sample x the discharge (m^3/s). The macroinvertebrates retained by *P. crassipes* during the hydrological connectivity of the floodplain were collected using a 35 cm diameter net, with 500 µm mesh size and 962 cm^2 area (Poi de Neiff & Carignan, 1997, US EPA, 2002). A net with a 1.5 m long handle, operated from a boat, was inserted vertically under the roots (approximately 70 cm depth) by lifting it to a horizontal position. Triplicate samples were taken from each floodplain lake in December 2015 (total of 15 samples). In the laboratory, aquatic plants were thoroughly washed to remove the macroinvertebrates, and the resulting suspensions were filtered through 1 mm and 500 µm sieves and preserved in 70 % ethanol. The macroinvertebrates were counted and identified to the lowest practical taxonomic level (usually family), using the keys from Michat et al. (2008), Domínguez & Fernández (2009), Ramírez (2010) and Libonatti et al. (2011). Macroinvertebrate abundance was expressed as the number of individuals per square meter. Genera or species level was used for taxa richness and family level for the relative abundance of macroinvertebrates.

Statistical analysis

We used the total species richness and beta diversity estimated by the Whittaker index (βw) to answer the first question and, cluster analysis based on similarities between sites and the specific diversity estimated with the Shannon-Wiener and Evenness indexes for the second question. Beta diversity (βw) was estimated with the Whittaker index using the modification introduced by Harrison (Magurran, 2004):

$\beta w = \{(S/\alpha) - 1\}/(N-1).100$, where: S = total number of species, α = mean species richness, and N = number of sites (n=5). The value of this index ranges from 0 (no turnover) to 100 (each sample has a unique set of species).

Cluster analysis was performed based on the

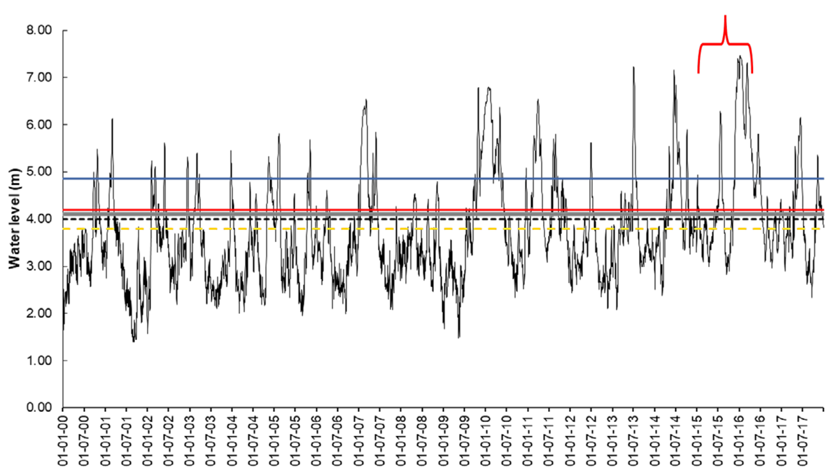

Figure 2. Water level fluctuations of the Paraná River at the Puerto Corrientes gauge between 2000 and 2017. The sites were connected to the Paraná River above the level indicated by the horizontal lines. Site 1: 3.10 m (yellow dashed line); Site 2: 4 m (black dashed line); Site 3: 4.10 m (grey line); Site 4: 4.20 m (red line); Site 5: 4.85 m (blue line). The red mark indicates the period January – December 2015. *Variaciones del nivel del agua del río Paraná en el hidrómetro del Puerto de Corrientes entre los años 2000 y 2017. Los sitios estuvieron conectados con el río Paraná por encima del nivel indicado por las líneas horizontales. Sitio 1: 3.10 m (línea de puntos amarilla); Sitio 2: 4 m (línea de puntos negra); Sitio 3: 4.10 m (línea gris); Sitio 4: 4.20 m (línea roja); Sitio 5: 4.85 m (línea azul). La marca roja, indica el período enero-diciembre de 2015.*

similarity of the relative abundances of microcrustaceans and macroinvertebrates measured by Euclidean distance using the unweighted pair group method with arithmetic mean (UPGMA). Prior to analysis, all abundance data were transformed using the Hellinger transformation (square root of abundance), which is appropriate for beta diversity assessment (Legendre & De Cáceres, 2013). The Hellinger transformation allows reducing the relative weight of abundant species in the analysis. To assess α diversity of microcrustacean and macroinvertebrate assemblages at each study site, Shannon-Wiener and evenness were calculated. Evenness refers to the absolute distribution of the relative abundance of species (Shannon-Wiener index) divided by ln S (species richness). The non-parametric Kruskal–Wallis test was used to detect significant differences between lakes with different connectivity, taking into account the water condition and the total abundance of macroinvertebrate and microcrustaceans during the sampling period. The same test was used to determine significant differences in diversity and evenness between lakes. Simple linear regressions were used to test the relationships between connectivity and water condition and ln of richness. Statistical analysis was performed using PAST 2.08 (Hammer et al., 2001) and InfoStat (Di Rienzo et al., 2013) software.

RESULTS

Connectivity analysis and water condition

The hydrological series of Paraná River between 2000 and 2017 showed an irregular sequence of floods and emergent soil periods in each site, reaching an absolute maximum of 7.47 m in January 2016 (Fig. 2). In this hydrological series, the river only three times exceeded 7.41 m. The analysis with PULSE 2.0 (Table 1) showed that, during 2015, the number of days connected to the Paraná River decreased from site 1 (205) to site 5 (64) and the frequency of pulses was high in the most connected sites (Table 1). This is due to geomorphological differences in the floodplain and bed slope.

Water temperatures were high and pH values were neutral or slightly acidic (Table 2). Comparisons with the Kruskal–Wallis test indicated that electrical conductivity increased significantly ($H=13.5$, $p=0.008$) and, dissolved oxygen concentrations ($H=13.5$, $p=0.009$) and suspended solids decreased significantly ($H=13.13$, $p=0.01$)

Table 1. Hydrological connectivity of five sites with the Paraná River during the period January-December 2015. Frequency of pulses is the number of complete pulses (potamophase and limnophase). *Conectividad hidrológica con el río Paraná de los cinco sitios durante el período enero-diciembre de 2015. La frecuencia de pulsos es el número completo de pulsos (potamofase y limnofase).*

	Site 1	Site 2	Site 3	Site 4	Site 5
Number of days connected with the Paraná River	205	172	145	117	64
Number of days in isolation	160	193	220	248	301
Frequency of pulses	7	9	9	5	3

Table 2. Physical and chemical characteristics of the water entering the floodplain during the hydrological connection of the study sites with the Paraná River (Average ±Standard error). *Características físico-químicas del agua que ingresa a la planicie de inundación durante la conexión hidrológica de los sitios estudiados con el río Paraná (promedio ±error estándar).*

	Paraná River	Site 1	Site 2	Site 3	Site 4	Site 5
Temperature (°C)	19.8	20.1	20	21	19.3	20.5
Dissolved oxygen (mg/l)	6.7±0.4	7.9±0.21	5.8±0.26	3.9±0.3	4.7±0.15	3.2±0.1
pH	7.5±0.02	7.1±0.01	6.9±0.01	7.0±0.02	6.4±0.01	6.2±0.01
Conductivity (µS/cm)	110±1.5	80±1.5	96±0.58	122±1.7	152±2.0	175±0.5
Depth (m)	8	4.8	3.5	2.5	2.2	2.1
Transparency (Secchi cm)	0.05	0.19	0.25	0.33	0.42	0.48
Suspended solids (mg/l)	953±247	451±100	317±27.5	176±9	19.1±2.3	15.8±1.6

from the most connected to the least connected sites (Table 2). The flood and the early isolation period of these floodplain lakes were characterized by a very dynamic behavior of nutrient concentrations. Dissolved inorganic nitrogen concentrations increased to high levels (125-330 µg/l) in the floodplain lakes after the floods, while phosphate concentrations changed little during and after the flood (80-106 µg/l). Additional information on the nutrient dynamics in these lakes can be found in the study previously performed by Carignan and Neiff (1992). The hydrological connectivity of the floodplain lakes (expressed as the number of days connected to the Paraná River) was correlated with specific conductivity ($R^2 = 0.97$, $F = 433.3$, $p < 0.0001$) and suspended solids ($R^2 = 0.83$, $F = 47.6$, $p < 0.0008$).

Microcrustacean assemblages

A total of 62 species of microcrustacean (48 cladocerans and 14 copepods) were identified (Table S1, Supplementary information, available at https://www.limnetica.net/en/limnetica). High species richness (Fig. 3a) was recorded at sites 1, 2 and 3, and the lowest at sites 4 and 5. The beta diversity (βw) of microcrustacean species richness across the five floodplain lakes was 14.63 %. The Shannon–Wiener diversity and the evenness indexes (Table S1) did not show significant differences ($H=4$, $p=0.9999$) between sites. Significant differences were found when Kruskal-Wallis test was used to compare microcrustacean abundance between sites with different hydrological connectivity ($H=12.4$, $p<0.01$). The linear regression between hydrological connectivity and the ln of microcrustacean species richness was not significant ($R^2 = 0.73$, $F = 8.28$, $p < 0.06$).

In the main channel of the Paraná River, species richness reached to 30 and the cladocerans *Bosmina hagmanni*, *Bosminopsis deitersi* and *Cerdiodaphnia dubia* had more than 8 % of the total abundance during the flood period (Table S1). Among copepods, *Diaptomus corderoi*, *Microcyclops finitimus* and *Attheyella fuhrmanni* were recorded only in the Paraná River.

Bosminopsis deitersi, *Alona monocantha*, *Chydorus pubescens*, *Euryalona orientalis*, *Graptoleberis testudinaria*, *Onchobunops tuberculatus*, *Pseudosida ramosa*, *Ceriodaphnia cornuta*, *Cerdiodaphnia dubia* and *Simocephalus serrulatus* were found at the six sites surveyed (Table S1). *Bosmina hagmanni*, *B. deitersi*, *C. dubia* and *Chydorus strictomarginatus* were abundant in the meander ponds (sites 1, 2 and 3) and have low or scarce representation in the oxbow lakes (sites 4 and 5). *Diaphanosoma brevirreme*, *Ceriodaphnia cornuta* and copepods (*Microcyclops anceps anceps*, *Microcyclops anceps pauxensis* and *Microcyclops* sp.) were abundant in sites 4 and 5.

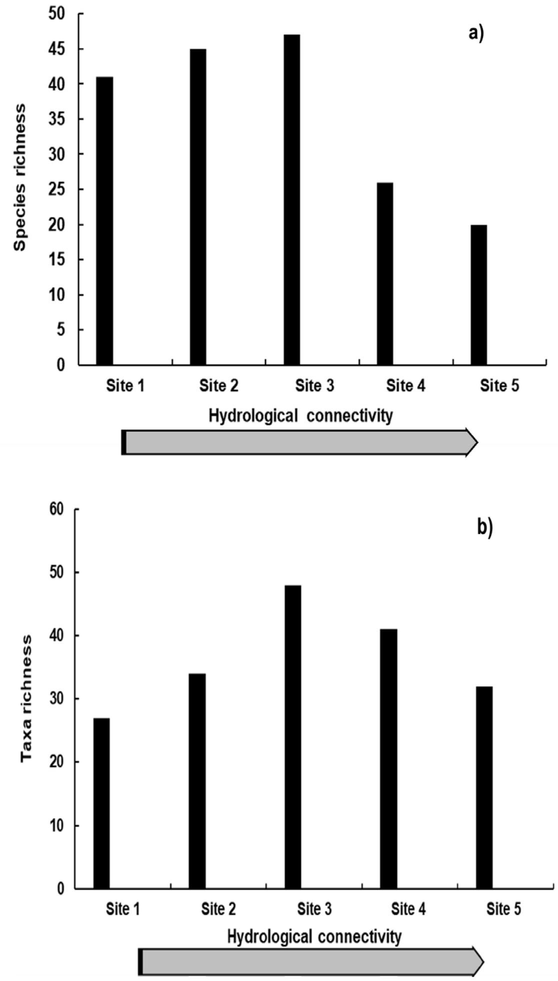

Figure 3.a. Species richness of microcrustaceans entering floodplain lakes during the connectivity gradient in the Paraná River. b. Taxa richness of macroinvertebrate assemblages retained by *P. crassipes* roots floating meadows of floodplain lakes during the connectivity with the Paraná River. *Riqueza de especies de microcrustáceos que ingresan a los lagos de la planicie de inundación durante la conectividad con el río Paraná. b. Riqueza de taxa de los ensambles de macroinvertebrados retenidos por las raíces de* P. crassipes *en las lagunas de la planicie de inundación durante la conectividad con el río Paraná.*

Cluster analysis, based on relative abundance, provided a good interpretation (Cophenetic correlation coefficient 0.9875) of the relative effect of hydrological connectivity (Fig. 4a). The three sites more connected and directly connected sites (1, 2 and 3) were grouped and separated from the less connected sites (4 and 5). Microcrustaceans from sites 1 and 2 showed a high degree of similarity.

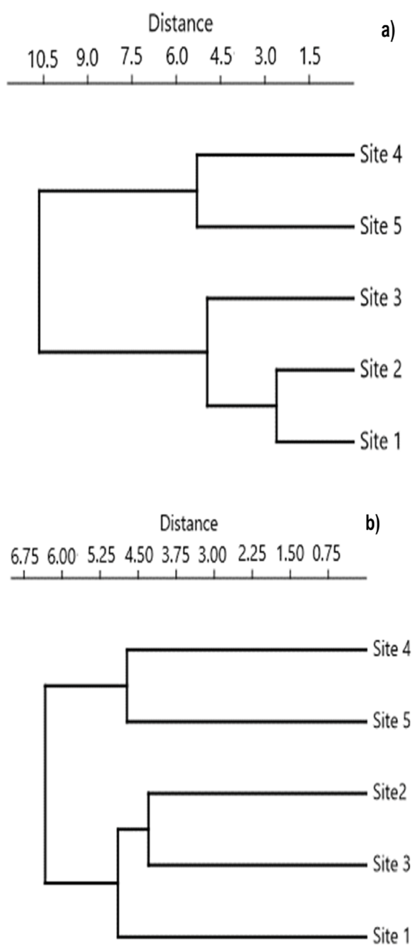

Figure 4.a. Cluster analysis based on Euclidean distance (UPGMA method) of the relative abundances of microcrustacean species in the five floodplain lakes. b. Cluster analysis based on Euclidean distance (UPGMA method) of the relative abundances of macroinvertebrate taxa in the five floodplain lakes. a. *Análisis de conglomerados basado en la distancia euclideana (método UPGMA) de la abundancia relativa de especies de microcrustáceos en cinco lagos de la planicie de inundación. b. Análisis de conglomerados basado en la distancia euclideana (método UPGMA) de la abundancia relativa de los taxa de ma*croinvertebrados en cinco lagos de la planicie de inundación.

Macroinvertebrate assemblages

A total of 63 macroinvertebrate taxa were identified in the *P. crassipes* floating meadows (Table S2, Supplementary information, available at https://www.limnetica.net/en/limnetica). The richness of taxa retained by the roots was lowest in the most connected lake (site 1), increased to 48 taxa in site 3, and decreased in sites 4 and 5 (Fig. 3b). Beta diversity (βw) was 16.9 %. Species diversity measured with the Shannon-Wiener (Table S2) and the evenness indexes (Table S2) did not show significant differences (H=4, p=0.9999) between sites.

No significant differences were found when Kruskal-Wallis test was used to compare the total abundance of macroinvertebrate between sites with different hydrological connectivity (H=1.65, p=0.8022). Note the large standard deviation values between samples for the total abundance of macroinvertebrates retained in plant roots (Table S2). Taxa richness, species diversity and abundance of macroinvertebrate were weakly correlated with hydrological connectivity.

Results showed a high abundance of Chironomidae larvae at all sites and similar proportions of Amphipoda (*Hyalella curvispina*) and Trichoptera larvae (*Cyrnellus* sp., Table S2). The dendrogram derived from the cluster analysis of relative abundance of macroinvertebrate taxa identified two groups (Fig. 4b). The grouping allowed a good interpretation (Cophenetic correlation coefficient= 0.7671) of the relative effect of hydrological connectivity. Macroinvertebrates living on aquatic plants from sites 4 and 5 were grouped and separated from the rest. The floating plants had a high proportion of Oligochaeta (Naididae) and high richness of Coleoptera adults and Diptera larvae (Table S2).

Sites 1, 2 and 3 were grouped by the abundance of molluscs (*Heleobia parchappii* and Ancylidae), ostracods (*Cytheridella ilosvayi*) and Baetidae larvae (*Callibaetis* sp.) which, at site 3, represented 7.8 % of the total (Table S2). Macroinvertebrate assemblages from site 1, which has low taxa richness (Table S2) and high proportion of Chironomidae larvae, were also segregated from sites 2 and 3 (Fig. 4b).

DISCUSSION

The results of the present study showed a transverse gradient in the physical and chemical characteristics of the water entering the floodplain during the hydrological connection from the most connected to the most isolated sites, with an increase in the electrical conductivity and a decrease in dissolved oxygen and suspended solids in the most isolated sites. However, in the least connected sites, dissolved oxygen did not decrease to the values (1.3 mg/l) recorded during the isolation period (Poi de Neiff et al., 2006), and electrical conductivity was lower than that registered in the oxbow lakes during isolation when it reached 340 µS/cm (Neiff et al., 2009). The high correlation between hydrological connectivity and, electrical conductivity and suspended solids indicated that there was no homogenization effect of the flood in the studied sites. In other floodplain lakes, the increased connectivity during floods led to high similarities in the physical, chemical and biological features between aquatic habitats (Thomaz et al., 2007).

Species richness and Beta diversity (Whittaker index)

The flood water that entered the floodplain lakes transported a high number of species of microcrustaceans, especially of Cladocera. The typical littoral family (Chydoridae) contributed most to the species richness. The number of species recorded in this study was high considering that it was a single sampling period and that, in previous extensive spatial and temporal surveys, 90 species were reported for the middle Paraná floodplain (Paggi & José de Paggi, 1990) and 63 for different environments of the upper Paraná floodplain (Serafim et al., 2003).

The number of microcrustacean species transported by floodwaters was higher in the three lakes most connected to the Paraná River (between 41 and 47 species) than in the least connected ones (between 20 and 26 species). Studies of zooplankton conducted in floodplain lakes of the Upper Paraná River reported higher rotifer (Aoyagui & Bonecker, 2004) and crustacean species richness (Alves et al., 2005) in connected lagoons which are subject to greater water exchange than isolated lagoons. In contrast, José de Paggi & Paggi (2008) found that species richness and abundance of zooplankton are greater in isolated lagoons of the middle Paraná River.

Macroinvertebrate taxa richness peaked at sites with intermediate connectivity, thus potentially supporting the intermediate disturbance hypothesis (Connell, 1978), assuming that the connectivity gradient partly equates to disturbance in riverine floodplains (Ward et al., 1999). According to previous studies in different floodplain rivers, taxa richness of benthic macroinvertebrates peaks at sites with intermediate level of lateral hydrological connectivity (Gallardo et al., 2014).

The low βw for both microcrustaceans (14.63 %) and macroinvertebrates (16.9 %) indicated that taxa richness varied little between sites with different hydrological connectivity. In this section of the Paraná River floodplain, other assemblages such as aquatic plants, have also been found to have low βw -between 12 % and 16 %- (Neiff et al., 2014). Conversely, in the same floodplain lakes studied (Neiff et al., 2009), fish had a high spatial species turnover (βw) = 40.33 %.

Similarity based on relative abundance, species diversity and evenness

The relative abundance of microcrustaceans and macroinvertebrates was related to the connectivity gradient, reflected by the similarities indicated by cluster analysis of the quantitative data (measured by Euclidean distance). In both assemblages, sites with less connectivity (and indirect connection) were separated from sites with more direct connectivity to the Paraná River. However, the similarity distances between the two groups were greater in the microcrustaceans than in the macroinvertebrates.

Microcrustaceans transported by the flood had a high relative abundance (%) of species, some of which (*Bosminopsis deitersi*, *Bosmina hagmanni* and *Cerdiodaphnia dubia*) were abundant in the Paraná River and in the sites more connected. These species have been reported as constants for zooplankton in floodplain lakes of the Upper Paraná River (Serafim et al., 2003). Instead, other species (such as *Chydorus strictomarginatus*) were abundant only in the most connected sites and probably came from the washes of the littoral areas surrounding these water bodies. The retention of macroinvertebrates by aquatic plants during hydrological connectivity led to a different composition of assemblages in lakes located on a connectivity gradient. Previous studies carried out in the least connected sites with high coverage of *P. crassipes* (Poi et al., 2020) also found abundance of Chironomidae larvae, high proportion of oligochaetes (Naididae) and high richness of coleopteran adults.

Our results suggest that, in our study sites, species richness followed different patterns in different assemblages across the lateral connectivity gradient and that the relationship between species richness and connectivity was not linear. A previous study performed in the Danubio floodplain showed that different biotic components attain peaks at different sites situated along a transect (Tockner et al., 1998).

The flood water coming from the Paraná River contributed with different proportions of microcrustaceans to the floodplain lakes, depending on their degree of connectivity. Furthermore, the results also suggest that the hydrological connectivity was more related to the similarity analysis based on the relative abundance of each assemblage than to the classical measures of βw (Whittaker index), which only consider species richness.

Because in both assemblages (microcrustaceans and macroinvertebrates) the Shannon-Wiener species diversity and evenness did not vary significantly between sites, these indexes were not useful to measure changes across a connectivity gradient in the floodplain lakes studied.

The high total species richness of microcrustaceans and macroinvertebrates is mainly a result of the spatial arrangement of the different floodplain lakes across the gradient of hydrological connectivity. Disturbances to the pulse hydrological regime reduce biodiversity (Pringle, 2003) by altering the connectivity with the river channel and reducing the duration of the flood phase. The conservation of these vegetated wetlands requires the maintenance of effective widths of connectivity that provide diverse habitats over time.

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