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The Application of Chironomid Pupal Exuvial Technique (CPET) for Ecological Analysis in a Neotropical Large River System

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Abstract

This field ecological study, based on the chironomid pupal exuvial technique (CPET), is new for the Paraná River and proposes an efficient tool to be used in future ecological approaches and biomonitoring. Drifting of pupal exuviae in a river-floodplain system of the Middle Paraná River floodplain was represented by 34 Chironomidae taxa, being the characteristic association obtained from the CPET: *Lopescladius*, *Onconeura*, *Paralauterborniella*, *Polypedilum*, and *Harnischia* complex. Diversity, richness, dominance, total density, and density of dominant taxa were different between the longitudinal and lateral dimensions but not between hydrologic phases, with a greater diversity and richness in the main channel of the river and higher density and dominance in floodplain habitats. The species turnover is the dominant process in structuring studied assemblages in spatial and temporal analysis, increasing in the floodplain habitats and in low-water phase. The results obtained showed that drifting exuviae in the longitudinal axis were coming from different assemblages and environments of a wider area (regional), while exuviae recorded in the connections of the floodplain environments in the lateral dimension could reflect the local assemblages. We demonstrated the ecological value of CPET studies to interpret the attributes of Chironomidae assemblage in river-floodplain systems of large rivers in an integrated way.

Introduction

One of the central issues for the integral understanding of river ecology and management of large rivers is to elucidate the diversity and distribution patterns in relation to the hydrological regime (Thomaz *et al* 2007). In large rivers with a floodplain like the Paraná River, besides the longitudinal upstream-downstream transport, it is fundamental to consider the interchange in the lateral dimension (Marchese *et al* 2002, Ezcurra de Drago *et al* 2007), because the hydrosedimentologic dynamics, with recurrent flood and drought pulses, generate a bidirectional interchange of matter and energy from the river to the floodplain and vice versa, determining their populations' characteristics (structure, distribution, abundance, adaptations, etc.) (Junk *et al* 1989, Neiff 1999, Montalto & Paggi 2006, Ezcurra de Drago *et al* 2007, Mesa *et al* 2012).

Chironomidae are among the most abundant and diverse insect families in aquatic ecosystems. The present discussions around the ecology of larval Chironomidae assemblage was oriented to elucidate the species diversity and the mechanisms involved in processes that structure assemblages in

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relation to environmental and spatial factors under a metacommunity concept framework (Heino 2005, Siqueira *et al* 2008, 2009, Anderson & Ferrington 2013, Petsch *et al* 2015). In Argentina, studies on the ecology of Chironomidae from their larval stages have been oriented principally to bionomy and diversity in relation to different habitat characteristics (Montalto & Paggi 2006, Medina *et al* 2008, Scheibler *et al* 2008, Zilli & Paggi 2013, Mauad *et al* 2016).

After chironomid adult emergence, their pupal exuviae become part of the drifting of aquatic environments. Through their study, it is possible to know the species diversity in an environment because they show diagnostic characteristics more easily recognized than the larvae, in many cases, allowing better taxonomic determination (Hardwick *et al* 1995, Wilson & Ruse 2005).

The use of the chironomid pupae exuvial technique (CPET) has been mentioned by different authors in ecological studies, and its efficiency has been demonstrated in lotic and lentic environments in other regions (Raunio & Muotka 2005, Raunio *et al* 2007a, Raunio *et al* 2011). These studies have been carried out, even in coincidence, with analyses of the larvae recorded in the benthos, with a higher number of taxa associated with the pupal exuvial technique (Ruse 1995, Raunio *et al* 2007a, 2007b).

The CPET also has been used widely to evaluate different Chironomidae assemblage attributes in relation to environmental conditions (Fend & Carter 1995, Hardwick *et al* 1995, Wilson & Bright 1973, Ruse 1995, Raunio *et al* 2007a), trophic state of habitats (Raunio *et al* 2010), and for biomonitoring purposes either as a complement to or a replacement for traditional benthic samplings (McGill *et al* 1979, Ferrington *et al* 1991, Raunio & Muotka 2005, Wilson & Ruse 2005, Raunio *et al* 2007b). However, in the Neotropical region, there are some contributions for diversity and phenology of Chironomidae for Patagonia (Argentina) (García & Añón Suarez 2007) and São Carlos (Brazil) (Siqueira *et al* 2008).

The purpose of this study was to evaluate the application of chironomid pupal exuvial technique (CPET) for an ecological analysis in a Neotropical large river system, considering longitudinal and lateral dimensions and hydrological phases.

Material and Methods

Study area

This study was carried out in five environments of the Middle Paraná River floodplain, located in the transversal section of Santa Fe and Paraná cities, Argentina (31°41′S, 60°43′W) (Fig 1). The regional climate is humid subtropical with hydric excess during the entire year and with a seasonal hydrograph influenced mainly by rainfall pattern, a high-level stage during the first 6 months of the year and maximum peaks in March–April. Low-water phase occurs in the second half of the year, with minimum flows in September–October. The Middle Paraná is included in the geographical region Chaco-Pampa plain (Marchese *et al* 2002, Iriondo 2007).

The Paraná River is 4400 km long from the headwaters of the Grande River in Brazil to the Río de la Plata, constituting the second longest river in South America, the second in relation to its area, 1.51 million km^2, and the third in relation to its mean annual flow into the ocean, approximately 470 km^3, with 16,000–19,000-m^3/s mean flow, reaching 60,000 m^3/s maximum (Paoli & Schreider 2000). Middle Paraná River has built a fringing floodplain with a surface of circa 20,000 km^2 including different habitats principally regulated by the flood pulse regime and the hydrological connectivity (Drago 2007). The environments studied include the right margin of the main channel of the Paraná River, the channel linking with a floodplain lake, and the connection of this lentic environment with a secondary channel.

The studied floodplain lake is connected permanently to the main channel and presents an irregular shape with a maximum depth of 4.6 m and a surface of 0.39 km^2. The connection channel with the main channel is 0.74 km long (Mesa *et al* 2012). Mini River is a secondary channel of the Paraná River system with ≈ 100-m^3/s flows.

The studied environments are characterized by dissolved oxygen ranging between 4.6 and 9.8 (ppm), conductivity of 65–540 µS/cm, pH of 6.2–7.6; temperature ranged between 13 and 28°C with Secchi disc of 0.07–0.65 m. The current velocity (m/s) ranged between 0.57 and 1.77 m/s for the main channel and < 0.8 m/s for minor secondary channels (Ezcurra de Drago *et al* 2007).

The five sampling stations were as follows: 1. right bank of the main channel of the Paraná River upstream from the connection with the channel to the floodplain lake (USP); 2. right bank of the main channel Paraná River downstream from the connection with the channel to the floodplain lake (DSP); 3. beginning of the connection channel of the Paraná River main channel with the floodplain lake (P-Ch); 4. connection between the channel and the floodplain lake (Ch-L); and 5. connection between the floodplain lake and the secondary channel (L-SCh) (Fig 1).

Collection and processing of chironomid pupal exuviae

Nets of 250 µm mesh, 0.10 m in diameter, and 0.30 m long, on the water surface, were used for sampling for 10 min (Ferrington 1987, Fend & Carter 1995, García & Añón Suarez 2007). At each station, three samples (replicates) were taken in the sites located in the longitudinal axis (USP, DSP) and lateral axis (P-Ch, Ch-L, L-SCh). Samplings were carried out during the high- and low-water phases (March and December 2010), respectively. For high-water phase, L-SCh could not be sampled.
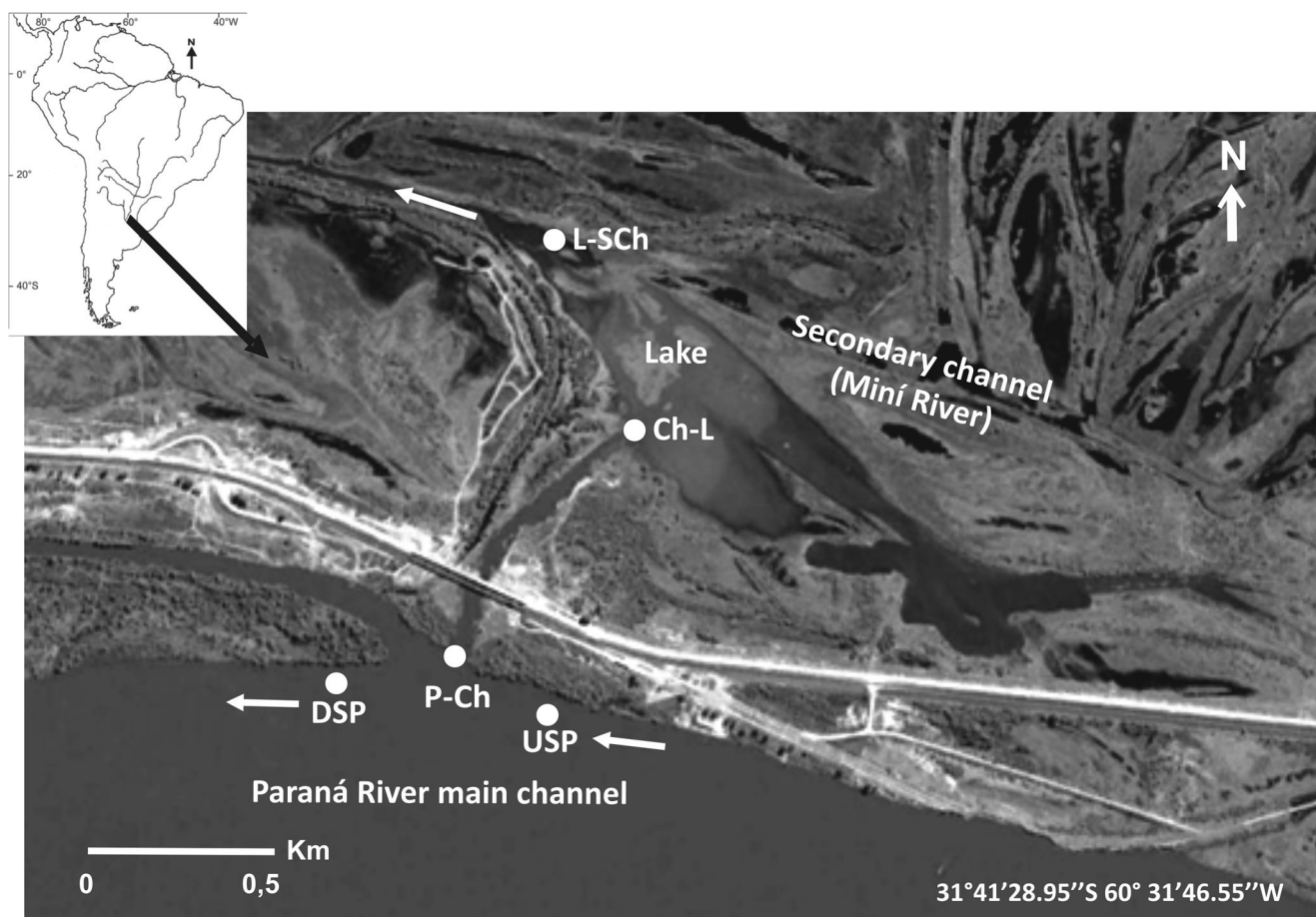
Fig 1 Study area showing sampling sites in the Paraná River system (Argentina). Abbreviations: *USP* Paraná River main channel, upstream from the connection with the floodplain lake channel; *DSP* Paraná River main channel, downstream from the connection with the floodplain lake channel; *P-Ch* beginning of the connection channel of the Paraná River main channel with the floodplain lake; *Ch-L* connection between the channel and the floodplain lake; *L-SCh* floodplain lake and the secondary channel connection.

Because chironomids have different daily emergency rhythms with changes in the frequency drift (Raunio & Muotka 2005), samples were all collected in the same time period (8–16 h) to minimize variability between samples. Drifting exuviae were fixed in situ with 10% formalin.

At each sampling station, depth (with a hand-held probe), transparency (Secchi disk), pH, temperature, conductivity, and dissolved oxygen (Horiba U-10) were determined in situ. To assess the flow velocity (m/s), an AOTT flowmeter was used; then, the volume of sampled water at each time interval was calculated.

The separation of the pupal exuviae was performed under a 10× stereomicroscope and the material was preserved in 70% alcohol for qualitative and quantitative analysis. All pupal exuviae were dehydrated by means of successive immersion in 80%, 96% and 100% ethanol and mounted in Euparal® (Paggi 2009). The exuviae were stained with fuchsine acid when necessary. Exuviae were identified under an optical microscope at the lowest possible taxonomic level following the keys of Wiederholm (1986), Cranston (1996), and Wiedenbrug (2000).

Data analysis

For drifting samples, the density of pupal exuviae (exuviae.m³/h) was calculated as the quotient between the number of exuviae in a sample and the volume of sampled water. This volume was calculated by multiplying the area of the submerged net, the current velocity at the net mouth, and the sampling time.

Chironomid density, density of dominant taxa, taxonomic diversity (*H*, Shannon-Wiener), rarefied richness values, and dominance (*D*, Simpson) between the different sampling dates, and between the high- and low-water phases at the longitudinal and lateral axes were analyzed as descriptors of Chironomidae assemblage. Richness was rarefied by individuals (density values) using Past (ver 1.89) (Hammer *et al* 2009).

The beta diversity partitioning the spatial turnover and nestedness was performed (Baselga 2010). Analyses were carried out in R betapart package (R Development Core Team) using the functions beta-multi.R to obtain β_{SOR} (total beta diversity), β_{SIM} (spatial turnover), and β_{NES} (nestedness) components (Baselga & Orme 2012).

Table 1   Physical and chemical parameters recorded in the sampled sites during high-water (HW) and low-water (LW) phases. Abbreviations: *USP* Paraná River main channel, upstream from the connection with the floodplain lake channel; *DSP* Paraná River main channel, downstream from the connection with the floodplain lake channel; *P-Ch* beginning of the connection channel of the Paraná River main channel with the floodplain lake; *Ch-L* connection between the channel and the floodplain lake; *L-SCh* floodplain lake and the secondary channel connection.

	Current velocity (m/s)	Depth (m)	Secchi (m)	T (°C)	pH	Conductivity (µS/cm)	O$_2$ (ppm)
HW							
USP	0.59	9.2	0.12	27	6.88	89.9	7.9
DSP	0.87	9.3	0.34	27	6.56	90.0	5.9
P-Ch	0.61	10.3	0.59	27	6.51	91.0	4.4
Ch-L	0.51	4.4	0.34	27	6.63	93.0	2.2
LW							
USP	1.06	9.6	0.24	26	7.11	54.5	8.9
DSP	1.06	8.0	0.26	26	7.20	58.4	7.4
P-Ch	0.18	6.5	0.24	26	7.34	54.6	7.5
Ch-L	0.52	6.3	0.24	26	7.31	54.1	7.1
L-SCh	0.46	3.2	0.26	28	7.14	54.9	8.8

The Mann-Whitney test was applied to compare total density, density of dominant taxa, rarefied richness, and diversity according to the hydroperiod (low- and high-water phases) and between the main channel (USP, DSP, P-Ch) and floodplain habitats (Ch-L, L-M) (longitudinal-lateral dimensions) (Past ver 1.89, Hammer *et al* 2009).

In order to relate the environmental parameters and assemblage metrics, Spearman correlation (Rho) was performed. Previously, we tested environmental multicollinearity and selected environmental variables for analysis (Past ver 1.89, Hammer *et al* 2009).

Results

Spatial and temporal analysis of Chironomidae exuviae

In general, main channel habitats were characterized by higher depth, current velocity, and dissolved oxygen. In the high-water phase, the lowest values of current velocity, pH, and dissolved oxygen (included two anoxia values in floodplain habitats) were recorded. Moreover, during the low-water phase, we observed a reduction in Secchi disc and conductivity values (Table 1).

The total richness of the Chironomidae taxa recorded was 34, with dominance of Chironominae (23 taxa, 20 Chironomini and 3 Tanytarsini), followed by Orthocladiinae (6 taxa) and Tanypodinae (4 taxa). Table 2 shows the occurrence (%Oc) and dominance (%N) of all the taxa in the different studied sites of the Paraná River during high- and low-water phases. An occurrence frequency of 100% of the studied environments in both phases was represented by *Paralauterboniella* sp., *Polypedilum* sp. 3 or 5, and

Onconeura sp. 1. The highest dominance (%N) was represented by *Lopescladius* sp. 1 and 2 and *Paralauterborniella* sp*.* In the present study, a pupal exuviae of *Robackia* was reported for the first time in the main channel of the Middle Paraná River (Table 2).

For rarefied richness and Shannon diversity values, significant differences were recorded between longitudinal and lateral dimensions (river/floodplain) (p = 0.05 and p = 0.028, respectively) but not between hydrologic phases (p > 0.05). The stations that showed the highest rarefied richness values were upstream Paraná River (USP) in both phases. Shannon diversity values ranged between 0.74 (Ch-L in high water) and 2.74 (USP in low water) (Table 3). No significant differences (p > 0.05) were found in the dominance (D) values between the hydrologic phases, although significant differences were recorded between river and floodplain environments (p = 0.028), with higher values in the floodplain habitats (Table 3).

The total density of exuviae and the density of dominant taxa showed significant differences only between the longitudinal and lateral dimensions (river/floodplain) (p = 0.037 and p = 0.028). The highest total density values were recorded in the floodplain environments (Ch-L: 698.72 and 615.09 exuviae.m^{-3}/h in high- and low-water phases, respectively) (Fig 2). The dominant taxa (density and occurrence) in the different sites and both hydrologic phases were *Onconeura* spp., *Lopescladius* spp., *Polypedilum* spp., *Paralauterborniella* sp., and *Harnischia* spp.; Chironomidae association characteristics of the lotic habitats were *Lopescladius* spp., *Onconeura* spp., *Polypedilum* spp., *Harnischia* spp., *Paralauterboniella* sp., *Coelotanypus* sp., *Axarus* sp., and *Rheotanytarsus* sp., while in the floodplain habitats, *Lopescladius* spp., *Paralauterboniella* sp.,

Table 2 Chironomid pupal exuviae density (ind.m^3/h), during high-water (HW) and low-water (LW) phases, occurrence frequency (%Oc), and numerical percentage (%N). Density: 1: 1–10 ind.m^3/h, 2: > 10–100 ind.m^3/h, 3: > 100 ind.m^3/h. Abbreviations: *USP* Paraná River main channel, upstream from the connection with the floodplain lake channel; *DSP* Paraná River main channel, downstream from the connection with the floodplain lake channel; *P-Ch* beginning of the connection channel of the Paraná River main channel with the floodplain lake; *Ch-L* connection between the channel and the floodplain lake; *L-SCh* floodplain lake and the secondary channel connection; *(Un)* unidentified.

Sites	USP		DSP		P-Ch		Ch-L		L-SCh	%N	%Oc
Hydrologic phase	HW	LW	HW	LW	HW	LW	HW	LW	LW		
Taxa											
Chironominae											
Chironomini											
Axarus sp.		1	1	1	2					0.55	44.44
Chironomus sp.								1		0.09	11.11
Cladopelma sp.								1		0.18	11.11
Crytpochironomus sp. 1									1	0.08	11.11
Crytpochironomus sp. 2		1			1		1	1	1	0.36	55.56
Crytpochironomus sp. 3									1	0.03	11.11
Cryptochironomus sp. 4	1		1			1			1	0.5	44.44
Dicrotendipes sp.						1				0.13	11.11
Harnischia complex sp. 1[a]	1	1	1			1		2		0.57	55.56
Harnischia complex sp.	1									0.04	11.11
Harnischia complex sp. 2	2	1	2	1	1	2	2	2		6.98	88.89
Harnischia complex sp. 3		1		1		2		1		1.10	44.44
Nilothauma complex sp.[a]		1								0.01	11.11
Parachironomus sp.								1		0.05	11.11
Paralauterborniella sp.	2	1	1	1	2	1	1	2	3	14.01	100
Polypedilum sp. 3 or 5[a]	2	1	2	1	2	2	1	2	1	6.03	100
Polypedilum sp. 6[a]	2	1	1	1	1		1	2	3	6.74	88.89
Robackia sp.			1							0.01	11.11
Saetheria sp.			1							0.01	11.11
Stenochironomus sp.								1		0.02	11.11
Tanytarsini											
Cladotanytarsus sp.					1			1		0.22	22.22
Tanytarsus sp.	1				1					0.08	22.22
Rheotanytarsus sp.	1		1		2		1			0.98	44.44
Orthocladiinae											
Cricotopus sp.								1		0.05	11.11
Lopescladius sp. 1	2	1	3		1	2	3	2		13.36	77.78
Lopescladius sp. 2	2	1	3	1	1		3	3		40.88	77.78
Onconeura sp. 1	2	1	2	1	2	2	1	1	1	5.05	100
Onconeura sp. 2	2	1						1		0.69	33.33
Thienemanniella sp.									1	0.08	11.11
Tanypodinae											
Ablabesmyia sp.			1							0.05	11.11
Coelotanypus sp.	1	1	2	1	1					0.66	55.56
Labrundinia sp.	1					1				0.23	22.22
Procladius sp.		1	1	1						0.13	33.33
Chironomidae (Un)		1								0.05	11.11

[a] Wiedenbrug (2000).

Table 3   Rarefied richness, diversity (*H*), and dominance (*D*) of Chironomidae pupal exuviae assemblage. Abbreviations: *USP* Paraná River main channel, upstream from the connection with the floodplain lake channel; *DSP* Paraná River main channel, downstream from the connection with the floodplain lake channel; *P-Ch* beginning of the connection channel of the Paraná River main channel with the floodplain lake; *Ch-L* connection between the channel and the floodplain lake; *L-SCh* floodplain lake and the secondary channel connection.

	High-water phase				Low-water phase				
	USP	DSP	P-Ch	Ch-L	USP	DSP	P-Ch	Ch-L	L-SCh
Rarefied richness	9.13	6.18	8.73	3.28	12.52	12.00	7.40	6.20	3.30
Shannon (*H*)	2.21	1.50	1.89	0.74	2.74	2.25	1.89	1.41	0.82
Dominance (*D*)	0.14	0.34	0.20	0.41	0.22	0.37	0.19	0.63	0.56

Polypedilum spp., *Harnischia* spp., and *Onconeura* spp. were dominant (Table 2 and Fig 3).

The beta diversity values indicated that species turnover (β_{SIM}) was higher than nestedness (β_{NES}) in all environments and phases. Spatial total dissimilarity (β_{SOR}) was higher in floodplain habitats and nestedness (β_{NES}) was lower in main channel habitats. In relation to hydrological phases, the highest values of β_{SIM} and the lower value of β_{NES} were obtained in the low-water phase. On the other hand, in the high-water period, higher values of β_{NES} were recorded (Fig 4).

The chironomid total density, richness (*S*), and dominance (*D*) were not significantly correlated with the different analyzed environmental variables. However, habitat depth was positively related to Shannon diversity and negatively related to the density of dominant taxa (Rho 0.69, *p* = 0.044; Rho –0.67, *p* = 0.050).

Discussion

In relation to the taxonomic composition, the 34 taxa of Chironomidae found from drifting pupal exuviae corresponding to Chironominae, Orthocladiinae, and Tanypodinae subfamilies were the prevailing taxa in the subtropical habitats. The genera and morphotypes from larval benthic stages found in the present study coincide with those recorded for the Paraná system by other authors (Marchese *et al* 2002, Marchese & Paggi 2004, Montalto & Paggi 2006, Ezcurra de Drago *et al* 2007, Mesa *et al* 2012, Zilli & Paggi 2013). On the other hand, the genus *Robackia* recorded in the present study in the main channel was cited only in its larval stage in ecological studies from Upper Paraná (Paggi *et al* 1998, Pinha *et al* 2013). In future researches, the development of exhaustive samplings could lead to the collection of immature and adult stages which, added to laboratory breeding, would allow the detailed study of the taxonomy, distribution, and lifecycle of this genus.

In the present study, which analyzed the diversity of heterogeneous environments with a great diversity of microhabitats, the CPET application made possible the recording of an important number of taxa from these different environments, as has been registered by other authors for other latitudes (Raunio *et al* 2007a).

Fig 2 Density of Chironomidae pupal exuviae in sample sites in the Paraná River system during high- and low-water phases. Abbreviations: *USP* Paraná River main channel, upstream from the connection with the floodplain lake channel; *DSP* Paraná River main channel, downstream from the connection with the floodplain lake channel; *P-Ch* beginning of the connection channel of the Paraná River main channel with the floodplain lake; *Ch-L* connection between the channel and the floodplain lake; *L-SCh* floodplain lake and the secondary channel connection.
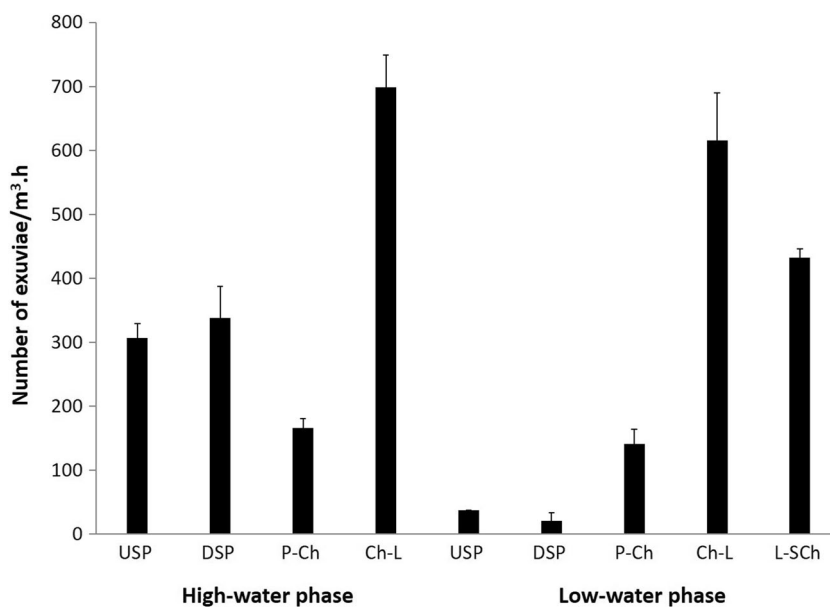
Fig 3 Relative density of dominant taxa of Chironomidae pupal exuviae in sample sites in Paraná River system during high- and low-water phases. Abbreviations: *USP* Paraná River main channel, upstream from the connection with the floodplain lake channel; *DSP* Paraná River main channel, downstream from the connection with the floodplain lake channel; *P-Ch* beginning of the connection channel of the Paraná River main channel with the floodplain lake; *Ch-L* connection between the channel and the floodplain lake; *L-SCh* floodplain lake and the secondary channel connection.
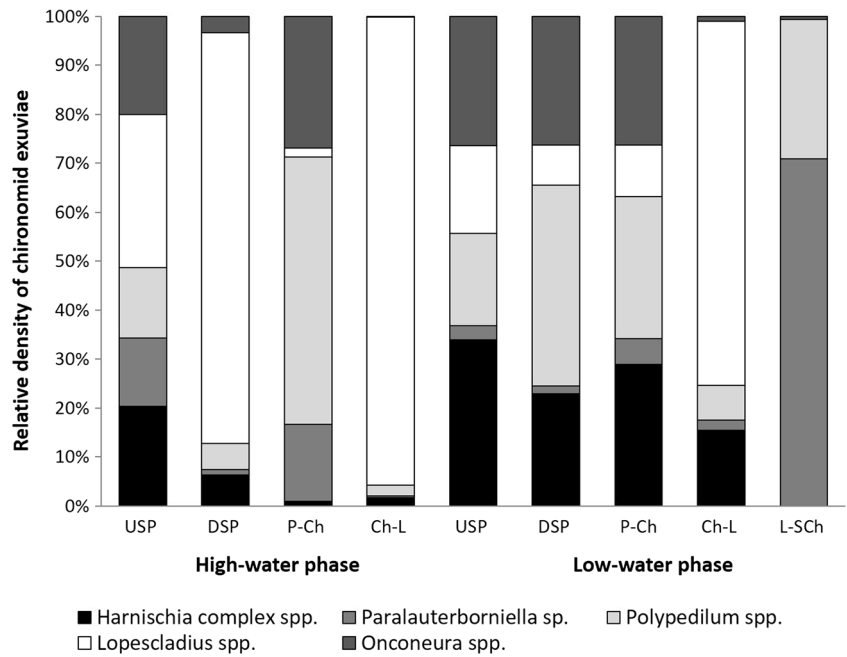
For the same system, Mesa *et al* (2012) recorded 33 taxa of chironomid larval assemblage, similar to 34 taxa found in this study with CPET, but considering a higher number of sampling stations (three in Paraná River, three in the connection channel, and four in the lake) in both water phases of two hydroperiods. In this sense, it is clear that, in order to find a similar number of Chironomidae taxa from the benthic larval stages, 75% more samples were required with the consequent additional effort of extraction, separation, and determination.

According to the results obtained for a river-floodplain system, the CPET more easily obtained information about the Chironomidae inhabiting different assemblages in waterbodies of different habitats

Fig 4 Partition of beta diversity (β_{SOR}) in two components: spatial turnover (β_{SIM}) and nestedness (β_{NES}) to Chironomidae assemblages using CPET.
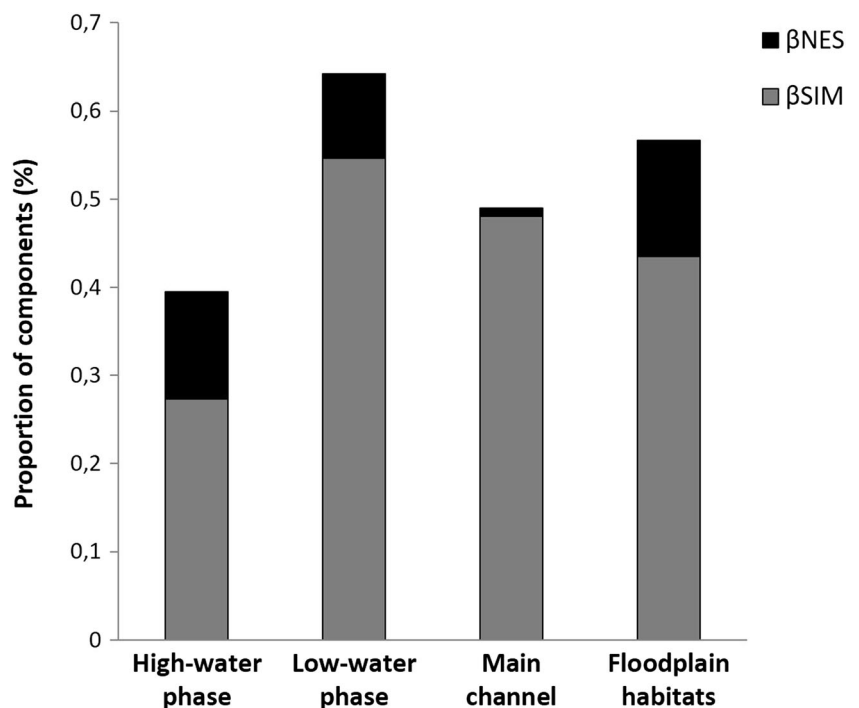
(pleuston, benthos, drifting, etc.). In addition, this technique allows for the sampling of wider areas than those obtained from benthic larvae as has been observed by other authors, providing a better knowledge of the regional pool of species (Ruse 1995, Raunio *et al* 2007a). This constitutes a great advantage with respect to other sampling techniques because sampling larval stages from benthos, pleuston, and drifting demands a greater effort and operational costs to obtain results comparable to those attained with the CPET.

Diversity and dominance indices allowed distinguishing of the environments in relation to the longitudinal and lateral dimensions (river/floodplain), with higher values of diversity and richness in the Paraná River sites and higher dominance values in all the floodplain environments. Thus, the diversity and density of dominant taxa were related only to depth of habitats, showing differences between the main channel sites (high depth and current velocity) and floodplain sites.

None of the diversity indicators presented differences between hydrologic phases. The pupal exuviae are the result of the adult emergence, a process which develops mainly in the spring-summer months. In the studied period, the samplings of the low- and high-water phases correspond to the beginning and end of summer, respectively, with similar temperatures, which could correspond to the similar values in this attribute for both phases.

In the present study, the highest values of richness and diversity were found in the main channel, indicating that drifting exuviae in the longitudinal axis come from different environments of a wider area (regional), while exuviae recorded in the connections of the floodplain environments in the lateral dimension could reflect the local assemblages.

The beta diversity values indicate that the species turnover is the dominant process in structuring studied assemblages in spatial and temporal analysis, increasing in the lateral axis in the floodplain habitats and in the low-water phase, being higher in relation to the contribution of the turnover component in environments with very different characteristics (main channel vs. floodplain lake and secondary channel). During the low-water phase, when the connectivity between the environments was low, the sampling stations showed a higher replacement. These results are coincident with Montalto and Paggi (2006) and Marchese *et al* (unpublished data), who carried out studies with larval chironomid assemblages in floodplain environments under a flood pulse regime and found the greatest richness and diversity in the lateral dimension environments with a higher heterogeneity and environmental dynamics (lakes, marginal wetlands, etc.). In concordance with our results, Thomaz *et al* (2007) concluded that the increment of dissimilarities in different large river floodplain habitats during low water levels is due to local forces that generate high inter-habitat

heterogeneity (high beta diversity), and conversely, the homogenization produced by flood is derived from increased connectivity (regional driving force) during high waters.

The results obtained through CPET application allowed ecologic interpretations, because they showed differences in the sites located in the longitudinal and lateral axes, in the main channel and its floodplain, demonstrating the efficiency of this technique for the evaluation of the attributes of the drifting pupal exuviae assemblage in an integrated way. Finally, this study based on CPET is new for the region and proposes an efficient tool to be used in ecological studies as well as in biomonitoring.

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