INFLUENCE OF ORDER AND THE HYDROGRAPHY IN IBICUÍ RIVER BACIN, AND SOME ENVIRONMENTAL FACTORS OF LOCAL SCALE IN STRUCTURE AND SPATIAL DISTRIBUTION OF COMMUNITIES OF MOLLUSKS

INFLUÊNCIA DA ORDEM DA BACIA HIDROGRÁFICA E DE ALGUNS FATORES AMBIENTAIS EM ESCALA LOCAL NA ESTRUTURA E DISTRIBUIÇÃO ESPACIAL DAS COMUNIDADES DE MOLUSCOS

INFLUENCIA DEL ORDEN DE LA CUENCA HIDROGRÁFICA Y DE ALGUNOS FACTORES AMBIENTALES A ESCALA LOCAL EN LA ESTRUCTURA Y DISTRIBUCIÓN ESPACIAL DE LAS COMUNIDADES DE MOLUSCOS

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Abstract: Landscape factors may determine differences in macroinvertebrate communities. For limnic mollusks, the influence of these factors is not well known yet. In this context, this study analyzed the community structure of limnic mollusks among different microbasins and sections of different stream orders, in a region with temperate climate, in southern Brazil. Additionally, the diversity of mollusks and the influence of some small scale environmental factors on the structure of communities were also studied. The samples were taken in four microbasins located in the middle course of the Ibicuí River, where segments of 1st, 2nd, 3rd and 4th orders were sampled. Twelve species were found, occurring dominance of the gastropod *Uncancylus concentricus* and of the bivalve *Pisidium dorbignyi*. The results showed the richness of communities was influenced by microbasins and orders. The greatest number of species was recorded in two microbasins located in low areas, with predominantly sandy substrate and more aquatic macrophytes, and also in higher orders sections (3rd and 4th). However, proportional composition and abundance of species in the communities were not influenced by these two factors.
Keywords: freshwater mollusks; ecology; landscape factors; Neotropical Region.

Resumo: Fatores da paisagem podem determinar diferenças nas comunidades de macroinvertebrados. Para moluscos límnicos, a influência desses fatores não é bem conhecida. Nesse contexto, este estudo analisou a estrutura da comunidade de moluscos límnicos em diferentes microbacias e trechos de diferentes ordens fluviais, em uma região de clima temperado no sul do Brasil. Adicionalmente, a diversidade de moluscos e a influência de alguns fatores ambientais de pequena escala na estrutura das comunidades também foram estudadas. As amostras foram coletadas em quatro microbacias localizadas no curso médio do rio Ibicuí, onde foram amostrados trechos de 1ª, 2ª, 3ª e 4ª ordens. Doze espécies foram encontradas, com dominância do gastrópode Uncancylus concentricus e do bivalve Pisidium dorbignyi. A riqueza das comunidades foi influenciada pelas microbacias e ordens fluviais. O maior número de espécies foi registrado em duas microbacias localizadas em áreas baixas, com substrato predominantemente arenoso e maior presença de macrófitas aquáticas, e também em trechos de ordens superiores (3ª e 4ª). Contudo, a composição proporcional e a abundância das espécies nas comunidades não foram influenciadas por esses dois fatores. As espécies encontradas na área

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estudada são tolerantes às variações ambientais determinadas por esses fatores da paisagem, que não foram suficientemente marcantes para produzir variações na composição de espécies. Em escala espacial local, apenas o tamanho das partículas influenciou a composição de espécies das comunidades, mas não a abundância proporcional das espécies.
Palavras-chave: Moluscos de água doce, Riacho, Ecologia, Fatores da Paisagem, Região Neotropical.

Resumen: Los factores del paisaje pueden determinar diferencias en las comunidades de macroinvertebrados. Para los moluscos límnicos, la influencia de estos factores no es bien conocida. En este contexto, este estudio analizó la estructura de la comunidad de moluscos límnicos en diferentes microcuencas y tramos de distintos órdenes fluviales, en una región de clima templado en el sur de Brasil. Adicionalmente, también se estudiaron la diversidad de moluscos y la influencia de algunos factores ambientales de pequeña escala en la estructura de las comunidades. Las muestras fueron recolectadas en cuatro microcuencas localizadas en el curso medio del río Ibicuí, donde se muestrearon tramos de 1º, 2º, 3º y 4º órdenes. Se encontraron doce especies, con dominancia del gastrópodo Uncancylus concentricus y del bivalvo Pisidium dorbignyi. La riqueza de las comunidades fue influenciada por las microcuencas y los órdenes fluviales. El mayor número de especies se registró en dos microcuencas localizadas en áreas bajas, con sustrato predominantemente arenoso y mayor presencia de macrófitas acuáticas, así como en tramos de órdenes superiores (3º y 4º). Sin embargo, la composición proporcional y la abundancia de las especies en las comunidades no fueron influenciadas por estos dos factores. Las especies encontradas en el área estudiada son tolerantes a las variaciones ambientales determinadas por estos factores del paisaje, que no fueron suficientemente marcados como para producir variaciones en la composición de especies. A escala espacial local, solo el tamaño de las partículas influyó en la composición de especies de las comunidades, pero no en la abundancia proporcional de las especies.

Palabras-clave: Moluscos de agua dulce, Arroyo, Ecología, Factores del paisaje, Región Neotropical.

1 INTRODUCTION

The community structure of freshwater macroinvertebrates may vary across the study spatial scale, principally in temperate regions (Downes et al., 1993). On landscape scale, for example, different microbasins, if they were in different basins or geomorphological regions, may determine differences in communities (Wu & Legg, 2007; Salvarrey et al., 2014). Differences in the stream orders may also influence the macroinvertebrate communities, especially when correlated to different geographical heights. Studies conducted along longitudinal gradients in streams show that not only the composition as richness increases with the increase of the order. (Jacobsen, 2004; Ramirez et al., 2004; Boyero, 2005; Salvarrey et al., 2014).

For freshwater mollusks, the landscape factors influence is poorly known. Studies are scarce and restricted to lentic environments, and have shown that larger areas can positively affect richness and composition of species. However, higher altitudes can affect negatively. (Oertli et al., 2002; Sturm, 2007; Maltchick et al., 2010; Sá et al., 2013; Hausdorf, 2002).

The influence of order of the rivers on freshwater mollusk communities has received some attention, but usually the studies are restricted to a single river or stream (Pérez-Quintero, 2007; Martello et al., 2014), not being analyzed at a landscape scale, which takes into account habitat connectivity and landscape fragmentation and heterogeneity. Generally, in a long gradient (1st to 6th order), communities change from upstream to downstream, causing variation in the composition and decrease of richness (Allan & Castillo, 2007). However, for small gradients, especially in homogeneous areas, mollusks communities do not seem to respond to the order (Tietze & De Francesco, 2010). Substratum granulometry and presence of macrophytes, for example, have been considered important factors related to variations in composition and community richness (Gonçalves et al., 1998). However, factors such as riparian vegetation and intermittency, important to structuring macroinvertebrate communities (Rios & Bailey, 2006; Bonada et al., 2008), have been less investigated for freshwater mollusks (Pereira, et al., 2000a). Furthermore, freshwater mollusks play an important role in aquatic ecosystems. They participate in the food chain because they represent an important food item of the diet of many fish and other vertebrates (Cummins & Bogan, 2006). Bivalves can represent a large portion of the biomass of invertebrates and are also primarily responsible for the filtration of water (Mcmahon & Bogan, 2001). Limnic mollusks are among the most threatened groups of animals worldwide (Bogan, 2006). Wetland drainage and channelization, sediment loading, in addition to pollution of water bodies and introduction of exotic species of mollusks are among the factors responsible for this problem (Ricciardi & Macisaac, 2000; Mansur et al., 2003).

The Ibicuí River basin has 290 km, approximately 35,439 km² drainage areas and annual flow ranging from 900 to 1000m^3/s (IBGE, 2002). The basin is located within the Pampa biome and, to a lesser extent, in a transitional zone with the Atlantic Forest, characterized by deciduous seasonal forest formations in areas of steeper slopes and more rugged terrain. The native vegetation is predominantly composed of grasslands, shrubs, and small, isolated forest patches. Nevertheless, the riparian forests are currently highly fragmented as a result of extensive agricultural expansion, primarily for rice and soybean cultivation, along with cattle and sheep farming (IBGE, 2002; De Nardin & Robaina, 2009). Furthermore, the waters of the river basin Ibicuí has been utilized for public supply, wastewater, urban drainage, industry, navigation, mining, agriculture, fisheries and recreation (Paiva et al., 2000). In the Central Depression, the original vegetation has been replaced by rice paddies along rivers and streams (Quadros & Pillar, 2002), and the slope of the plateau, for various types of plantation such as soybeans, and has been used for breeding bovine and ovine animals (Alves, 2008; De Nardin & Robaina, 2009).

This study aims to understand how landscape-scale factors, such as stream order, altitude, connectivity, and land use, influence the composition and richness of freshwater mollusk communities in the Ibicuí River basin. Given the scarcity of research addressing these factors for mollusks in lotic environments, especially in impacted areas like the Ibicuí basin, this work is essential to generate information that contributes to the conservation of these species, which play a key ecological role and are among the most threatened animal groups worldwide.

2 METHODOLOGY

2.1 STUDY AREA

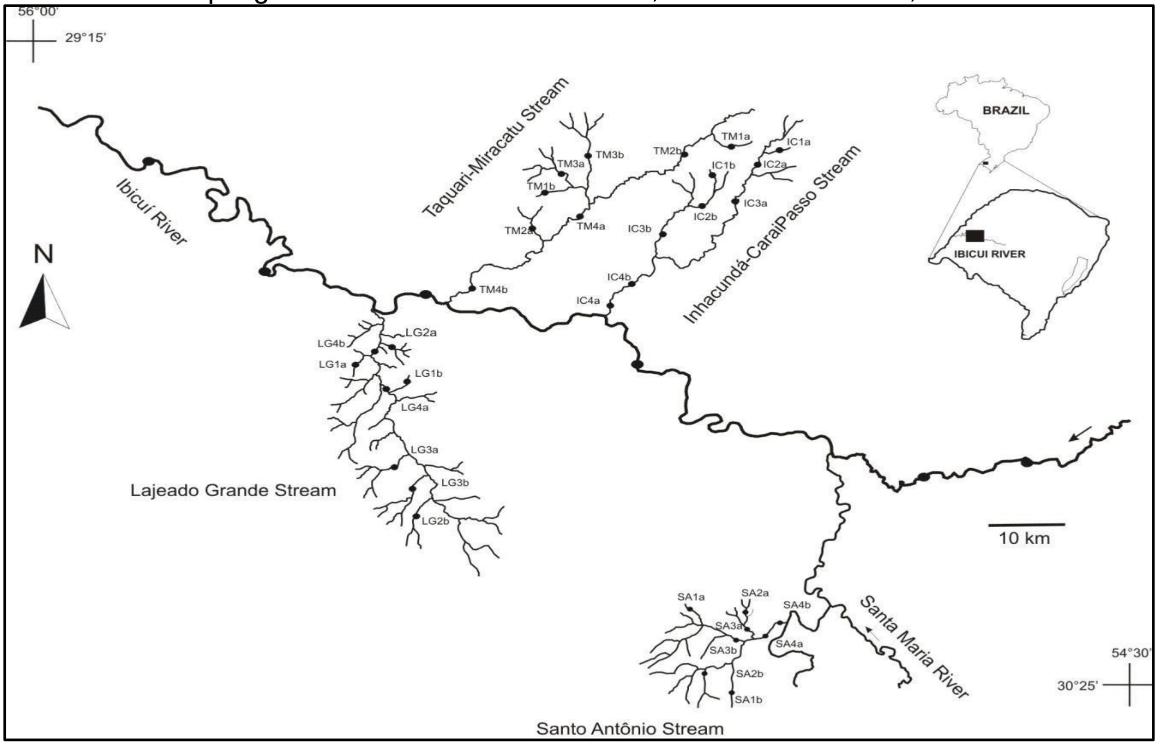
The Ibicuí River basin is located in southern Brazil (Rio Grande do Sul), and the main channel flows east to west until it flows through the Uruguay River basin (Figure 1). In the eastern sector, the basin covers an area of plain, and runs through the Central Depression physiographic region, characterized by soft undulations and low altitudes, ranging from 19 to 90m. In the western sector, many tributaries run through the basin physiographic region of the Serra Geral plateau and its slopes, where the altitude ranges from 300 to 400m (Robaina et al., 2010). In the main channel, the flow is slow, the substrate is sandy, and the floodplain is wide, which leads to the occurrence of floods during rainy periods (IBGE, 2002).

The climate in the region is subtemperate, with well defined seasons and average annual temperature of 18.1°C to 22°C, with 13ºC mean temperature of the coldest month. Rainfall is well distributed and reaches values greater than 1400mm annually (Maluf, 2000). Considering this hydric balance, the region is included between the humid and sub-humid climate (Maluf, 2000). This river is located in the Pampa biome, characterized by vegetation composed of grasses, low plants and some trees and shrubs found near watercourses, in lowlands. The vegetation is characterized by fields (Marchiori, 2002).

2.2 SAMPLINGS SITES

Samples were collected at four microbasins of the middle Ibicuí River: Inhacundá-Caraí Passo, Taquari-Miracatu, Lajeado Grande and Santo Antônio (Figure 1), and have a catchment area of approximately of 340 km², 540 km², 488 km² and 90 km², respectively. The microbasins Taquari-Miracatu and Inhacundá Caraí-Passo located north of Ibicuí River have altitudes ranging from 102.1m to 285.3m and 73m to 358.14m respectively, and upstream sectors are on the basalts of the Serra Geral Formation and downstream on the arenites of Botucatu and Guará Formations (De Nardin & Robaina, 2009; Robaina et al., 2010). The microbasins Lajeado Grande and Santo Antônio are located south of the Ibicuí River in lower areas, 48.5m to 161.84m and 35.96m to 141.12m respectively. Both flow over the arenites of Botucatu and Caturrita formations (Robaina et al., 2010), but the downstream sectors of the first roam on the basalts of Serra Geral Formation. The land use in the microbasins is characterized by various livestock and plantations, although the Santo Antônio microbasin is the one with the predominance of rice cultivation. In each watershed were sampled segments of 1st, 2nd, 3rd and 4th orders, totaling eight sampling sites per microbasin totaling 32 sampling sites (Figure 1). For each site, environmental factors such as hydroperiod, the dominant substrate grain size, presence of riparian vegetation and aquatic macrophytes were described. The location and environment characterization of each location are shown in Table 1.

Figure 1 - Map showing location of study area, with the four microbasins studied, including all sampling sites in the Ibicuí River basin, Rio Grande do Sul, Brazil.



Source: The authors, 2012. The image was created in the Corel Draw program.

2.3 SAMPLING AND IDENTIFICATION

Samples were collected in April and May 2010. In each site, the mollusks were collected manually with the help of sieves with mesh size of 1mm, in three quadrats of 1 x 5m. When there were macrophytes, these were removed and then examined individually to verify the presence of mollusks. These, if found, were added to samples. When macrophytes were absent, sampling was carried out only in the sediment of the site, following the defined sampling areas. Additional collecting, for comparison between the diversity of the streams and the Ibicuí River were made in the main course of Ibicuí (6th order). The collected individuals were preserved in 70% alcohol in plastic pots and taken to the laboratory for analysis. In the laboratory, individuals were identified to the taxonomic level of species, with the help of literature, and/or experts, and counted. After identification, the specimens were deposited in the Invertebrate Collection, Department of Biology, Universidade Federal de Santa Maria (UFSM).

2.4 DATA ANALYSIS

The diversity of the as a whole, of each microbasin and each set of segments of the same order were analyzed using descriptors such as composition, richness (S), number of specimens (N), and relative frequency/dominance (%). The diversity was also estimated by the Shannon-Wiener index (H'), obtained by the program PAST (Hammer et al., 2001). This index ranges from 0 (when the sample contains only one species) and a maximum value corresponding to the S species, but rarely exceeds the value of 4.5 (Margalef, 1972). The cumulative species richness in streams of the middle Ibicuí River was calculated using the mean curve of the collector, based on 500 curves generated by random addition of samples. The analysis was performed for total area, using the program Estimate S, version 8.2 (Colwell, 2006). To compare the estimated richness of the communities among the four basins and four sets of orders, rarefaction curves were constructed. The number of species was estimated for 94 individuals (for comparative analysis among the four microbasins) and 88 individuals (for comparative analysis among the four sets of orders) and the average was measured based on 1000 interactions, using the program ECOSIM, version 7.72 (Gotelli & Entsminger, 2009). The species composition and abundance of mollusks communities are determined by an interaction of different landscape factors, and analysis of similarities was done (ANOSIM) (Clarke & Warwick, 2001).

Analysis of similarities of two-factor cross (ANOSIM - *Two-way crossed*) was used to test differences between the two factors: i) samples of segments of different orders, and ii) samples of different microbasins. For the factor of different orders were included four levels (segments of 1st, 2nd, 3rd and 4th orders) and the microbasins factors, the four microbasins (Santo Antônio, Taquari-Miracatu, Inhacundá-Caraí Passo and Lajeado Grande). ANOSIM uses a test statistic (*R*) based on the difference between the average of all the rank dissimilarities between objects between groups and the average of all the rank dissimilarities between objects within groups. *R* is scaled to be within the range +1 to –1. Differences between groups would be suggested by *R* values greater than zero where objects are more dissimilar between groups than within groups. *R* values of zero indicate that the null hypothesis is true. Negative *R* values indicate that dissimilarities within groups are greater than dissimilarities between groups (Clarke & Warwick, 2001).

Table 1 - Location and environmental characterization of sampling sites in the Inhacundá-Caraí Passo (IC), Taquari-Miracatu (TM), Lajeado Grande (LG) and Santo Antônio (SA) microbasins, middle course of Ibicuí River basin, RS, Brazil. 1= Myriophyllum aquaticum (Vell., 1825), 2= Pontederia lanceolata (Nuttall, 1818), 3= Polygonum hidropiperoides (Michaux, 1803), 4= Cyperus sp. (Linnaeus, 1753), 5= Ludwigia sp. (Linnaeus, 1753), 6= Thalia geniculata (Linnaeus, 1763), 7= Nymphaea sp. (Linnaeus, 1753), 8= Eichhornia azurea (Kunth, 1853).

Streams/Sites	Coordinates	Altitude(m)/width(m)	Orders	Hydroperiod	Land use	Substratum	Riparian forest	Macrophytes
IC1a	29°23'08''; 55°00'55''	358/1.5	1st	Intermittent	Pasture	Gravel/silt	Present on both sides	1
IC1b	29°25'54''; 55°07'41''	113/1.5	1st	Intermittent	Pasture	Sand/gravel	Absent	Absent
IC2a	29°25'23''; 55°03'21''	230/6	2nd	Permanent	Pasture	Sand/gravel	Present on both sides	Absent
IC2b	29°27'48''; 55°07'14''	153/8.4	2nd	Permanent	Plantation	Sand/silt	Absent	Absent
IC3a	29°25'79''; 55°04'12''	166/8.5	3rd	Permanent	Pasture	Sand/gravel	Present on both sides	Absent
IC3b	29°31'02''; 55°10'43''	324/8	3rd	Permanent	Plantation	Sand	Present on both sides	Absent
IC4a	29°35'28''; 55°14'01''	77/12	4th	Permanent	Pasture	Sand/silt	Present on both sides	Absent
IC4b	29°36'22''; 55°14'10''	73/13	4th	Permanent	Pasture	Sand	Absent	1
TM1a	29°33'35''; 55°04'33''	297/1	1st	Intermittent	Pasture	Gravel/silt	Present on both sides	Absent
TM1b	29°26'25''; 55°18'30''	135/2	1st	Intermittent	Pasture	Sand/gravel	Present on both sides	Absent
TM2a	29°28'30''; 55°18'05''	112/5	2nd	Permanent	Pasture	Sand/gravel	Present on both sides	Absent
TM2b	29°23'33''; 55°08'28''	145/5	2nd	Permanent	Plantation	Sand/gravel	Present on both sides	Absent
TM3a	29°25'06''; 55°18'47''	149/6	3rd	Permanent	Pasture	Sand/gravel	Present on both sides	Absent
TM3b	29°24'36''; 55°16'01''	123/6	3rd	Intermittent	Pasture	Sand/gravel	Absent	Absent
TM4a	29°28'42''; 55°16'39''	102/14	4th	Permanent	Pasture	Sand	Present on both sides	Absent
TM4b	29°35'13''; 55°25'23''	285/14	4th	Permanent	Pasture	Sand	Present on both sides	Absent
LG1a	29°40'43''; 55°33'03''	49/1.5	1st	Intermittent	Pasture	Sand/gravel	Present on both sides	Absent
LG1b	29°43'30''; 55°30'57''	93/3	1st	Intermittent	Plantation	Sand	Present on both sides	2
LG2a	29°39'54''; 55°31'53''	71/2	2nd	Intermittent	Pasture	Sand	Present on both sides	Absent
LG2b	29°54'55''; 55°29'31''	162/3	2nd	Intermittent	Pasture	Sand/gravel	Absent	Absent
LG3a	29°49'34''; 55°30'44''	154/4	3rd	Permanent	Plantation	Sand/gravel	Present on one side	2
LG3b	29°51'16''; 55°29'19''	117/3	3rd	Permanent	Pasture	Sand/gravel	Present on both sides	Absent
LG4a	29°43'18''; 55°32'03''	115/8	4th	Permanent	Pasture	Sand	Present on both sides	Absent
LG4b	29°40'42''; 55°32'58''	48/12	4th	Permanent	Pasture	Sand/silt	Present on both sides	Absent

Table 1 - Continuity.

Streams/Sites	Coordinates	Altitude(m)/width(m)	Orders	Hydroperiod	Land use	Substratum	Riparian forest	Macrophytes
SA1a	30°15'58''; 55°01'10''	112/3	1st	Permanent	Pasture	Sand/silt	Absent	3, 4
SA1b	30°19'52''; 54°59'39''	98/5	1st	Permanent	Plantation	Silt	Absent	4, 5, 6
SA2a	30°16'08''; 54°58'45''	141/2	2nd	Permanent	Pasture	Sand	Absent	7
SA2b	30°19'32''; 55°01'05''	36/2	2nd	Permanent	Plantation	Sand/Silt	Absent	3, 5, 1
SA3a	30°17'42''; 54°59'17''	100/3	3rd	Permanent	Pasture	Sand	Absent	8
SA3b	30°18'46''; 54°59'49''	98/4	3rd	Permanent	Pasture	Sand	Present on both sides	2, 8
SA4a	30°17'01''; 54°57'43''	79/5	4th	Permanent	Pasture	Sand	Present on both sides	Absent
SA4b	30°16'46''; 54°57'31''	96/7	4th	Permanent	Pasture	Sand	Present on both sides	Absent

Source: The authors, 2012.

To analyze the influence of small factors on composition and abundance of communities were carried out tests similarity between communities. These were based on the presence and absence of mollusk species and abundance proportional species. In the first case, the similarity is calculated by Coefficient of Geographic Resemblance (CGR). This index is equivalent to the Sörensen, DICE and Czekanowski indexes (Magurran, 2004). In the second case, the similarity was calculated using the Coefficient of Similarity of Morisita-Horn or MORISIT2. Both tests were carried out in the NTSYS PC 2.10s program. Subsequently, the similarity matrices are represented by cluster analysis (clustering) with the weighting of the means of pairs of groups (WPGMA), to avoid the effect of sample size (species richness in different communities) on analyses. Possible distortions in the graphic representation of similarity matrices by cluster analysis were evaluated by the cophenetic correlation coefficient (r). Values of *r* near 1, the lower are the distortion. We considered clusters with similarity over 75%. Data sets that show a spatially structured character may have a relationship due to geographical proximity, known as spatial autocorrelation (Legendre & Legendre, 1998). To test this relationship, we constructed a matrix of geographical distance between the localities, obtained from the coordinates of sampling sites, since mollusks can be dispersed by terrestrial vertebrates (Brown, 2007).

The correlation between the geographic distance matrix with the matrices of similarity in community composition was determined by the Mantel test (Manly, 2000). This test correlates matrices, based on the statistic Z, where Z is dependent on the magnitude of the number of elements in the matrix to be compared. Thus, it is necessary a normalization to convert Z in coefficient (*r*) ranging from 1 a -1 (Valentin, 2000). The significance of Z was determined by a permutation test of Monte Carlo, using 5000 permutations. Mantel and Monte Carlo tests were performed in the NTSYS PC 2.10s program.

The spatial distribution of mollusks communities focus on composition and abundance proportional of their species was correlated with four matrices of hypotheses based on environmental factors such as presence of macrophytes, presence of riparian vegetation, grain size substrate and hydroperiod, to determine a correlation.

1) Matrix hypothesis of macrophytes: postulates that communities at sites with presence of macrophytes are more similar among themselves than those in areas without macrophytes. Constituted a binary matrix, in which pairs of sites with the same condition, whereas pairs of sites with different conditions received the value 0.

2) Matrix hypothesis of riparian vegetation: postulates that the communities present in sites with presence of riparian vegetation are more similar among themselves than of sites without riparian vegetation. This matrix is based on observation *in loco* of the occurrence of riparian forest and is considered riparian forest sites with the presence of forest on one or both sides. We have established a binary matrix in which pairs of sites with the same condition, whereas pairs of sites with different conditions received value 0.

3) Matrix hypothesis of substrate: postulates that the communities present in sites with similar granulometry of the substrate are more similar among themselves than those of sites with different granulometry. The granulometry was determined subjectively, based on the observation *in loco*. We considered two types of predominant granulometry (>70%) at site: gravelly, with a predominance of clasts greater than 6 mm and sandy, with a predominance of clasts smaller than 2 mm. To

obtain the similarity matrix, the pairs of sites with different granulometry receive value 0 and the pairs of locations with the same granulometry given the value 1.

4) Matrix hypothesis of hydroperiod: postulates that the communities present at sites with the same hydroperiod are more similar among themselves than those of sites with different hydroperiod. This matrix is based on intermittent streams marked on topographic charts, scale 1:50,000. We have established a binary matrix in which pairs of sites with the same condition of hydroperiod, whereas pairs of sites with different hydroperiods received value 0.

Mantel's test partial was used to test the correlation between the similarity matrix in the composition and abundance proportionality of communities and the four matrices hypothesis using the distance matrix to remove the effect of the geographical proximity. The similarity and clustering analysis, and Mantel and partial Mantel tests were performed using the software NTSYSpc 2.10S.

3 RESULTS

A total of 707 individuals were collected in the studied microbasins, assigned to eight families, 11 genus and 12 species (Table 2).

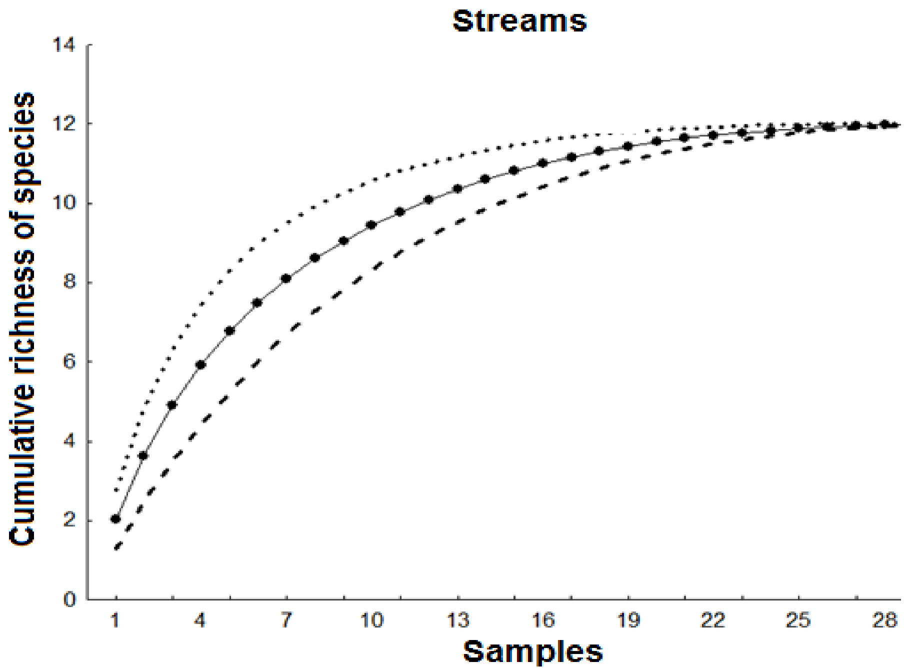
Table 2 - Species founded in streams Taquari-Miracatu (TM), Lajeado Grande (LG), Santo Antonio (SA) and Inhancundá-Caraí Passo (IC) and in the four groups formed by sum of segments of the same order (1st, 2nd, 3th and 4th) of the four streams, in the middle course of Ibicuí River, Rio Grande do Sul.

	Streams				Orders			
Species	**TM**	**LG**	**SA**	**IC**	**1st**	**2nd**	**3th**	**4th**
GASTROPODA								
Ampullariidae								
Pomacea canaliculata (Lamarck, 1804)	0	22	83	9	50	54	10	0
Lymnaeidae								
Lymnaea columella (Say, 1817)	0	11	3	6	1	12	7	0
Planorbidae								
Biomphalaria tenagophila (Orbigny, 1835)	1	40	36	12	38	4	12	35
Ancylidae								
Uncancylus concentricus (Orbigny, 1835)	104	52	22	58	185	8	42	1
Hebetancylus moricandi (Orbigny, 1837)	0	0	3	0	0	0	3	0
BIVALVIA								
Hyriidae								
Diplodon delodontus (Lamarck, 1819)	0	0	0	9	0	0	9	0
Mycetopodidae								
Anodontites trapesialis (Lamarck, 1819)	0	0	12	0	10	0	0	2
Anodontites trapezeus (Spix, 1827)	0	0	1	0	0	0	0	1
Corbiculidae								

Corbicula fluminea (Müler, 1774)	0	0	16	0	0	0	0	16
Pisidiidae								
Eupera klappenbachi (Mansur & Veitenheimer, 1975)	0	0	5	0	0	1	4	0
Pisidium dorbignyi (Clessin, 1879)	97	48	49	0	23	122	19	30
Musculium argentinum (Orbigny, 1835)	0	3	5	0	0	0	5	3
Total	202	176	235	94	307	201	111	88
S	3	6	11	5	6	6	9	7
H'	0,049	0,105	0,124	0,08	0,08	0,073	0,127	0,091
Sterile sites	4	3	2	2	3	3	2	3
Sterile plots	14	13	8	16	10	12	13	16

The collector curve reached an asymptote showing that few species can be found, if the sampling effort is increased (Figure 2). Gastropods comprised 41.66% of species and 65.34% of collected individuals, and bivalves comprised 58.34% of species and 34.65% of collected individuals. The gastropod *Uncancylus concentricus* and the bivalve *Pisidium dorbignyi* were dominant in the area, corresponding to 33.38% and 27.43% respectively, of the total of individuals.

Figure 2 - Cumulative curve of species of mollusks found in four microbasins in the middle Ibicuí River, RS, Brazil.



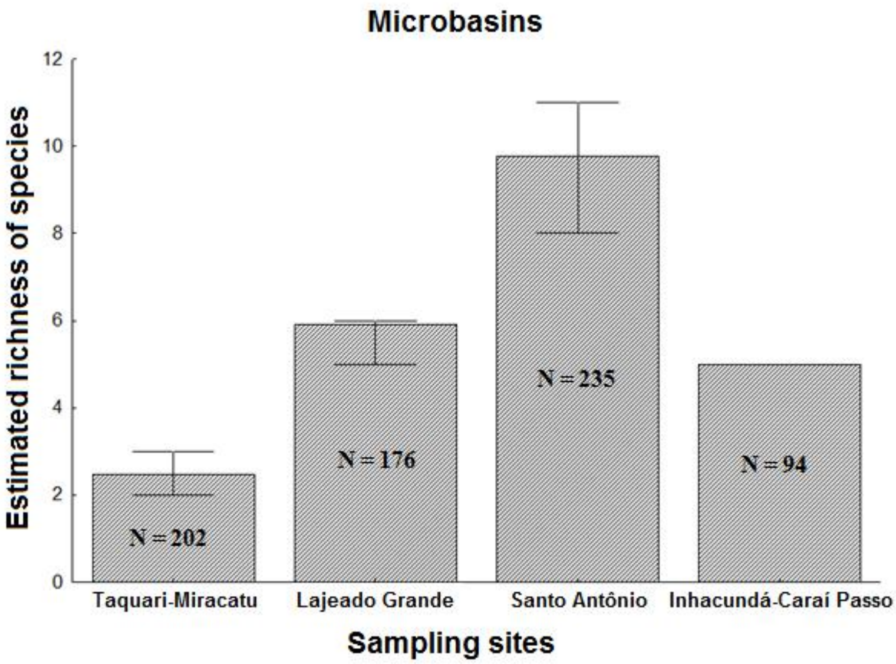
In additional samplings made in the main channel of the Ibicuí River were found the species *P*. *canaliculata*, *D*. *delodontus*, *A*. *trapezeus* and *C*. *fluminea*. The species *C*. *inflata*, *M*. *siliquosa*, *M*. *corrientesensis*, *C*. *largillierti*, *P*. *megastoma*, *C*. *martensi*, *F*. *fossiculifera* and *L*. *blainvilleana* were found exclusively in the main channel, being *P*. *megastoma*, *C*. *martensi*, *F*. *fossiculifera* and *L*. *blainvilleana* represented only by shells.

The microbasin of Santo Antônio stream showed the highest abundance, richness and diversity Index value among the four studied microbasins (Table 2). The dominant species were *P*. *canaliculata* (35.31%) and P. *dorbignyi* (20.85%). Among the 12 sampled species in the whole area, the only one who didn't sample in this stream was *D*. *delodontus*. Taquari-Miracatu obtained the second highest abundance, however, had the smallest value of diversity index (Table 2). In this microbasin *U*. *concentricus* represented more than a half of found species (51.48%) followed by *P*. *dorbignyi* (48.01). The microbasin Lajeado Grande showed the third highest abundance and the second highest value of diversity index (Table 2). *U*. *concentricus* (29.54%) and *P*. *dorbignyi* (27.72%) were the abundant species, which together represented almost all individuals found. Inhacundá-Caraí Passo stream showed the smaller abundance and the second smaller richness, and a small value of diversity index. *U*. *concentricus* represented more than a half of individuals sampled in this microbasin (61.70%) as in Taquari-Miracatu stream.

The methods for estimating richness (Figure 3) demonstrated that the Santo Antônio stream showed higher estimating richness for a sample of 94 individuals randomly collected. The microbasin Taquari-Miracatu showed the smallest estimating richness, followed by Inhacundá-Caraí Passo microbasin. Those results confirmed the differences obtained in terms of raw values observed.

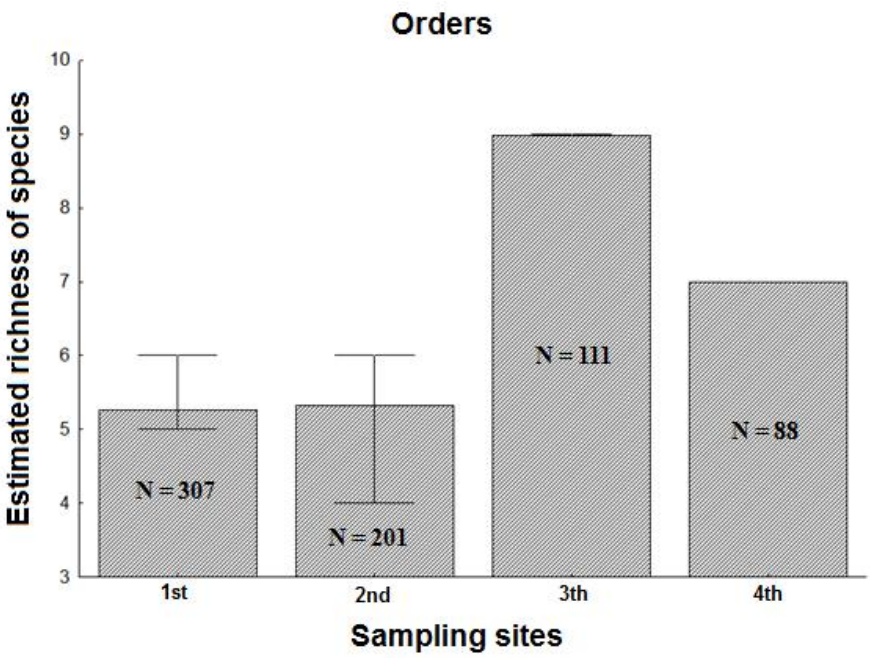
The group formed by sites of 1st order showed the higher abundance, followed by the 2th order group. However both showed smaller richness and low values of diversity index (Table 2). In the group of sites of 1st order, *U*. *concentricus* was the dominant species (60.26%) and in the group of sites of 2th order was *P*. *dorbignyi* (60.69%).

Figure 3 - Comparison of estimated richness of mollusks species among studied microbasins in the middle course of Ibicuí River basin, RS, April/May, 2010. Vertical bars indicate the variation around the mean.



The group formed by sites of 3th order showed the highest richness and the value of diversity index; however, it had the second smallest abundance (Table 2). The dominant species in this group were *U*. *concentricus* (37.83%) and *P*. *dorbignyi* (17.11%). The group formed by sites of 4th order showed the second highest richness however, abundance and the value of diversity index were low. In additional samplings made in the main channel of the Ibicuí River were found the species *P*. *canaliculata*, *D*. *delodontus*, *A*. *trapezeus* and *C*. *fluminea*. The species *C*. *inflata*, *M*. *siliquosa*, *M*. *corrientesensis*, *C*. *largillierti*, *P*. *megastoma*, *C*. *martensi*, *F*. *fossiculifera* and *L*. *blainvilleana* were found exclusively in the main channel, being *P*. *megastoma*, *C*. *martensi*, *F*. *fossiculifera* and *L*. *blainvilleana* represented only by shells.

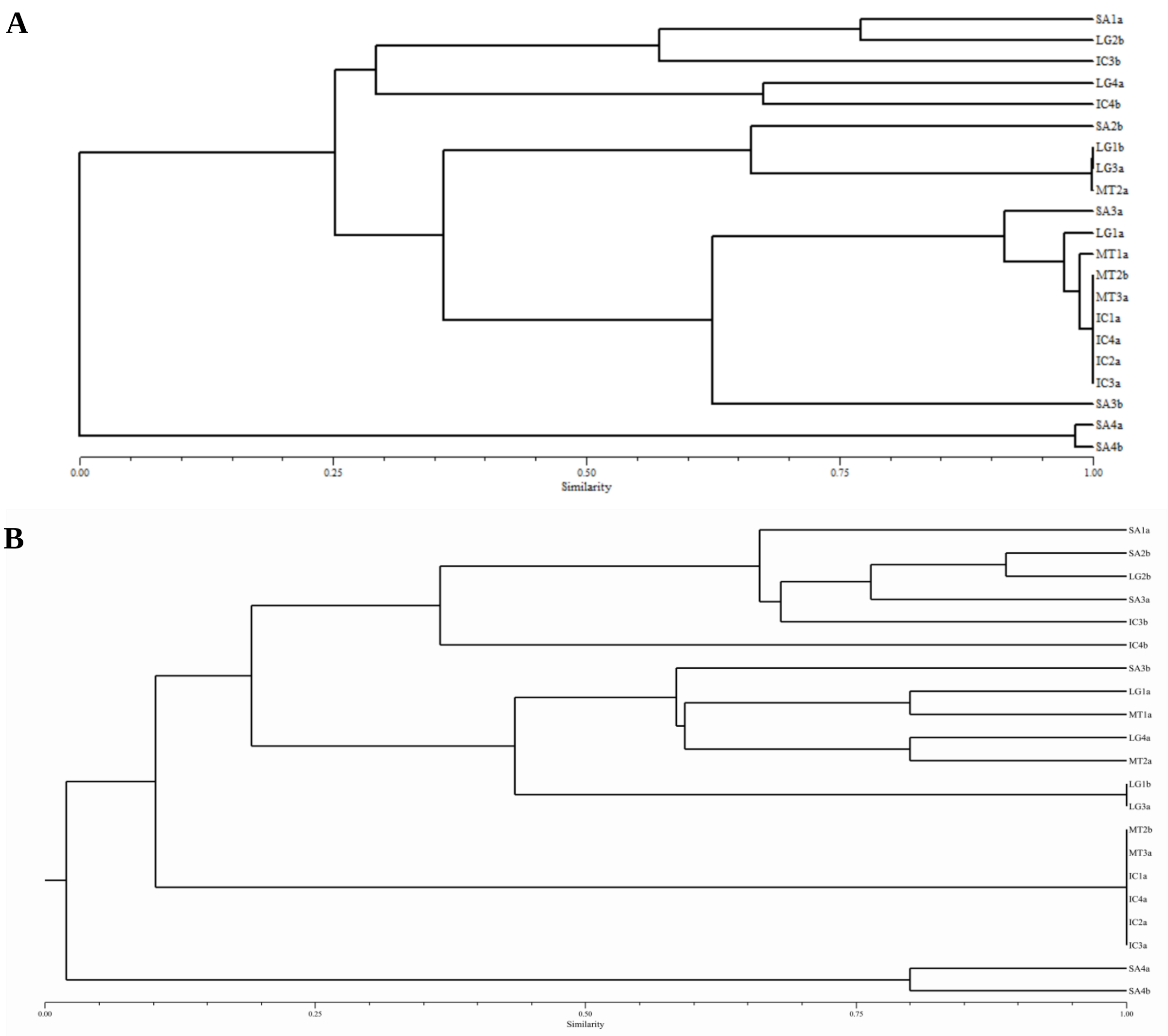
Figure 4 - Comparison of estimated richness of mollusks species among sampled groups of orders in the middle course of Ibicuí River basin, RS, April/May, 2010. Vertical bars indicate the variation around the mean.



The Mantel test showed that the influence of geographic distance in mollusks communities similarity, both in terms of point of view of comparison such as the proportional abundance of species was low and not significant (Table 3). The analysis of grouping of communities of local scale showed that when the composition is considered four groups with similarity over 75% are formed; three of them with similarity over 90% (Figure 5A). When the abundance of community species is considered six groups with similarity over 75% are formed, but only two of them have similarity over 90% (Figure 5B). Furthermore, this test showed that the presence of riparian vegetation and macrophytes did not correlate with structure of mollusks communities (Table 3). However, substrates had a similar and significant correlation ($p<0.01$, after correction of Bonferroni) with composition, but not with abundance of community species (Table 3). The correlation was positive for substrate, indicating that higher is the similarity in granulometry, higher is the similarity in community composition. Although, intermittence and macrophytes were marginally significant for composition.Table 3 - Influence of distance and correlation between composition (presence/absence) and abundance of species of mollusks communities and environmental factors such as intermittence, presence of macrophytes and riparian vegetation and grain size substrate, in streams of middle course of Ibicuí River.

	Presence/Absence	Abundance
Distance	*r* = -0.01895; *p* = 0.4249	*r* = 0.03016; *p* = 0.3463
Intermittence	*r* = -0.11623; *p* = 0.0610	*r* = -0.10353; *p* = 0.0982
Macrophytes	*r* = 0.13856; *p* = 0.0496	*r* = 0.07157; *p*= 0.1430
Riparian vegetation	*r* = 0.02135; *p* = 0.3715	*r* = -0.00023; *p* = 0.4275
Substrate	***r* = 0.026643; *p* = 0.0040**	*r* = 0.20702; *p* = 0.0170

Figure 5. Similarity (Coefficient of Geografical similarity CGR) in mollusks communities of N sites of four microbasins in the middle course of Ibicuí River. A) Species composition (r = 0.83339; p = 1.0000) and B) proportional abundance of species (r = 0.89083; p = 1.0000). r represents the correlation coefficient cophenetic. The abbreviations of sites were defined in table 1.



4 DISCUSSION

Previous studies carried out in streams in southern Brazil show the existence of a malacofauna represented by about 9 to 23 species (Martello et al., 2006; 2014; Pereira et al., 2011; Sá et al., 2013). Therefore, in this study, the richness found was one of the lowest in the region. The greater diversity found in streams in the state was obtained in the headwaters of the Gravataí River, an area characterized by a wetland with the presence of macrophytes, favorable conditions for the establishment of mollusks, especially gastropods (Martello et al., 2008). Thus, the predominance of bivalves species obtained in this study, as in Martello et al. (2006) and Pereira et al. (2011), may be related to environments more sandy and with less macrophytes.

The dominant species, *U*. *concentricus* and *P*. *dorbignyi*, seem to reflect the environmental conditions present in the study area. *U*. *concentricus* have a preference for non-polluted environments, mainly living on leaves or stems of aquatic plants and decomposition leaves. This species prefers lentic environments or low current, and is often found on stones in streams (Lanzer, 1996; Santos, 2003). Individuals of the genus *Pisidium* (Studer, 1820) occupy various types of substrate, with sediments and macrophytes (Pfeifer & Pitoni, 2003), but with preference for granulometry sandy with silt (Mansur & Pereira, 2006; Pereira et. al., 2011).

B. *tenagophila* was well distributed in the study area and is one of the most common planorbids in wetlands of Rio Grande do Sul. The species is associated with aquatic macrophytes (Pereira et al., 2000a; 2000b; 2011; Pfeifer & Pitoni, 2003) in wetlands, shallow areas of lakes, dams, irrigation canals and in calm waters on the shores of small streams and rivers in sandy substrate (Tuan, 2009), these features were similar to those found in this study. Another important characteristic of this species is that it survives long drying time and is thus of advantage in dry periods, being found in two sites intermittently of Lajeado Grande microbasin.

P. *canaliculata* was not a dominant species in the streams studied, but was common. This species occurs in the sandy sediment and in aquatic macrophytes from lentic and lotic ecosystems in the Rio Grande do Sul (Pereira et al., 2000a; 2000b). In streams, it has a habit of staying immovable on stones or in association with macrophytes and especially in periods of dry season, remains buried in the sediment (Estebenet & Martín, 2002).

The Shannon-Wiener index (H ') for the study area as a whole, as well as most of the microbasins was low, indicating high variation in the proportional abundance of species. A study conducted at Touro Passo River, which used the same sampling method adopted here (Martello et al., 2006), values of the Shannon-Wiener index found for biocenosis mollusks ranged from 0.07 to 0.137. These values are similar to those found in this study, showing a possible tendency of low diversity of mollusks in the streams of the State. In general, the malacofauna found in the study area can be considered typical of streams; it presents low diversity of Unionoida, which is usually found in rivers of higher order (Strayer, 1999). In Rio Grande do Sul, these bivalves are abundant and diversified in segments of high order, such as Jacuí River (Pfeifer & Pitoni 2003), Sinos River (Mansur & Pereira 2006) and Uruguay River (Castillo et al*.*, 2007).

In this study were present in greater numbers in the main channel of the Ibicuí River (additional material), where are found living specimens of *C*. *inflata*, *A*. *patagonicus*, *A*. *trapezeus*, *F*. *fossiculifera* and *M*. *corrientesensis*. In the microbasins, the Unionoida was not significant and was collected specimens of the *A*.

trapesialis, A. *trapezeus* and *D*. *delodontus* (Table 2) in stretches of 3rd and 4th orders of Santo Antônio and Inhacundá-Caraí Passo microbasins. The richness of mollusks communities depends on several environmental factors related to the type and variety of substrates, providing an increase in record of species (Pfeifer & Pitoni, 2003; Copatti et al., 2010). Frequently rivers or plateaus located in in slope areas have best preserved riparian vegetation, water velocity faster, better oxygenated water, substrate consisting of coarser sediment, and lower temperature than the lowland of rivers (Martel et al., 2007; Maloney & Weller, 2011).

In the study area, the Santo Antônio microbasin, located in the lowland area, showed the highest abundance and richness of mollusks, with seven families and 11 species, differentiating itself from others by having greater diversity of macrophytes. Only the sites of 4th order, with exclusively sandy substrate showed no macrophytes. Most Unionoidea was found in this microbasin, possibly by the dominance of sand in the substrate, which facilitates the establishment of these mollusks. Due to the sedentary habit, these mollusks live mainly buried in muddy or sandy substrates (Baldigo et al., 2002; Vaughn et al., 2004). The microbasin Lajeado Grande, which is also located on the plain, with mainly sandy substrate, had the second highest richness. However, in general, the four microbasins shared dominant species *U*. *concentricus* and *P*. *dorbignyi*. In the Santo Antônio microbasin, *P. canaliculata* was also dominant. These species have a preference for sites with presence of macrophytes. Moreover, *H*. *moricandi*, *A*. *trapesialis*, *A*. *trapezeus* and *C*. *fluminea* were unique to the microbasin. *H*. *moricandi* prefer environments with water current and presence of macrophytes (Santos, 2003). Anodontites species exhibit a preference for sandy substrate and are very rarely found among stones and gravels (Machado et al., 2008). *C*. *fluminea* similarly has a preference for sandy substrate (Viana & Avelar, 2010).

The lowest richness presented by Taquari-Miracatu microbasin can be attributed to the absence of macrophytes in all locations sampled. *P*. *dorbignyi* and *U*. *concentricus* occurred in great abundance in this microbasin. The substrate in this microbasin was predominantly a mixture of sand and gravel, often used by these species, although *P. dorbignyi* occurs very frequently associated with macrophytes. In this microbasin predominated riparian forest in the areas sampled, and may be a determining factor in the colonization of these species. Some studies show the importance of the relationship of riparian forest with the composition of macroinvertebrate communities (Rezende, 2007; Hoverman et al., 2011), however, for mollusks, knowledge is still scarce. According to the 'River Continuum Concept' segments of medium order have a higher environmental variability, including greater diversity of food resources and a daily variation in a wider temperature. Therefore, sections of mean size would provide the establishment of a wider variety of organisms and also determine a change in the proportion of functional groups. Predators, collectors and scrapers are more frequent in small orders (1st and 2nd), the last being dominant in these sections. In medium-sized orders (3rd and 4th), most commonly occurring predators and collectors, the latter being predominant.

The sets of sites of 3rd and 4th order showed greater richness (nine and seven species, respectively) than sites from 1st and 2nd order (six species each). The set of 3rd order segments, where the greatest richness was found, showed the greatest diversity of substrate in relation to the granulometry and macrophytes. Rare species *A. trapezeus* and *H. moricandi* were found only in the 4th and 3rd orders, respectively. Increase in the number of species of mollusks, from upstream to downstream was also observed for bivalves in the Ayui Grande stream and the Sinos River (Mansur & Pereira, 2006), and gastropods in the Camaquã River (Pereira al., 2000a), and gastropods and bivalves in the Velhaco River (Pereira et al., 2011). However, the abundance of species was lower in sets of 3rd and 4th orders. *U. concentricus* and *P. dorbignyi* that were the most abundant in the study area occurred in the four sets of orders, but *B. tenagophila* and *P. canaliculata*, were more abundant in sites of the 1st and 2nd orders, explaining the decrease in abundance in the other sets. In general, middle orders segments provide the occurrence of a greater variety of habitats than those of small orders, mainly providing conditions favorable for establishing groups of endofauna and filterers, such as bivalves. Different microbasins may also have differences in their landscapes regarding geology, environmental preservation and land use, for example, and also between the macroinvertebrate communities (Paavola et al., 2000; Salvarrey et al., 2014). However, in the present study there was no influence of landscape structure order and microbasin in communities of mollusks. Microbasins and orders tended to share the dominant species.

The absence of influence of landscape in question on the community structure of mollusks can be related on the one hand, the lack of marked environmental differences, important for the species of mollusks found among the studied microbasins and orders. Although it has found a higher presence of macrophytes and fine sediments in the microbasin Santo Antonio, in places of 3rd and 4th orders, these differences were not sufficiently intense between the analyzed factors for the occurrence of differences in community structure. Moreover, the species, especially the most abundant, as *P. dorbigniy*, *U. concentricus*, *P. canaliculat*a and *B. tenagophila*, appear to be tolerant of some variation in local-scale factors, which may vary with change orders and microbasins, such as granulometry, presence of macrophytes and hydraulic condition (Kotzian & Simões, 2006). In addition, the bivalves, and especially Unionoida, which are responsible for much of the diversity of this group, occur especially in habitats with lower current and higher orders (Strayer, 1999). The Unionoidea were quite rare in the study area. In southern Brazil, are more diverse in streams of higher order (Mansur & Pereira, 2006; Castillo et. al., 2007), and are associated with granulometry consisting of a mixture of sand and mud (Cummins & Bogan, 2006). The size of their shells in relation to other mollusks, also involves the necessity of larger areas for survival. Thus, microbasins with lower orders would not be appropriate for the occurrence of Unionoida.

The influence of the substrate and the presence of macrophytes on the communities of mollusks in southern Brazil is well known (Pereira et al., 2000a). However, in the study area, only the granulometry influenced the composition of communities, but not the abundance of its species. In general, in southern Brazil, communities formed by the simultaneous presence of *D. delodontus*, *A. trapesialis* and *A. trapezeus* can be found in streams (Mansur et al., 1994; Pereira et al., 2000b). In sandy substrates were *C. fluminea* and species of *Pisidium*, gravelly substrates are common species of Ancylidae and *P. canaliculata* (Pereira et al., 2000b; Santos, 2003; Avelar, 2010).

In the three groups similarity analysis based on species composition was predominantly sandy substrate. In the larger group was found only *U. concentricus* suggesting a preference for environments with lower current and presence of macrophytes (Pereira et al., 2000a). The group formed by sites LG1b, LG3a and TM2a also had grain size predominantly sandy, however the species composition at these sites was characterized by the occurrence of *P. dorbignyi*, which is preferred for this type of substrate. The sites SA4a and SA4b contain only sandy substrate, and the species share *A*. *trapesialis* and *C*. *fluminea*. The species found at these two sites also showed preference for substrates less coarse. Macrophytes are usually an important factor in local scale community composition of mollusks (Rios & Bailey, 2006). In Rio Grande do Sul, in general, Pisidiidae and Ancylidae species, as well as *B. tenagophila* are associated with the presence of macrophytes (Santos, 2003; Martello et al., 2008). For mollusks of the streams of the middle Ibicuí River, riparian vegetation and hydroperiod have tended to influence the communities. The importance of the presence of riparian vegetation and macroinvertebrate community composition is well known mainly for Insecta. The presence of riparian vegetation avoids silting, reduces autotrophic production by shading, affecting the growth of periphyton, and contributes large amounts of allochthonous detritus (Giuliatti & Carvalho, 2009). Adding to this, the management of riparian vegetation influences the habitats characteristics and in sites of the lowest order; their influence tends to be stronger (Ormerod et al., 1993; Callisto et al., 2001).

The intermittency or hydroperiod affects the macroinvertebrate community, as they respond to seasonal factors and the successional stage of the ecosystem leading to wide variations in population densities. Rainy periods generate an increase in current velocity, and species can be loaded either passively by the current, as generated by changes in the sediment, and dry periods may favor the permanence of species more tolerant to desiccation (Pimpão, 2007; França et al., 2007; Tuan, 2009). The absence of close correlation between abiotic factors and abundance of species proportion of communities may be related to ecological preferences of each species. Thus, although many species may occur at certain locations, as discussed above, it is possible to establish their populations requires the predominance of certain conditions, or a set of conditions not found in the study area or not discussed here.

5 CONCLUSION

Although landscape factors like stream order and microbasins can influence macroinvertebrate communities, this pattern was only partially observed for freshwater mollusks in the middle Ibicuí River basin. Only species richness was affected, with higher richness in 3rd and 4th order streams due to greater habitat diversity, mainly related to granulometry and the presence of macrophytes. Differences in richness between the Santo Antônio (sandier) and Lageado Grande (gravelly) microbasins were driven by geological differences. However, species composition and abundance did not vary with stream order or microbasin, indicating high tolerance to these factors. At a local scale, grain size and macrophyte presence influenced species composition but not abundance, reinforcing the role of habitat structure in mollusk distribution while highlighting their tolerance to substrate variations.

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