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Landscape and habitat characteristics associated with fish occurrence and richness in southern Brazil palustrine wetland systems

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Abstract We investigated the influence of environmental factors in fish communities of 146 palustrine wetlands, covering a wide range of altitude and wetland surface area in Neotropical region. Two questions were analyzed: (1) Are wetland altitude, area, habitat diversity, hydroperiod (permanent and intermittent), ecoregion, and macroinvertebrate richness good predictors of occurrence, richness, abundance and composition of fish species? and (2) Are the predictors of fish richness similarly applicable to different ecoregions in Southern Brazil? Our data showed that fish richness was related to habitat diversity and macroinvertebrate richness, and fish occurrence was influenced by wetland area and macroinvertebrate richness. Fish abundance was influenced by altitude, hydroperiod and macroinvertebrate richness, and the fish composition was jointly associated with ecoregion, and hydroperiod. The predictors of fish richness were not similarly applicable to different ecoregions. Our results showed that the habitat diversity, macroinvertebrate richness, altitude and hydroperiod were the environmental predictors that potentially structure and maintain the fish occurrence and richness in southern Brazil palustrine wetlands. Such information is essential to develop wetland conservation and management programs in this region, where more than 90 % of wetland systems have already been lost and the remaining ones are still at high risk due to the anthropogenic activities.

Keywords South American fishes · Ecoregions · Ichthyofauna · Biodiversity conservation

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Introduction

Understanding patterns and factors that influence species occurrence, richness and distribution are among the main goals of community ecology and conservation biology (Ricklefs 1987; Zimmerman and Simberloff 1996). Area is a core component of modern explanations for the presence and absence of species across a landscape. The relationship between area and species richness predicts that larger areas harbor more species than smaller ones (Arrhenius 1921; Rosenzweig 1995). The positive relationship between area and species richness can be considered a valid generalization to freshwater fishes (Ricklefs and Lovette 1999; Zhao et al. 2006; Latta et al. 2008; Lubinski et al. 2008; Kruk et

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al. 2009), which includes the coastal freshwater wetlands of southern Brazil (Maltchik et al. 2010a).

Several hypotheses have been proposed to explain the positive relationship between area and species richness (Neigel 2003). The habitat heterogeneity hypothesis suggests that as the sampled area increases, the richness of microhabitats rises, incorporating more species to the assemblages (Andren 1996). In addition to area and habitat diversity, other factors such as altitude, hydroperiod, and availability of food resources also are recognized as determinants of fish assemblage structure (Angermeier and Schlosser 1989; Abell et al. 2008; Lévêque et al. 2008; Pinto et al. 2009). Ruetz et al. (2005) indicated that seasonal drought shapes spatio-temporal patterns of wetland fish populations, since many aquatic species do not have adaptations for tolerating long dry periods (Williams 2006).

In a broad spatial scale, freshwater ecoregions are defined as large areas encompassing one or more freshwater systems with a distinct assemblage of natural freshwater communities and species (primarily fishes) (Abell et al. 2008). In this sense, freshwater ecoregions capture the broad patterns of fish species associated to ecological and evolutionary processes generated primarily by continental (mountain building, speciation and glaciation) and regional scale filters (broad climatic and physiographic patterns, and regional catchments). Understanding the effects of landscape and habitat on fish species richness and distribution have important conservation implications since ecological patterns observed within a local community might be considerably different from those found over broader areas such as landscapes or regions (Angermeier and Schlosser 1989).

Wetlands are vanishing from many landscapes and becoming smaller in area due to the human occupation (Gibbs 2000). Almost half of the wetlands of the world disappeared in the last century due to agricultural and urban development (Shine and Klemm 1999). In Southern Brazil, many of the wetlands have been drained, leaving behind fewer, more isolated wetland fragments in an agricultural landscape (Maltchik 2003). This can be expected to affect Neotropical freshwater fishes – the most diversified freshwater fish fauna of the world (Reis et al. 2003) – especially fish species which are more sensitive to environmental variations due to relatively short life spans. An important fraction of this diversity (including endemic and threatened species) is exclusive to wetland systems (Costa 2002, 2008; Volcan et al. 2009, 2010) and it is concentrated in Brazil (Buckup et al. 2007). In this sense, understanding fish species composition and richness patterns in fragmented and natural wetlands is a priority for developing biodiversity conservation strategies in this region.

The biodiversity patterns in fragmented wetlands were analyzed in several communities in Southern Brazil, e.g. macrophytes (Rolon and Maltchik 2006; Rolon et al. 2008), macroinvertebrates (Stenert and Maltchik 2007; Stenert et al. 2008) and waterbirds (Guadagnin et al. 2005, 2009; Guadagnin and Maltchik 2007). However, the effects of the landscape fragmentation on fish communities in Southern Brazil wetlands are still little known (Fernandes et al. 2009; Maltchik et al. 2010a). In this study, we investigated 146 palustrine wetlands over an extensive area of the Neotropical region (~280,000 km^2, Southern Brazil), covering a wide gradient of altitude and wetland surface areas. Two questions were analyzed: (1) Are wetland altitude, area, habitat diversity, hydroperiod (permanent and intermittent), ecoregion, and macroinvertebrate richness good predictors of occurrence, richness, abundance and composition of fish species? and (2) Are the predictors of fish richness similarly applicable to different ecoregions in Southern Brazil?

Materials and methods

Study area

The state of Rio Grande do Sul is located in Southern Brazil and has an area of 282,184 km^2 (Fig. 1). The Moist Subtropical Mid-Latitude Climate prevails in this region; the altitude ranges from sea level to 1,200 m (NE). The annual precipitation varies between 1,200 and 1,800 mm, being relatively well distributed without the existence of a dry period (Köppen 1931). The mean temperature varies between 12 °C, in winter, and 26 °C, in summer (Radambrasil 1986). The vegetation is characterized by small fragments of forest, and temperate and tropical grassland areas. The forest is represented by three major types: temperate summer-green and mixed ever-green deciduous forests, and temperate mountainous coniferous forest. The grasslands are represented by savanna, steppe, and pioneering formations (Radambrasil 1986; Rambo 2000).

Rio Grande do Sul has approximately 3,441 wetlands, with a total inundation area of approximately 30,332 km^2

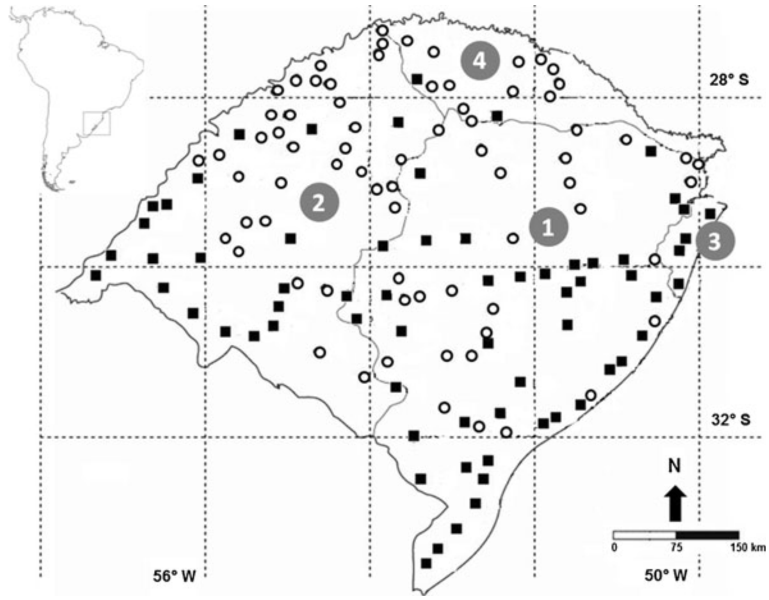

Fig. 1 Wetlands sampled in southern Brazil, distributed in freshwater ecoregions according Abell et al. (2008). Occurrence of fishes (*black square*); No records of fish (*white circle*). 1-Laguna dos Patos; 2-Lower Uruguay; 3-Tramandaí-Mampituba; 4-Upper Uruguay

(10.7 % of the total area of the state) (Maltchik 2003). Approximately 72 % of the wetlands have an area smaller than 1 km^2 (Maltchik 2003). The study area is composed by four ecoregions: Laguna dos Patos, Lower Uruguay, Upper Uruguay and Tramandaí-Mampituba (Abell et al. 2008). Delineating ecoregions required compiling and synthesizing information on the distribution of fish species. The freshwater species, dynamics, and environmental conditions within a given ecoregion are more similar to each other than to those of surrounding ecoregions. The criteria applied to the ecoregion delineation in the study area was based on the major habitat types (temperate, tropical and subtropical coastal rivers, and tropical and subtropical upland rivers), and fish species lists (Abell et al. 2008). The higher number of sampled wetlands was located in Laguna dos Patos and Lower Uruguay since these two ecoregions cover approximately 90 % of study area (Fig. 1).

Data collection

A total of 146 palustrine wetlands distributed over 250,000 km^2 were selected from topographic maps based on two criteria: (1) area smaller than 10 ha; and (2) fairly even distribution of the wetlands across Southern Brazil (Fig. 1). Palustrine wetlands include marshes, bogs, fen, wet meadows and seasonally wet woods (Tiner 1999). The water depth of palustrine wetlands studied was low (shallow wetlands) and its hydrology was affected mainly by precipitation, surface water runoff, groundwater discharge, and inundation from small streams and lakes. Terrestrial ecosystems have a great influence on the studied palustrine wetlands. Sampling was performed initially in the eastern portion of the state of Rio Grande do Sul, and then moved towards the western portion. Each wetland was sampled once from March to October 2002 always during the period with surface water. Since each wetland was sampled once from March to October (8 months), the influence of the sampling period (seasonal variations) on fish occurrence and richness was analysed to identify if our results reflect the effects of studied variables rather than sampling seasonality.

Wetland area was measured in the field. Wetland boundaries were determined based on (1) visual observations of the watermarks, drift line and/or owners' information, and (2) vegetation indicators (e.g. plants with morphological, physiological or reproductive adaptations to prolonged saturation/inundation, and the proportion between aquatic and terrestrial species in the plant community). Wetland location and altitude were determined using a GPS satellite receiver in the field (Garmin, GPS III Plus, Hsin Tien, Taiwan). The wetlands surveyed were categorized according the degree of water permanence (permanent or intermittent – henceforth "hydroperiod"). Permanent wetlands retain water for the entire hydrological cycle, whereas intermittent wetlands eventually dry up, retaining water for at least 4 months of the year (Maltchik et al.

2004). Information about hydroperiod was obtained with the landowners.

Habitat diversity in each wetland was quantified by counting the number of distinct habitats of dominant vegetation. The dominant vegetation was investigated in the field, and separated into five habitat types: woody vegetation (tree/shrub), floating-leaf stands, *Eryngium* spp. Stands, emergent vegetation, and submerged vegetation. The habitat diversity was the cumulative number of vegetation and open water habitats in the wetland systems. The minimum size to consider a habitat was 10 % of the total area of each wetland.

A multi-habitat method was used to sample major habitats in proportional representation within a wetland (adapted from EPA, Barbour et al. 1999). Fishes and macroinvertebrates were collected systematically from all available wetland habitats (water depths of less than 50 cm) with a dip net (D-shaped, 30 cm width, 400 μm mesh) by kicking up the substrate and then sweeping above the disturbed area to capture dislodged or escaping fishes and macroinvertebrates (Nielsen and Johnson 1983; Plafkin et al. 1989; Rosenberg et al. 1997). Mesh size was small enough to retain small and juvenile fishes and macroinvertebrate, and large enough to prevent excessive clogging by fine sediments (Rosenberg et al. 1997; Batzer et al. 2001). The sampling method used is efficient to capture fishes in southern Brazil coastal wetlands since the studied palustrine wetlands had reduced surface area, high densities of macrophytes, and low water depths (Maltchik et al. 2010a). Although the time required for fish and macroinvertebrate sampling varied from 10 min in the wetlands with no more than 1 ha to 60 min in wetlands of 10 ha, the sampling effort was the same for all wetlands, represented by 25 sweeps of 1-m over all habitat types. For example, if the habitat types in the sampling wetland were 20 % floating-leaf stands and 80 % emergent vegetation, then 20 % or 5 sweeps were completed in floating-leaf stands, and 80 % or 20 sweeps were completed from emergent habitats. We decided to use the same effort due to the broad scale of our study (± 250,000 km^2). The large scale of this study did not allow for temporal sampling. Sweeps were pooled into one sample per wetland (3.5-L plastic bucket), and the organisms were euthanized with lethal dose of phenoxyethanol, and preserved in situ with 10 % formaldehyde.

In the laboratory, each sample was washed through a 400 μm sieve and leaves, stems, and other debris were removed. Macroinvertebrate were separated from fish and were preserved with 80 % ethanol. Macroinvertebrates were identified under 7× magnification, according to Lopretto and Tell (1995), Merritt and Cummins (1996), and Fernández and Domínguez (2001). In most cases, macroinvertebrates were identified to family level. Macroinvertebrate richness corresponded to the number of families collected in each sampled wetland. Chironomids, hyalellids, and molluscs were identified to genus level, and the ostracods were identified to species level.

Fishes were identified to the lowest taxonomic level possible according to Reis et al. (2003). Voucher specimens were deposited at the reference collection of Laboratório de Ecologia e Conservação de Ecossistemas Aquáticos (LECEA) - UNISINOS and Museu de Ciência e Tecnologia da Pontíficia Universidade Católica do Rio Grande do Sul (MCT/PUCRS).

Data analyses

The influence of the sampling period (seasonal variations) on fish occurrence and richness was analysed to identify if our results reflect the effects of studied variables rather than sampling seasonality. So, two partial mantel correlation tests were used to verify the correlation of faunal dissimilarity with: 1) space, controlling sampling period correlation; and 2) sampling period, controlling geographical correlation. Three distance matrices were constructed: A) Faunal distance matrix based on species abundance (dependent variable); B) Time distance between samples, based on months in which each wetland was sampled once (predictor variable), and C) Geographic distance matrix based on latitude and longitude coordinates (predictor variable). While the faunal matrix was constructed using Bray-Curtis distance, the time and geographic matrices were constructed using Euclidean distance. For each partial correlation (A x B (C) and A x C (B)), a Mantel test using the Pearson rank correlation method was used to answer the following question: Do wetlands that are close together or sampled in similar months exhibit similar faunal composition?? The analyses were performed using R statistical program version 2.9.0 (R Development Core Team 2009). The significance of correlations was tested by permutations (9999 permutations).

Fish richness and abundance per wetland were estimated as the total number of species and the number of individuals in each wetland. Frequency of occurrence was calculated as the number of sites where a given species occurred divided by the total number of sites where fish occurred (n=71). The environmental variables analyzed were: area, altitude, habitat diversity, macroinvertebrate richness, ecoregion and hydroperiod. The correlation between the environmental variables was tested using the Pearson's correlation coefficient. The relationship between fish occurrence and environmental variables was tested by logistic regression using all wetlands surveyed (n=146). The influence of environmental variables on the fish richness and abundance was verified by General Linear Models using only wetlands with fish presence (n=71). The influence of environmental variables on fish richness was tested in a separate analysis for each ecoregion by General Linear Models; Laguna dos Patos (n=42) and Lower Uruguay (n=23). The best fit models of logistic and linear regressions were selected by AIC criteria (Akaike 1974) and by selection method of both directions. The value of fish abundance was square root transformed and wetland area was log-transformed.

A Non-Metric Multidimensional Scaling (NMDS) was used to assess the variation of abundance of fish species among wetlands of the two main ecoregions (Laguna dos Patos and Lower Uruguay). The analysis was performed with the Bray-Curtis dissimilarity index using two axes in the R statistical program version 2.9.0 (R Development Core Team 2009). Only species occurring in more than two collections were included in the analysis and the abundance was square root transformed. Wetlands with only one species were considered outliers and were removed from the ordination analysis. The NMDS is used to graphically represent the similarity in species composition in multiple dimensions. NMDS is unconstrained by environmental variables so the ordination of sites is driven only by species composition. Then, the environmental variables (altitude, area, habitat diversity, macroinvertebrate richness, ecoregion, and hydroperiod) were fitted to the ordination by *envfit* function of vegan package (Oksanen et al. 2009) in the R statistical program version 2.9.0 (R Development Core Team 2009).

The Permutation Multivariate Analysis of Variance was used to compare differences in fish species composition between the most representative ecoregions (Laguna dos Patos and Lower Uruguay), hydroperiod (permanent and intermittent) using vegan package (Oksanen et al. 2009) in R statistical program version 2.9.0 (R Development Core Team 2009). The Permutation Multivariate Analysis of Variance was an useful tool for the analysis and partitioning of sums of squares of a multivariate data set using a distance matrix (metric or semimetric) and permutations for the hypothesis test. To assess differences in fish composition the Bray-Curtis distance matrix was used with 499 permutations.

An Indicator Species Analysis (Dufrene and Legendre 1997) was performed to determine which fish species discriminated the main ecoregions (Laguna dos Patos and Lower Uruguay), and hydroperiod (permanent and intermittent). The analysis was performed using labdsv package (Roberts 2007) in R statistical program version 2.9.0 (R Development Core Team 2009) and the significance of the discriminating power (< 0.05) was obtained by 9999 permutations.

Results

A total of 1,633 individuals distributed among 5 orders, 13 families and 42 species were collected (Table 1). Characiformes was the most diverse order with 20 species, followed by Cyprinodontiformes (9 species). Siluriformes represented 7 species, Labriformes, 5; and Synbranchiformes, only 1 species. The most diverse family was Characidae with 13 species. Cichlidae had 5 species and Callichthyidae, Poeciliidae and Rivulidae had 4 species each. Several families were represented by only one species: Erythrinidae (*Hoplias* aff. *malabaricus*), Lebiasinidae (*Pyrrhulina autralis*), Heptapteridae (*Rhamdia* aff. *quelen*), Synbranchidae (*Synbranchus marmoratus*) and Anablepidae (*Jenynsia multidentata*).

Poeciliidae was the family that presented the highest number of individuals (67.05 %), followed by Characidae (22.71 %), Rivulidae (2.75 %), Crenuchidae (2.51 %), Cichlidae (1.4 %) and Loricariidae (1.1 %). The remaining families represented 2.48 % of the total individuals collected. *Cnesterodon brevirostratus* (31.9 %), *Phalloceros caudimaculatus* (21.86 %) and *Cnesterodon decemmaculatus* (13.22 %) were the most abundant species representing approximately 66 % of the fish individuals collected. Some species were represented only by a single individual: *Cyphocharax voga, Characidium* sp., *Characidium* aff. *zebra, Cnesterodon* sp., and *Laetacara dorsigera* (Table 1).

Fishes occurred in 71 of the 146 wetlands surveyed. Fish richness ranged from 1 to 8 species per wetland.

Table 1 Distribution (Ecoregion), abundance (n), dominance (percentage-P(n)%), and frequence index (percentage-P(F)%), of the fish species captured in 71 wetlands of southern Brazil. 1-Laguna dos Patos; 2-Lower Uruguay; 3-Tramandaí-Mampituba; 4-Upper Uruguay

Taxa	Ecoregion	Abundance	P(n)%	P(F)%
Order Characiformes				
Family Curimatidae				
Cyphocharax voga (Hensel, 1869)	2	1	0,06	2,38
Steidachnerina brevipinna (Eigenmann & Eigenmann, 1889)	2	6	0,37	7,14
Family Crenuchidae				
Characidium rachovii Regan, 1913	1,2	39	2,39	26,19
Characidium aff. *zebra* Eigenmann, 1909	1	1	0,06	2,38
Characidium spp.	2	1	0,06	2,38
Family Characidae				
Aphyocharax anisitsi Eigenman & Kennedy, 1903	2	7	0,43	11,90
Astyanax cf. *eigenmanniorum* (Cope, 1894)	1,2	24	1,47	16,67
Astyanax jacuhiensis Cope, 1894	1	4	0,24	9,52
Astyanax sp.	3	2	0,12	2,38
Charax stenopterus (Cope, 1894)	1,2	2	0,12	4,76
Cheirodon ibicuhiensis (Eigenmann, 1915)	2	11	0,67	7,14
Cheirodon interruptus (Jenyns,1842)	1,2	95	5,82	47,62
Hyphessobrycon igneus Miquelarena, Menni, López & Casciotta, 1980	1	74	4,53	26,19
Hyphessobrycon boulengeri (Eigenmann, 1907)	1	9	0,55	7,14
Hyphessobrycon luetkenii (Boulenger, 1887)	1,2	102	6,25	52,38
Hyphessobrycon meridionalis Ringuelet, Miquelarena & Menni, 1978	2	2	0,12	4,76
Pseudocorynopoma doriae Perugia, 1891	2	4	0,24	2,38
Serrapinnus calliurus (Boulenger, 1900)	1,2	35	2,14	11,90
Family Erythrinidae				
Hoplias aff. *malabaricus* (Bloch, 1794)	1,2	4	0,24	9,52
Family Lebiasinidae				
Pyrrhulina australis Eigenmann & Kennedy, 1903	1,2	3	0,18	7,14
Order Siluriformes				
Family Heptapteridae				
Rhamdia aff. *quelen* (Quoy & Gaimard, 1824)	1	3	0,18	4,76
Family Callichthyidae				
Callichthys callichthys (Linnaeus, 1758)	1,2	3	0,18	7,14
Corydoras paleatus (Jenyns, 1842)	1,2	3	0,18	4,76
Corydoras undulatus Regan, 1912	2	3	0,18	7,14
Hoplosternum littorale (Hancock, 1828)	1	6	0,37	2,38
Family Loricariidae				
Hisonotus charrua Almiron & Azpelicueta, 2006	2	13	0,80	2,38
Otocinclus arnoldi (Regan, 1909)	2	5	0,31	2,38
Order Synbrachiformes				
Family Synbranchidae				
Synbranchus marmoratus Bloch, 1795	1	3	0,18	4,76
Order Cyprinodontiformes				
Family Anablepidae				
Jenynsia multidentata (Jenyns, 1842)	1	5	0,31	7,14

Table 1 (continued)

Taxa	Ecoregion	Abundance	P(n)%	P(F)%
Family Rivulidae				
Austrolebias periodicus (Costa, 1999)	2	4	0,24	2,38
Cynopoecilus fulgens (Costa 2002)	1	11	0,67	2,38
Cynopoecilus melanotaenia (Regan, 1912)	1	5	0,31	7,14
Cynopoecilus nigrovittatus (Costa 2002)	1	25	1,53	7,14
Family Poeciliidae				
Cnesterodon brevirostratus Rosa & Costa, 1983	1,2,4	521	31,90	11,90
Cnesterodon decemaculatus (Jenyns, 1842)	1,2	216	13,23	23,81
Cnesterodon sp.	1	1	0,06	2,38
Phalloceros caudimaculatus (Hensel, 1868)	1,2,3,4	357	21,86	69,05
Order Labriformes				
Family Cichlidae				
Apistogramma borellii (Regan, 1906)	2	5	0,31	2,38
Cichlasoma dimerus (Heckel, 1840)	2	6	0,37	7,14
Cichlasoma portalegrensis (Hensel, 1870)	1	9	0,55	11,90
Crenicichla lepidota (Heckel, 1840)	2,3	2	0,12	4,76
Laetacara dorsigera (Heckel, 1840)	2	1	0,06	2,38
Total		1633	100	

The most widespread species were *Phalloceros caudimaculatus* (69 % of wetlands), *Hyphessobrycon luetkenii* (52.4 %), *Cheirodon interrupts* (47.6 %), *Hyphessobrycon igneus* (26.2 %), *Characidium rachovi* (26.2 %), *Cnesterodon decemmaculatus* (23.2 %) and *Astyanax* cf. *eigenmanniorum* (16.7 %). Fish species composition matrix was correlated with geographic distance matrix controlling time distance matrix (r=0.1253, P=0.002), but fish composition was not correlated with time controlling geographic distance matrix (r=0.049, P=0.125). This result showed that the geographical distribution patterns of fish species in southern Brazil wetlands were not influenced by the sampling period.

Wetland area was negatively correlated with altitude (P=0.005), but it was not correlated with habitat diversity (P=0.301). The other environmental variables were not correlated (P>0.05) (Table 2). Fish occurrence was determined by altitude, area and macroinvertebrate richness (R^2=0.383; P=0.001). Fish occurrence was positively related to wetland area and macroinvertebrate richness (z=1.580, z=2.800, respectively), and altitude was negatively related to fish occurrence (z=−3.755). In wetlands with fish occurrence, habitat diversity, macroinvertebrate richness and altitude explained 13.1 % of the variation in fish richness (F=4.524; P=0.005). Fish richness was positively associated with habitat diversity and macroinvertebrate richness (z=1.746, z=2.106, respectively), and negatively associated with altitude (z= −2.272). In the Lower Uruguay ecoregion, fish richness was positively influenced by wetland area (z=1.068) and habitat diversity (z=0.943) and it was negatively related to altitude (z=−0.007) (F=4.443; R^2_{adj}=0.32; P=0.016). In the Laguna dos Patos ecoregion, none of the environmental variables studied was associated with fish richness.

Fish abundance was influenced by altitude, hydroperiod and macroinvertebrate richness (F=4.254; R^2_{adj}=0.122; P=0.008). Fish abundance was higher in

Table 2 Pearson's correlation coefficients between environmental variables of studied wetlands in southern Brazil

	Altitude	Habitat diversity	Macroinvertebrates
Area (Log)	−0.341**	0.13	−0.016
Altitude	–	−0.187	0.039
Habitat diversity		–	−0.012

Values marked with asterisks are significantly different at the P<0.01(**) levels permanent than in intermittent wetlands and it was positively related to altitude (z=2.093) and macroinvertebrate richness (z=1.676). Fish assemblage similarity among wetlands was represented by two axes of NMDS ordination (stress=0.2188). The two axes of NMDS ordination were related to macroinvertebrate richness, ecoregion and hydroperiod (Fig. 2). Macroinvertebrate richness was positively correlated to first axis and negatively related to second axis (R^2=0.1095; P=0.041). While wetlands of Laguna dos Patos ecoregion were related to negative scores in first NMDS axis and with positive scores in second NMDS axis, wetlands of Lower Uruguay ecoregion were positively related to first axis scores and negatively to the second axis scores (R^2=0.0587; P=0.037). Intermittent wetlands were associated with positive scores of both NMDS axes and permanent wetlands were related to negative scores of both NMDS axes (R^2=0.0678; P=0.017).

Species composition changed between ecoregions (F=1.741; R^2=0.032; P=0.032) and hydroperiod (F=2.054; R^2=0.037; P=0.024). Indicator species analysis demonstrated that *Hyphessobrycon igneus* was associated with Laguna dos Patos ecoregion, and *Aphyocharax anisitsi*, *Serrapinnus calliurus*, *Steindachnerina brevipinna*, *Corydoras undulatus* and *Cichlasoma dimerus* were associated with Lower Uruguay ecoregion (P<0.05).

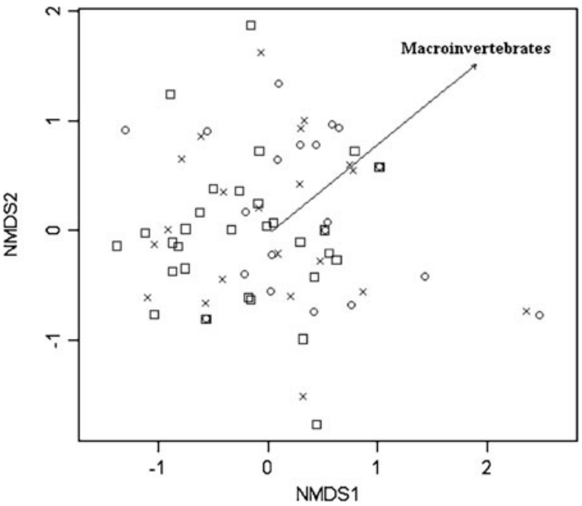

Fig. 2 Results from non-metric multidimensional scaling analysis (NMDS) of fish community dissimilarities in wetlands of southern Brazil, related with ecoregions and macroinvertebrate diversity. Lower Uruguay Ecoregion (*white circle*), Laguna dos Patos Ecoregion (*white square*), Species (×)

Discussion

Fish community structure

One of the main characteristics of the Neotropical fish fauna is the high dominance of the orders Characiformes and Siluriformes (Lévêque et al. 2008). Our results showed a different pattern from the expected. We observed a high richness and abundance of Cyprinodontiformes. This result may be related to the characteristics of the habitats studied – small wetlands (reduced area), high density of macrophytes, low water depth, susceptibility to water stress, and lack of sampling in streams and rivers. Such characteristics select species, especially from the families Poeciliidae and Rivulidae (Kruk et al. 2009; Maltchik et al. 2010a).

Fish response to wetland area and habitat diversity

Our results showed that while wetland area did not influence the fish richness in Southern Brazil and in Laguna dos Patos ecoregion, wetland area influenced the fish occurrence. Recent studies carried out in southern Brazil showed that despite the significant fish species-area relationship (Maltchik et al. 2010a), the slope of the log species/log area regression line was low (0.18) when compared with other studies (Eckmann 1995). Connor and McCoy (1979) reported that slopes lower than 0.2 (Preston 1960) may result from sampling of non-isolated areas. Zhao et al. (2006) claimed that the low relationship between lake area and fish richness was a consequence of human activities related to rice plantations. According to those authors, patterns of inundation and connectivity across rice fields and natural wetlands may have had an important impact on species-area relationships, enabling smaller wetlands to support a similar number of species compared to medium and even larger wetlands. The lack of relationship among fish richness and wetland area in our study may be the result of high concentration of rice fields in Southern Brazil, mainly in one of the studied ecoregions (Laguna dos Patos). Furthermore, the flat landscape and the low altitude of most of the Laguna dos Patos ecoregion favor the water exchange between natural and man-made wetlands, mainly after periods of heavy rain.

Area and habitat diversity are important attributes associated with species richness. However, it is difficult to assess the independent effect of each variable on richness because they are highly correlated (Ricklefs and Lovette 1999). Even though some studies suggested that the species-area relationship could result from an increase in habitat diversity with area (Williams 1964), this relationship has not been effectively studied in Southern Brazil wetlands. In our study, wetland area and habitat diversity were not correlated and habitat diversity was associated with fish richness. The habitat parameters chosen to quantify habitat diversity in our study play an important role in structuring fish assemblages (Carpenter and Lodge 1986; Dibble et al. 1996; Okada et al. 2003; Slade et al. 2005). The increase of substrate area offered by plants provides more abundant food due to the availability of substrates for prey, higher productivity and refuge (Miranda and Hodges 2000; Harrel and Dibble 2001). Agostinho et al. (2007) demonstrated that habitats containing macrophytes provide higher fish richness.

Fish response to wetland altitude

Species richness in most taxonomic groups generally decreases as an altitude increase (Gaston 2000; Heino 2002; Oertli et al. 2002; Rolon and Maltchik 2006). Our results showed the negative influence of altitude on fish occurrence and richness, corroborating previous studies (Matthews 1998; Bistoni and Hued 2002; Jaramillo-Villa et al. 2010). We think that altitude was a major limiting factor for fish occurrence in our study, since wetlands without fish occurrences were mainly located at higher altitudes (altitudes varying from 300 to 1,200 m). On the other hand, fish abundance was positively related to altitude due to the increase of the number of *C. brevirostratus* – a gregarious poeciliid fish – typical from headwaters – that form large schools (Rosa and Costa 1993).

Fish response to hydroperiod and macroinvertebrate richness

Several studies suggest that hydroperiod influences fish composition and structure in wetland systems (Baber et al. 2002; Ruetz et al. 2005; Fernandes et al. 2009; Macedo-Soares et al. 2010; Maltchik et al. 2010a). This influence is related to the harsh conditions of intermittent ecosystems and because many species do not have adaptations for tolerating or escaping the dry phase (Laufer et al. 2009). In our study, the hydroperiod did not influence the fish occurrence and richness; nevertheless, permanent wetlands presented higher abundance. The similarity in species richness might be due to the occurrence of four fish species of Rivulidae family. Rivulidae is the only Neotropical fish family that presents specialization for living in temporary waters (Nelson 2006). Most of Rivulidae species lives temporarily in wetlands formed during periods of heavy rains; the adults die when the ponds dry, but they leave their eggs buried in the substrate under diapause to eclode with the arrival of a new rain period (Costa 1998).

The macroinvertebrate community is a food source for several taxa (Stenert and Maltchik 2007), including endemic and endangered fishes (Costa 2009; Laufer et al. 2009). Fish occurrence and richness was positively related to macroinvertebrate richness. We think that the relationship between fish and macroinvertebrate was more associated with the response from invertebrates to the wetland environmental characteristics than the trophic relationships between fishes and macroinvertebrates, since most fish species observed also eat small fishes and small fragments of aquatic plants. Several studies carried with macroinvertebrates in Southern Brazil wetlands showed that wetland area, habitat diversity, altitude and hydroperiod also influence macroinvertebrate richness (Stenert and Maltchik 2007; Maltchik et al. 2009, 2010b). These results suggest that the landscape and environmental variables that determine fish and macroinvertebrates richness may be very similar.

Conclusions and conservation implications

The variation of species composition between ecoregions corroborated the statement proposed by Abell et al. (2008) for the identification of fish diversity in the study area. They used a database and numbers of endemic fish species to define freshwater ecoregions of the world. Although the species associated with Laguna dos Patos and Lower Uruguay ecoregions were not necessarily exclusive from these ecoregions, the composition observed was very similar from previous studies performed in both ecoregions (Reis et al. 2003; Buckup et al. 2007).

Current evidence suggests that aquatic species, in particular freshwater fish, are on high risk of extinction (Jenkins 2003; Olden et al. 2007). These researchers reinforced the need for understanding the ecological patterns that influence freshwater fish to decrease extinction risks. Our study represents an important research done at Neotropical broad scales that analyses the influence of environmental predictors and ecoregion in palustrine wetland fishes. Our data showed that fish richness was related to habitat diversity and macroinvertebrate richness, and fish occurrence was influenced by wetland area and macroinvertebrate richness. However, the fish composition was jointly associated with ecoregion and hydroperiod. Our results showed that habitat diversity, macroinvertebrate richness, altitude and hydroperiod were the environmental predictors that potentially structure and maintain fish occurrence and richness in southern Brazil palustrine wetlands. Such information is essential to develop wetland conservation and management programs in this region, where more than 90 % of wetland systems have already been lost and the remaining ones are still at high risk due to the anthropogenic activities.

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