Fish assemblage structure in altitude rivers under the effect of exotic species introduction, northeast of Rio Grande do Sul, Brazil

Estrutura da assembléia de peixes em um rio de altitude sob o efeito da introdução de espécie exótica no nordeste do Rio Grande do Sul, Brasil

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Abstract: Aim: Lack of knowledge about the ichthyofauna and its organization in streams of the Neotropical region makes it difficult to predict anthropogenic effects on these communities. The present study aimed at describing fish assemblages of three small rivers of high altitude and low order in Southern Brazil. **Methods:** Introduction of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), an exotic species, was studied as a disturbance factor of the autochthonous fish assemblage. **Results and conclusion:** The results revealed a reduction in species richness, abundance and biomass at sites stocked with trout, indicating harmful effects of introduction on indigenous ichthyofauna, leading to habitat restriction for some species, and probably competition for resources.

Keywords: autochthonous ichthyofauna, community structure, rainbow trout, lotic system, *Oncorhynchus mykiss*.

Resumo: Objetivo: O desconhecimento da ictiofauna e seus padrões de organização em riachos da região neotropical dificultam a predição de efeitos antrópicos sobre as mesmas. Este trabalho tem como objetivo descrever a assembléia de peixes de três rios de altitude e de baixa ordem no sul do Brasil. **Métodos:** A introdução da espécie exótica truta arco-íris (*Oncorhynchus mykiss* Walbaum, 1792) foi estudada como um distúrbio sobre a ictiofauna autóctone. **Resultados e conclusão:** Os resultados revelam uma diminuição na riqueza, abundância e biomassa nos locais povoados com trutas. Isso indica o efeito danoso da introdução sobre a ictiofauna autóctone, levando a restrição de habitats para algumas espécies e, provavelmente, competição por parte dos recursos.

Palavras-chave: ictiofauna autóctone, estrutura da comunidade, truta arco-íris, sistema lótico, *Oncorhynchus mykiss*.

1. Introduction

Many aspects of growth, maturity, habitat preferences and structure of Neotropical fish communities have been poorly studied. Particularly the ecology of small endemic species of low dispersion capacity is almost unknown (Pavanelli and Caramaschi, 2003; Perez-Júnior and Garavello, 2007; Santos and Caramaschi, 2007; Súarez, 2008; Montag et al., 2008). Anthropogenic impacts such as pollution, overfishing, habitat alterations, and changes in water cycles caused by deforestation significantly affected the autochthonous fish assemblage (Vari and Malabarba, 1998). In addition to these disturbances, the introduction of exotic species exerts pressure on already stressed ecosystems. Even being justified by short-term economical or social benefits, long-term effects may cause unsustainable conditions (Hall and Mills, 2000).

The exotic species may influence the structure and function of aquatic and terrestrial ecosystems, potentially being able to affect food supply and human health (Hall and Mills, 2000). Complex interactions with the autochthonous fauna includes competition (Blanchet et al., 2007), predation on native fish (Ogutu-Ohwayo, 1990; Leunda et al., 2008) or other vertebrates (Orizaola and Brana, 2006; Ortubay et al., 2006) and invertebrates, introduction of allochthonous parasites and diseases (Fazio et al., 2008) and hybridization (Kozfkay et al., 2007; Camarena-Rosales et al., 2008).

Rainbow trout is among the most dispersed species. The species was introduced in at least 82 countries (Cambray, 2003). The distribution of autochthonous species in the environment was studied by Larson and Moore (1985) who observed that the occupied area by *Salvelinus fontinalis* after rainbow trout introduction diminished 59.7% in 40 years. Ross (1991) revised studies in American rivers and concluded that 77% of introduction eliminated or diminished autochthonous populations. In 50% occurred change in resources use, and the interactions by predation and competition are frequent. There is little knowledge about the effect of exotic species in the community organization, and some of which can be a cascade through multiple trophic level, what makes it to underestimate the importance of the subtle impacts of biological invasion (Flecker and Townsend, 1994).

In Southern Brazil, at high altitude regions, the main land use is pasture and *Pinus* sp. cultivation. Tourism is yet an incipient activity in this area, but one of the principal attractions is rainbow trout (*O. mykiss* Walbaum, 1792) catch and release fly fishing. These fishes are introduced annually in catchable size of about 25 cm to increase number of visitors from other regions of Brazil and from abroad. The autochthonous ichthyofauna is not commercially important, due to the small body size. The lack of knowledge about ecological background makes it difficult to predict if competition and predation occurs and to what degree the interference negatively affects the autochthonous communities. Harmful effects of the introduction may be the loss of rare, unknown and endemic native species.

The present study intended to investigate the structure of the fish assembly from low orders streams in Southern Brazil, with presence and absence of rainbow trout to evaluate the influence of exotic species introduction in these ecosystems.

2. Material and Methods

The area is situated in São José dos Ausentes municipal district, Northeast of Rio Grande do Sul State, Brazil. The climate is Cfb (humid temperate climate) according to the Köppen classification. The mean of lowest air temperature was 12.0, 12.2 and 11.3 °C to 2001, 2002 and 2003 respectively; and the mean of hottest air temperatures was 22.2, 22.3 and 22.4 °C to 2001, 2002 and 2003 respectively (INMET, 2009). In winter temperature might reach –5 °C, with frequent formation of frost and occasional snow. The annual precipitation varies from 1500 to 2000 mm, with well distributed rainfalls during the year (Boldrini, 1997).

The sampling design included three streams in the Silveira basin of the northeast region of Rio Grande do Sul. The Silveira stream is a tributary of the upper reaches of the River Uruguay (Figure 1). Lajeadinho, (L1 sampling site) is a third order stream, which was never stocked with rainbow trout but showed adequate structure in terms of substrate, depth and flow for trout reproduction. The Marco (M1) is a fourth order stream, without introductions of trout since the 80's. This site was considered undisturbed reference site. The Silveira is a fifth order stream, where trout are introduced on an annual basis in autumn and winter

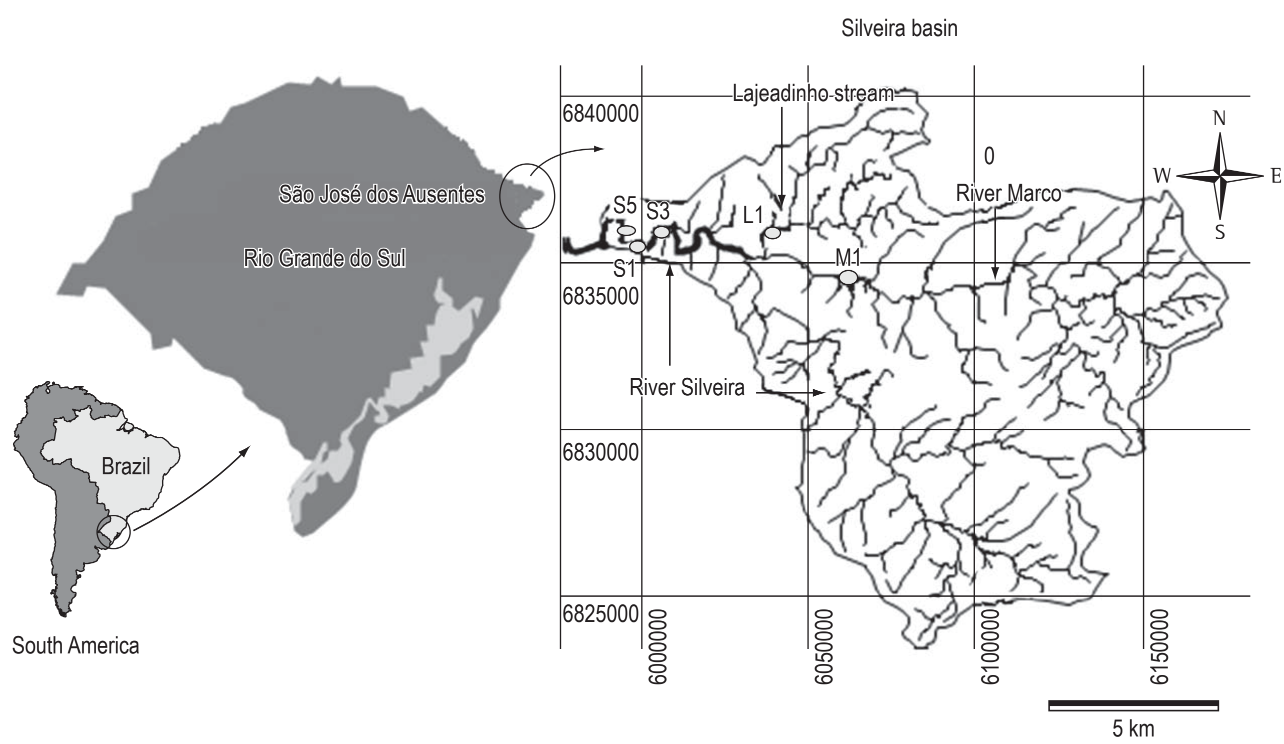

Figure 1. Map of the study area, showing the rivers Silveira (S1, S3 and S5), Marco (M1) and Lajeadinho (L1).

to sustain a put-and-take fishery. Three sampling sites were chosen at the Silveira (S1, S3 and S5).

No physical barriers impede trout dispersal from the Silveira to the Lajeadinho stream. In the last two sampling periods, were found trout in Lajeadinho stream, so from that period it was considered a trout stream. Natural dispersal from the stocking reach in Silveira to the reference site in the Marco stream was hampered due to several waterfalls and very shallow rapids.

Fish were collected by 3-pass electro-fishing. Sampling sites with an area of approximately 300 m^2 in each site were isolated up and downstream by 15 mm mesh size block nets. Electro-fishing followed the method described in Bozzetti and Schulz (2004).

The sampling period was between April/02 and August/03, through the four seasons of one year with approximately 3 months and half between the sampling trips, adding up to six collections at each site.

Captured fish were fixed in 10% formalin and preserved in 70% ethanol (Malabarba and Reis, 1987). After one week in the ethanol solution, fish were weighed with 0.01 g precision and the total length was measured to the nearest mm.

Species abundance was calculated for each side using the removal method by Carle and Strub (1978). The steadiness of occurrence was calculated as C = (number of samples with the species/total number of samples) × 100, where "constant species" are those that occurred in at least 50% of the sample, "accessory species" are those occurring in 25% to 50% of the samples, and "accidental" are those species found in less than 25% of the samples (Dajoz, 1983).

Biomass was calculated as $B = (B_s \times N)/C_s$, where B_s is the total weight of captured fish, C_s the total number of captured fish, and N is the population density (Agostinho and Penczak, 1995). The diversity of autochthonous ichthyofauna was determined by the Shannon-Wiener (H') and evenness (J) by the Pielou (1975) index, using program Divers (Smith, 1993). The specimens were classified in 2cm size classes. Class extension was defined according to Sturges apud Vieira (1991). The locations of the sampling sites were plotted against the variables most correlated to the axes, with the chord distance as a similarity measure between variables. Additionally, a Principal Components Analysis was performed using MULTIV (Pillar, 2004a).

To calculate the between-rivers similarity the variables used were water conductivity, pH, temperature and flow velocity, measured at each sampling site. The congruence between the abiotic and biotic datasets was tested with SYNCSA (Pillar, 2004b), using chord distance as similarity measure. The abiotic variables were submitted to a randomization test using the Gower index as similarity measure (Pillar and Orlóci, 1996).

Values of biomass, species richness, Shannon-Wiener diversity index and evenness were also submitted to a randomization (Pillar and Orlóci, 1996) to test for significant differences between sites, seasons and also for the presence/absence of trout. The test was carried out using MULTIV (Pillar, 2004a), and the chord distance was the similarity measure. For all other variables, the Euclidean distance was used. A cluster analysis was performed with biomass data, and grouping sharpness was tested by bootstrap (Pillar, 1999). The software used was MULTIV (Pillar, 2004a).

3. Results

The randomization test showed significant differences (P = 0.001) of abiotic data between seasons but not between sampling sites (P = 0.511). None of the tested abiotic variables was significantly related with the fish assemblage.

To evaluate trout influence on the ichthyofauna, the autochthonous fish assembly was described. Twelve species were identified in the study area. Characiformes dominated the fish fauna composition. The captured species and the constancy of occurrence are listed in Table 1. Species richness by sampling site is displayed in Figure 2. The richness remained constantly low throughout the study in the River Silveira. In the River Marco the highest richness was found in Autumn 2002, when both the accidental and accessory species occurred. Statistical analysis detected significant differences (P = 0.001) among the richness values in the River Silveira when compared to Lajeadinho and Marco. In Lajeadinho occurred a significant change in richness. Until Summer 2003 no trout were captured at this site and richness varied between 10 and 11 species. With trout present richness decreased significantly to 7 or 8 species (P = 0.001).

The abundance showed significant differences among the rivers (P = 0.001). In all samples, the S1 site in the River Silveira presented the lowest values for fish abundance richness and diversity. Abundance did not vary significantly among the different seasons of the year (P = 0.324).

The estimated biomass of native fish fauna was lowest in the River Silveira. Significant differences between seasons were not found (P = 0.680).

Cluster analysis (Figure 3) resulted in three clear groups. Group 1 included all sampling sites of the River Silveira, River Lajeadinho in Winter 2002 and River Marco in Spring 2002 and Autumn 2003. Group 2 was formed by the River Marco in all other dates as well as River Lajeadinho in Summer and Autumn 2003. Group 3 comprised all other dates in River Lajeadinho.

The diversity index, the autochthonous equitability of the native ichthyocenosis, as well as the abundance and estimated biomass of trout by sampling site are shown in Table 2. The diversity index was lower in the trout stocked River Silveira and differed significantly from rivers Lajeadinho and Marco (P = 0.004). Again, seasons did not affect the diversity index (P = 0.43), but trout presence drops the diversity index (P = 0.007). At the seasons that

Table 1. Autochthonous fish families and species occurring in Silveira Basin (L: Lajeadinho stream, M: Marco river, S: Silveira river) and the percentual frequency of occurrence.

Fish species	Percentual frequency of occurrence		
	L	M	S
Characidae			
Astyanax sp.	100.00	100.00	100.00
Astyanax brachypterygium (Bertaco and Malabarba, 2001)	60.00	33.33	0.00
Bryconamericus sp.	100.00	100.0	100.00
Curimatidae			
Steindachnerina ' (Braga and Azpelicueta, 1987)	0.00	16.66	5.55
Ciclidae			
Australoheros facetus (Jenyns, 1842)	60.00	83.33	72.22
Anablepidae			
Jenynsia eirmostigma (Ghedotti and Weitzman, 1995)	100.00	83.33	0.00
Poeciliidae			
Cnesterodon brevirostratus (Rosa and Costa, 1993)	80.00	66.66	5.55
Pimelodidae			
Rhamdia quelen (Quoy and Gaimard, 1824)	100.00	83.33	88.88
Trichomycteriidae			
Trichomycterus sp.	100.00	83.33	11.11
Loricariidae			
Rineloricaria sp.	100.00	0.00	0.00
Eurycheilichthys pantherinus (Reis and Schaefer, 1992)	20.00	100.00	77.77
Pareiorhaphis hystrix (Pereira and Reis, 2002)	100.00	100.00	72.22

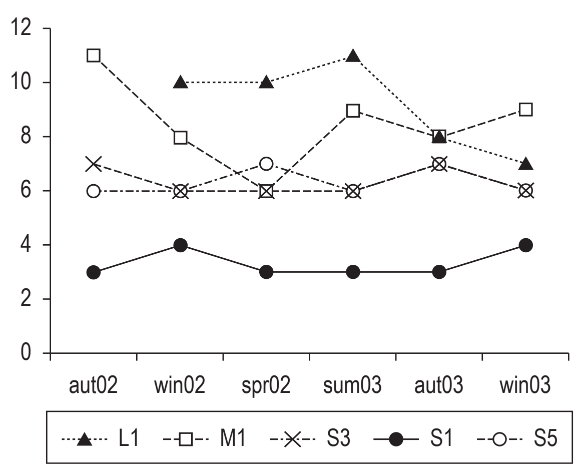

Figure 2. Species richness of each sampling site by season: aut02 = Autumn 2002; win02 = Winter 2002; spr02 = Spring 2002; sum03 = Summer 2003; aut03 = Autumn 2003; win03 = Winter 2003.

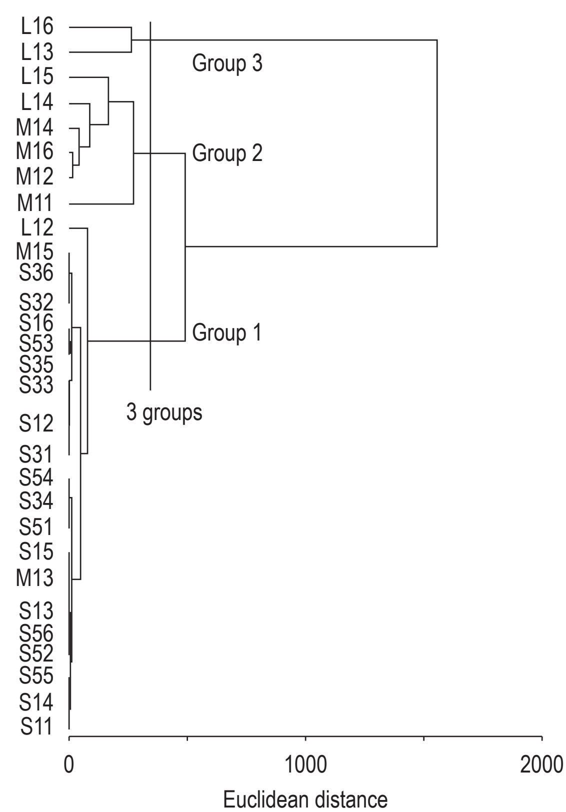

Figure 3. Cluster analysis dendrogram of autochthonous ichthyofauna biomass considering sampling sites. The letter and first number indicate the sampling site. The second number indicates the collection date: 1 Autumm 2002; 2: Winter 2002; 3: Spring 2002; 4: Summer 2003; 5: Autumm 2003 and 6: Winter 2003.

Lajeadinho presents trout, the diversity index dropped to values comparable to that found in the River Silveira.

Evenness indices were fairly constant throughout the sampling period, not showing any significant differences among rivers, seasons or trout presence (P = 0.162); the highest values were recorded in the summer in the rivers Silveira and Marco.

Table 2. Exotic and native estimated abundance (fish.ha^{-1}), exotic and native biomass (kg.ha^{-1}) and diversity index (H') and equitability (J) of native species.

Season	Site	Trout density (fish.ha^{-1})	Trout biomass (kg.ha^{-1})	Native ichthyofauna density (fish.ha^{-1})	Native ichthyofauna biomass (kg.ha^{-1})	H'	J
Autumn 2002	S1	55	13.26	860	1.15	0.50	0.45
	S3	9	2.51	2895	4.48	1.16	0.60
	S5	0	0.00	2031	3.27	1.34	0.75
	M1	0	0.00	3625	24.93	1.35	0.56
Winter 2002	S1	10	2.62	567	3.78	0.86	0.63
	S3	13	2.44	2635	6.06	1.13	0.63
	S5	0	0.00	1354	2.64	0.90	0.50
	M1	0	0.00	3699	11.30	1.32	0.64
	L1	0	0.00	2950	11.15	1.72	0.76
Spring 2002	S1	0	0.00	323	1.27	0.69	0.63
	S3	9	2.46	772	3.81	1.16	0.65
	S5	5	0.61	740	4.79	1.23	0.64
	M1	0	0.00	263	1.58	1.15	0.68
	L1	0	0.00	10538	60.44	1.81	0.79
Summer 2003	S1	0	0.00	214	0.45	0.48	0.45
	S3	0	0.00	865	3.75	1.52	0.85
	S5	0	0.00	816	2.71	1.73	0.98
	M1	0	0.00	3363	17.31	2.00	0.91
	L1	0	0.00	12192	21.19	1.89	0.79
Autumn 2003	S1	20	4.83	383	1.88	0.78	0.72
	S3	74	12.89	725	4.20	1.05	0.54
	S5	10	1.92	403	0.86	1.63	0.85
	M1	0	0.00	2137	6.61	1.41	0.68
	L1	102	31.63	8008	19.90	1.61	0.78
Winter 2003	S1	144	17.22	806	5.55	0.90	0.65
	S3	109	16.76	1731	5.83	1.09	0.61
	S5	342	40.23	744	2.71	1.03	0.58
	M1	0	0.00	2671	18.53	1.54	0.70
	L1	51	10.30	7833	33.08	1.25	0.64

The analysis of the distribution in size classes in the sites (Figure 4) showed that species such as *Astyanax* sp. show variations in size according to the season, with larger individuals present mainly in spring, independently the absence or presence of trout. Smaller classes of *Bryconamericus* sp., *Astyanax* sp. and *Australoheros facetus*, have greater influence in rivers with presence of trout. Principal components analysis (Figure 5) showed that the River Silveira was negatively related to axis 1 and positively related with axis 2. The variables most correlated with the second axis was big *Rhamdia quelen* (between 20.0 and 28.0 cm) and the smallest *Eurycheilichthys pantherinus* (between 2.0 and 4.0 cm), both presenting negative relation with the axis.

4. Discussion

Among the 12 species found, *Astyanax brachypterygium* and *Pareiorhaphis hystrix* were recently described (Bertaco and Malabarba, 2001; Pereira and Reis, 2002, respectively), while *Astyanax* sp., *Bryconamericus* sp., *Rineloricaria* sp., and *Trichomycterus* sp. have not yet been described, or are still in incomplete state of description.

The low diversity found was probably explained for the altitude. The sampling places at the Silveira basin were located at 1100 to 1200 m, with low temperatures, where despite the low order, riparian vegetation are composite mainly by scrub and grass. At the similar altitude, in Argentina, Bistoni and Hued (2002) found 15 species, being probably the physical and chemical conditions, with very low temperatures the principal factor that determines this low richness. At the literature, the species richness to low order river vary from 11 species found by Mazzoni (1998), 15 reported by Uieda and Barreto (1999) in third order streams, and 22 in fourth order water courses, 18 from Uieda et al. (1987) and 14 species in streams related by Penczak et al. (1994) working in tributaries of the River Paraná. The number of species found at the Silveira basin is smaller, but similar to those results. Bastos (2002) studying the fishes at Silveira basin found only six species. Probably

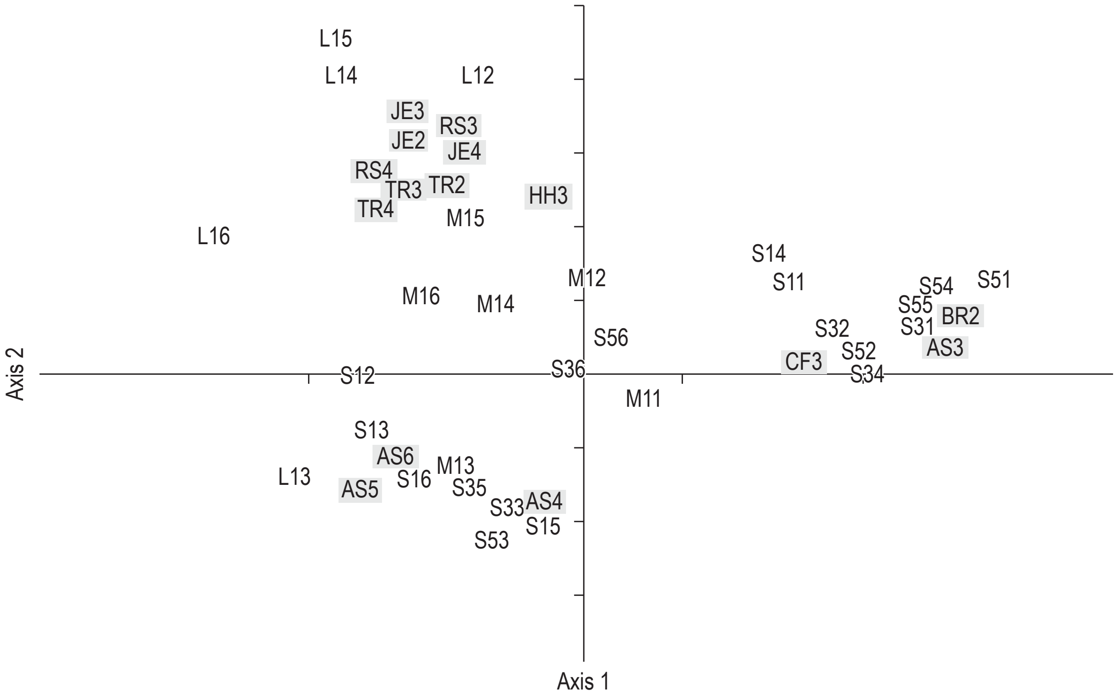

Figure 4. Biplot of native species by size classes against sampling dates. In bold are the variables more correlated with the axes; the letter gives description of species and the number gives the size class. AS: *Astyanax* sp.; BR: *Bryconamericus* sp.; CF: C. facetum; HH: *P. hystrix*; JE: *J. eirmostigma*, RS: *Rineloricaria* sp.; TR: *Trichomycterus* sp.; 2: between 2.0 and 3.9 cm; 3: 4.0-5.9; 4: 6.0-7.9; 5: 8.0 - 9.9; 6: 10.0 - 11,9 cm. Other symbols are: the sampling site and date, (the letter and the first number give the site and the second number gives the date) = 1: Autumn 2002; 2: Winter 2002; 3: Spring 2002; 4: Summer 2003; 5: Autumm 2003 and 6: Winter 2003.

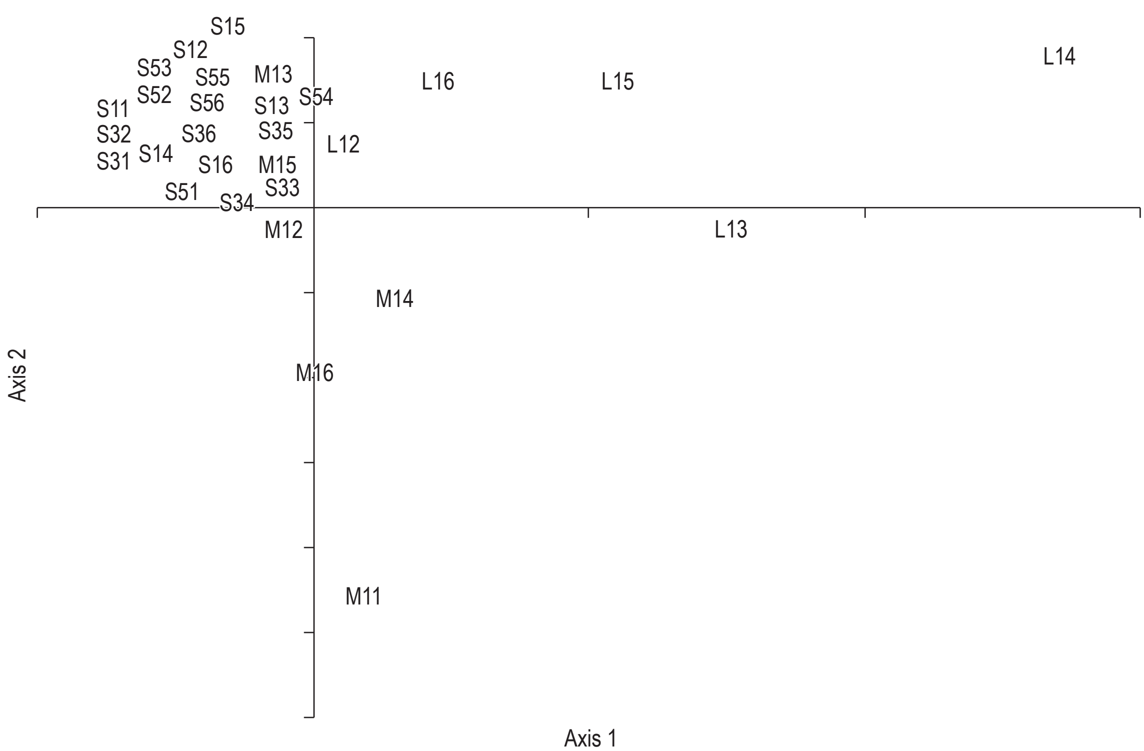

Figure 5. Principal component analysis plot, using correlation as similarity measure. The letter and the first number indicates the sampling site and the second number gives the date; 1: Autumn 2002; 2: Winter 2002; 3: Spring 2002; 4: Summer 2003; 5: Autumn 2003 and 6: Winter 2003.

the lower supply of food resulting from lower productivity in rivers of lower volume act as a filter species allowing a few species occupies those environments (Súarez, 2008).

Angermeier (1994) divided diversity values in native and artificial, defining the later as created by man through the addition of biotic elements to natural systems. Evaluation through artificial diversity usually induces to erroneous conclusions, with higher indices being found in habitats rich in exotic species. Therefore, at the present study, the presence of trout always was considered a disturbance and, thus not included in abundance, diversity and evenness analyses. Scott and Helfman (2001) pondered that an intermediate step to exotic invasion would involve the invasion by native species of other habitats in the river, provisionally promoting a diversity increase. This process was not observed in the River Silveira neither in the Lajeadinho, probably because the study area is isolated downstream by an unsurpassable waterfall. For this reason, the diversity indices calculated seemed to be reasonably sensible to the presence of exotic species.

Generally, fish abundances are used in relation to biomass values in fish community studies (Penczak et al., 1994); in the present study, however, the use of biomass values becomes useful due to the weight differences verified between sampled individuals. Moreover, since trout may reach a greater size compared to the native species, it could affect food availability for the latter, in a river with few food resources (Guadagnin et al., 1998). The present data set revealed a trend towards reduction in both number (ind.ha^{-1}) and biomass of the native species, wherever the trout was present, having some of the smallest individuals been found in trout sites.

According to Ross (1991) the establishment of an exotic species in a new environment does not reveal very much about the native community and its structure and the way this community is affected. However, the decrease of a certain species population, and perhaps the increase of others, may reflect alterations in growth rates, reproductive success or survival related to the regulation through biotic processes, where predation and competition may cause changes. At the River Silveira it is not possible to know if trout is established, but studies show that it can reproduce in this habitat (Winckler-Sosinski et al., 2005, 2006). Despite some between-river differences indicated by the data, the lack of information regarding the fish assembly before the introduction makes it difficult to describe any changes in structure. This is a common problem in introductions studies, as stated by Ross (1991). Changes in species abundance, with increase and predominance of introduced trout in rivers have been described by some authors (Cadwallader, 1979; Larson and Moore, 1985; Townsend, 1996). In Argentina, in rivers where trout were introduced in the beginning of the 20th century, Bistoni and Hued (2002) found only rainbow trout and *Bryconamericus iheringi* in first order streams, with trout appearing until third order streams (restricted to altitudes higher than 800 m), where richness increases from 14 to 31 species. Also in Argentina, Videla and Bistoni (1999) found rainbow trout from 900 m of altitude onwards, were they dominated the fish communities. The authors emphasized the transition between 1100 m of altitude, where trout comprised only 2.77% of the community, and 1400 m of altitude only 3 km upstream, where trout was the only fish species. Menni and Gomez apud Videla and Bistoni (1999) mentioned the displacement of one endemic species from its natural habitat, and Fernandez and Fernandes apud Videla and Bistoni (1999) reported great damage to *Trichomycterus* sp. caused by the introduction of trout in rivers from north Argentina. In the present study, both the diversity and biomass were lowest in the River Silveira. Also, some species of smaller sizes such as *Astyanax* sp., *Bryconamericus* sp., and *A. facetum* were found in this river. In the Silveira basin, the riparian vegetation is limited to shrubs and pasture for the most part of the river courses, except for those regions of higher declivities. For this reason the fish assemblage composition should remain across the sampling sites, varying only in different environments, such as riffles, runs and pools, which were well represented in the sampling effort in the Rivers Silveira, Marco and Lajeadinho. In the former, the decrease in species number observed at S1 may be explained by the dominance of runs, a type of where, according to Martin-Smith (1998), fish populations tend to have lower richness but similar abundance. The results, however, indicate that the presence of trout is affecting the native fish community qualitative as well as quantitatively.

Moyle and Light (1996) postulated that abiotic variables are the main factors of success for invading species in the new community. The same authors mention the great capability of most fish species to adjust to invaders, assuming organizational patterns characteristic of those from already communities coexisting for long. When extinction occurs, usually due to the presence of carnivorous fish, it may reflect the great number of introductions taking place. It was showed by Pelicice and Agostinho (2009) where the *Cichla kelberi* introduction caused sequences of extinction and colonization events and the small sized species disappeared. The River Silveira has received great amounts of trout (Winckler-Sosinski, 2004) since the 90s. However, due to the low survival of this species in this river, and the dominance of smaller individuals, it is not likely that predation is the main cause composition differences in the fish assemblies. Only large trout individuals have been related to predate on native fish at River Silveira (Winckler-Sosinski, 2004).

According to Herbold and Moyle (1986), competition is the main mechanism that dislocates native species. However, these consequences are not readily recognizable. Changes in consumption patterns for organisms of different trophic levels caused by the presence of fish with different behaviors or by predation promoted by them, can lead to changes in trophic cascade (Flecker and Towsend, 1994). At the Silveira and Marco rivers, studying the differences caused by trout introduction, Winckler-Sosinski et al. (2008) identify differences at the benthic macroinvertebrates community. This indicates that the ichthyofauna changes observed at the present study is related with changes at different trophic levels.

Due to the changes recorded in the fish assemblies in presence of exotic species, it may be assumed that these communities are mainly regulated by biological factors. The main pressure factor caused by increments in trout biomass in these rivers seems to be competition, since native fish only become a significant food source for trout of larger sizes, which are relatively rare in the observed systems. However, studies on the life history of as well as food webs dynamics of ichthyofauna are necessary to clarify the mechanisms causing the displacement of autochthonous species.

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