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OCCURRENCE OF NON-NATIVE FISH SPECIES IN A NEOTROPICAL RIVER UNDER THE INFLUENCE OF AQUACULTURE ACTIVITIES

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ABSTRACT

The introduction of non-native species is one of the greatest global changes and is a substantial threat to continental fish fauna. This study aimed to evaluate the composition and structure of the fish assemblage of the Azul River, a tributary along the left margin of the Piquiri River basin, to determine the occurrence, abundance and spatial distribution of non-native fish species. Sampling was carried out from February to November 2014 at three sites along the river using of electric fishing equipment. Thirty-two fish species belonging to 20 genera, 11 families and five orders were collected. Eight non-native fish species were recorded, and *Gymnotus inaequilabiatus* and *Oreochromis niloticus* were listed among the five most abundant species in the Azul River. The fish fauna followed a distribution pattern expected for the Neotropical region, with a predominance of Characiformes and Siluriformes. However, the high representativeness of the abundance of the non-native species of the Gymnotiformes and Perciformes orders highlights the potential negative impacts on the structure of the fish assemblages of the Azul River due to species introductions.

Key words: bioinvasion; invasive species; *Oreochromis niloticus;* fish farms; biotic homogenization.

OCORRÊNCIA DE ESPÉCIES DE PEIXES NÃO NATIVOS EM UM RIO NEOTROPICAL SOB A INFLUÊNCIA DA ATIVIDADE AQUÍCOLA

RESUMO

A introdução de espécies não nativas é uma das grandes mudanças globais e uma das principais ameaças a ictiofauna continental. Dessa forma, esse trabalho teve por objetivo avaliar a composição e estrutura da ictiofauna do rio Azul, um tributário da margem esquerda da bacia do rio Piquiri, a fim de determinar a ocorrência, abundância e distribuição de espécies de peixes não nativas. Para isso, quatro amostragens foram realizadas de fevereiro a novembro de 2014 em três locais ao longo do rio, por meio do uso de equipamento de pesca elétrica. Durante o período amostral, 32 espécies de peixes foram registradas, as quais pertenceram a 20 gêneros, 11 famílias e cinco ordens. Houve o registro de oito espécies de peixes não nativas, dentre elas *Gymnotus inaequilabiatus* e *Oreochromis niloticus* estiveram presentes entre as cinco mais abundantes no rio Azul. Desse modo, a ictiofauna do rio Azul seguiu o padrão de distribuição esperado para a região Neotropical, com predominância de Characiformes e Siluriformes. Por outro lado, o aumento de ocorrência de espécies de peixes não nativas, observado da cabeceira para a foz do rio, indica possível processo de homogeneização biótica, potencialmente intensificado pelo escape de peixes não nativos advindos de pisciculturas.

Palavras-chave: bioinvasão; espécies invasoras; *Oreochromis niloticus*; pisciculturas; homogeneização biótica.

INTRODUCTION

The introduction of fish species is highlighted as a factor that critically affects the distribution of fish populations in lotic environments (ORTEGA *et al*., 2015; FREHSE *et al*., 2016; COA *et al*., 2017). Introductions resulting from human interference are not new, as they have been recorded for approximately ten thousand years (PERRY and VANDERKLEIN, 1996). However, in the last few centuries, human activities have caused significant and drastic changes to biodiversity that are incomparable to natural or historical effects (VITULE *et al*., 2012; BARBIERI *et al*., 2016).

The accidental or deliberate release of non-native fish species has become one of the main problems for the conservation of continental fish fauna (GHERARDI, 2007; LEPRIEUR *et al*., 2008; PELICICE *et al*., 2017). Currently, aquaculture activity stands out as the main source of introduction of non-native fish species into freshwater ecosystems, and it is highlighted as the main contaminant and dispersing activity (DAGA *et al*., 2015) in Brazilian continental ecosystems (CASAL, 2006; VITULE *et al*., 2009; ORTEGA *et al*., 2015; LIMA *et al*., 2016; DAGA *et al*., 2016). The main pathways for non-native species introduction from aquaculture activities are escape via effluent water, inappropriate management, and the rupture or overflow of ponds after floods (ORSI and AGOSTINHO, 1999). Fish ponds that are located dangerously near riverbeds or in areas susceptible to floods lead to the repeated spread of non-native fish to the wild (ORSI and AGOSTINHO, 1999; MAGALHÃES *et al*., 2011).

Although aquaculture has positive aspects related to biodiversity conservation, such as the reduction of extractive fishing pressure, the current production model does not efficiently comply with the principles of sustainability and biosafety (DIANA, 2009), amplifying the introductions of species with high invasive risks (FORNECK *et al*., 2016). It is recognized that not all introductions result in negative effects on biodiversity (GOZLAN, 2008), but the biological attributes of species of zootechnical interest match the general attributes of species with high invasive potential, such as high adaptability to new environments, wide environmental tolerance, fast growth and early sexual maturation (RICCIARDI and RASMUSSEN, 1998).

Brazil has mega-diverse native fish fauna, but production is almost entirely based on non-native fish species (LIMA JUNIOR *et al*., 2012; PELICICE *et al*., 2014). As in other developing countries, the challenges involved in preventing new introductions and biological invasions can be particularly difficult. Rapid economic development means that decisions might be based on political issues or short-term economic demands, and the long-term consequences to the environment and the risks to conservation biology have been ignored (LIMA JUNIOR *et al*., 2012; PELICICE *et al*., 2014, 2017).

Hence, in this study, we aimed to evaluate the composition and structure of the fish assemblage of a Neotropical river under the influence of the aquaculture activity. The Azul River, a tributary on the left margin of the Piquiri River basin, Brazil, was studied to determine the occurrence, abundance and spatial distribution of non-native fish species. Specifically, we evaluated the effects of the presence of fish farms on spatial occurrence and distribution of the native and non-native fish assemblages. Therefore, we tested the hypothesis that aquaculture activity influences the occurrence, abundance and dispersion of non-native fish species. To achieve our objectives, we first analyzed the variations in the abundance and frequency of fish species occurrence at the spatial scale considered. Then, we evaluated the changes in the fish assemblage attributes and, finally, we described the variations in the composition and structure of the fish assemblage in the river basin.

METHODS

Study area

The Azul River belongs to the upper Paraná River basin, which is one of the major river basins of Brazil. It is characterized as a third-order river (sensu STRAHLER, 1957), has a drainage area of 337 km^2 and is 62 km in length (Figure 1). The Azul River microbasin stands out for its use in public supply and the dense number of aquaculture properties in its drainage area. Currently, the Azul River microbasin has 39 fish farms, totaling 216 ponds and an area of approximately 60 ha (ZACARKIM and OLIVEIRA, 2015). According to ZACARKIM and OLIVEIRA (2015), fourteen fish species are produced in the Azul River microbasin, belonging to four orders and 10 families. The Nile tilapia *(Oreochromis niloticus)* is the species with the highest storage at 82%, followed by Pacu *(Piaractus mesopotamicus)* at 7% and Piauçu *(Megaleporinus macrocephalus)* at 3%.

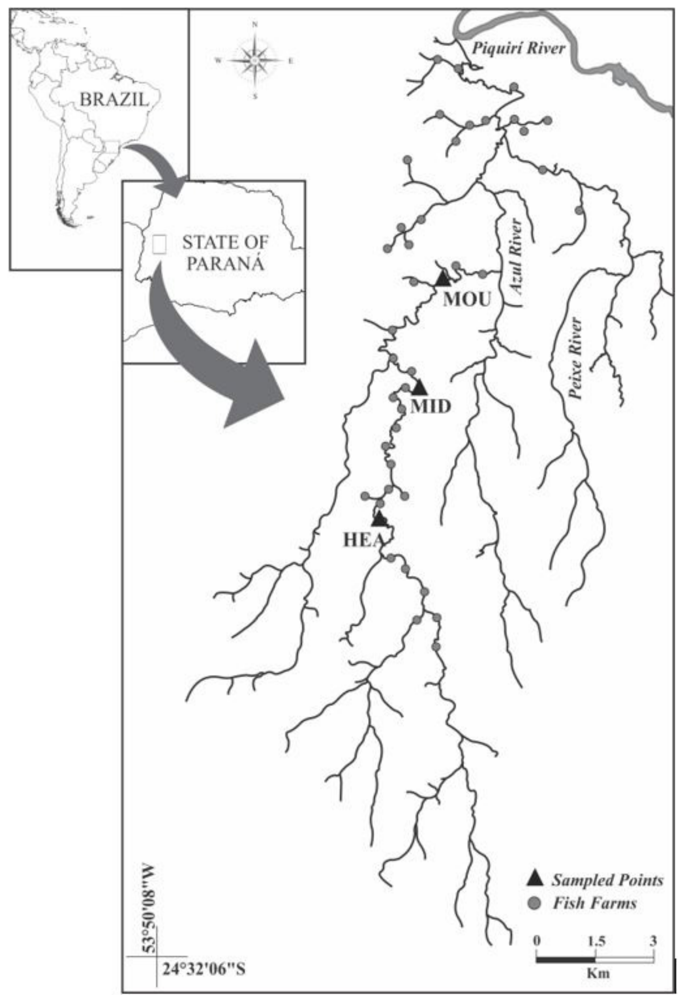

Figure 1. Delimitation of the Azul River microbasin, Piquiri River basin, State of Paraná, Brazil. • indicates the location of the sampled sites. ▲ indicates the location of the fish farms in the microbasin. HEA: headwater, MID: middle, MOU: mouth.

Of the total cultivated species, five are considered native in the upper Paraná River basin (*Leporinus friderici, Megaleporinus piavussu, P. mesopotamicus, Prochilodus lineatus* and *Rhamdia quelen*), three species are classified as native to other Brazilian basins (*Astyanax lacustris, Brycon amazonicus,* and *M. macrocephalus*), three species are results from hybridization (hybrid between *Pseudoplatystoma corruscans* and *Pseudoplatystoma* sp., hybrid between *Colossoma macropomum* and *Piaractus brachypomus* and hybrid between *C. macropomum* and *P. mesopotamicus*) and three species are native to other countries and continents (*Clarias gariepinus, Cyprinus carpio* and *O. niloticus*).

Non-native species account for 50% of the species produced, with a total storage of 86%, thus indicating a pattern of aquaculture breeding based on non-native species. Among the six native species produced, the percentage of storage is only 11% of the total cultivated storage.

Fish sampling

Fish sampling was conducted four times from February to November 2014 at three sites (headwater, middle and mouth; Figure 1) along a longitudinal gradient. Electric fishing equipment was used (AC portable generator, 2.5 kW, 400 V, 2 A, connected to voltage rectifier), and sampling was performed using successive removals in 50 meter stretches per sample site.

The captured fishes were anesthetized and euthanized with an overdose of benzocaine and were then fixed in 10% formalin. In the laboratory, all individuals except for *Trichomycterus* sp. and *Ancistrus* sp. were identified following GRAÇA and PAVANELLI (2007). Species classification followed ESCHEMEYER and FONG (2017) for higher taxa and REIS *et al*. (2003) for Neotropical families. Voucher specimens were deposited in the ichthyological collection of Nupélia at the Universidade Estadual de Maringá (UEM, 2017).

Abundance and frequency of fish species occurrence

The density values (number and weight of fishes ha^{-1}) of the collected species were determined on the basis of three successive removals at each sampled site and by applying Zippin's maximum likelihood method (ZIPPIN, 1965), which is related to the premise of effort and the efficiency of constant catches. For cases where there were restrictions to the methods, specifically $0 < R < ((S-1))/2$, where R is the restriction index and s is the number of catches, the AGOSTINHO and PENCZAK procedure (AGOSTINHO and PENCZAK, 1995) was used. The 10 most abundant species that were captured at headwater were fixed and represented graphically to describe the variations over spatial scales.

To determine the frequency of fish species occurrence at the sampled sites over the collection periods, Dajoz's constancy index (1973) was calculated using the equation: $C = (n / N) \times 100$, where C = constancy; n = number of times the species was captured; and N = total number of collections. The species is considered constant when $C \geq 50\%$, not relevant when $50\% > C \geq 20\%$ and accidental when $C < 20\%$.

Fish assemblage attributes

The richness of species (number of species), Shannon diversity index $H' = -\sum_{i=1}^{s} p_i * \ln p_i$, where s = species number and p_i = proportion of species i), and evenness ($E = H'/\ln S$, where H' = Shannon diversity index, and S = species richness) were calculated for each sample and for each site (MAGURRAN, 1988). One-way ANOVAs were used to assess whether spatial differences existed in species richness, diversity index, evenness, and density values (number and weight of fishes/ha) among sites (assumptions of normality and homoscedasticity were tested using the Shapiro-Wilk and Levene tests, respectively). When the one-way ANOVA was significant, a Tukey test was used to identify which categories differed. If the assumptions of the ANOVA were not met, the data were rank transformed (QUINN and KEOUGH, 2002), and the assumption of homoscedasticity of the variance was rechecked. If the assumption was met, one-way ANOVA was applied to the adjusted data (CONOVER and IMAN, 1981). If the assumptions for the ANOVA could not be met, a non-parametric Kruskal- Wallis test (ZAR, 1999) was used.

Composition and structure of fish assemblage

To summarize the composition and structure of the fish assemblage, principal coordinate analysis (PCoA; LEGENDRE and LEGENDRE, 2012) in a Bray–Curtis similarity matrix with 9,999 randomizations was applied. Principal coordinate analysis is a generalization of a principal component analysis, in which the eigenvalues are extracted from a similarity or distance matrix (BORCARD *et al*., 2011; LEGENDRE and LEGENDRE, 2012). The main advantage of this method is that it can be applied when the relationships between the variables are not linear. The axes with positive eigenvalues were retained for interpretation (BORCARD *et al*., 2011). To test for significant differences in the structure and composition of the fish assemblages among sites, we used a permutational multivariate analysis of variance (PERMANOVA; ANDERSON, 2001). Finally, we used a similarity percentage analysis (Simper) to determine the contribution of each fish species that accounted for the similarity within or the dissimilarity between sites (headwater, middle and mouth sites; CLARKE, 1993).

Species richness, Shannon diversity index, evenness, PERMANOVA and Simper were computed using PC-Ord® 5.0 (MCCUNE and MEFFORD, 2011). PCoA was performed using the "vegan" packages (OKSANEN *et al*., 2015) in the R software (R Development Core Team 2012). Analysis of variance was performed using Statistica™ 7.0. The level of statistical significance for all analyses was $p<0.05$.

RESULTS

Ichthyofauna survey

A total of 32 species, 20 genera, 11 families and 5 orders were collected, with 13 species classified as not relevant and 19 species as constants (Table 1). The highest richness values

Table 1. Biogeographical origin, frequency of occurrence, density and range of length of the species collected in the Azul River microbasin, Piquiri River basin, Brazil.

Orders/Families/Species	Biogeographical origin	Frequency of ocurrence*	Density (N.ha^{-1})		
			Headwater	Middle	Mouth
CHARACIFORMES					
Characidae					
Astyanax lacustris Lütken, 1875	Non-native	Constant	98.16	79.11	-
Astyanax bockmanni Vari and Castro, 2007	Native	Not relevant	-	18.65	-
Astyanax fasciatus (Cuvier, 1819)	Native	Constant	-	39.59	29.3
Astyanax paranae Eigenmann, 1914	Native	Constant	88.53	58.18	67.55
Piabarchus stramineus Eigenmann, 1908	Native	Constant	1027.02	140.68	143.43
Piabina argentea Reinhardt, 1867	Native	Constant	510.59	48.19	-
Serrapinus notomelas (Eigenmann, 1915)	Native	Not relevant	-	-	39.45
Crenuchidae					
Characidium gomesi (Travassos, 1956)	Native	Not relevant	-	893.52	-
Characidium zebra Eigenmann, 1909	Native	Not relevant	-	175.44	-
Erythrinidae					
Hoplias sp. 2	Native	Not relevant	-	-	39.45
Parodontidae					
Parodon nasus Kner, 1859	Native	Not relevant	-	58.31	-
GYMNOTIFORMES					
Apteronotidae					
Apteronotus aff. *albifrons* (Linnaeus, 1966)	Non-native	Constant	98.16	176.94	103.76
Gymnotidae					
Gymnotus inaequilabiatus (Valenciennes, 1839)	Non-native	Constant	4018.24	211.53	530.14
Gymnotus pantanal Fernandes, Albert, Daniel-Silva, Lopes, Crampton and Almeida-Toledo, 2005	Non-native	Not relevant	-	-	95.24
Gymnotus paraguensis Albert and Crampton, 2003	Non-native	Constant	-	30.08	47.62
Gymnotus sp.	Non-native	Not relevant	-	86.10	-
Gymnotus sylvius Albert and Fernandes-Matioli, 1999	Non-native	Constant	-	60.15	95.24
PERCIFORMES					
Cichlidae					
Crenicichla britskii (Kullander, 1982	Native	Constant	67.73	-	162.34
Oreochromis niloticus (Linnaeus, 1758)	Non-native	Constant	137.41	106.35	326.22
SILURIFORMES					
Cetopsidae					
Cetopsis gobioides (Kner, 1858)	Native	Constant	49.08	60.15	47.62
Heptapteridae					
Cetopsorhamdia iheringi (Schubart and Gomes, 1959)	Native	Not relevant	-	30.08	-
Imparfinis mirini Haseman, 1911	Native	Constant	-	48.19	62.02
Imparfinis schubarti (Gomes, 1956)	Native	Constant	-	-	78.9
Phenacorhamdia tenebrosa (Schubart, 1964)	Native	Not relevant	-	39.53	-
Rhamdia quelen (Quoy and Gaimard, 1824)	Native	Not relevant	-	-	78.98
Loricariidae					
Hypostomus ancistroides (Ihering, 1911)	Native	Constant	-	175.44	78.9
Hypostomus cf. *paulinus* (Ihering, 1905)	Native	Constant	39.59	991.75	146.42
Hypostomus sp.	Native	Constant	-	93.02	-
Otothyropsys sp.	Native	Not relevant	39.59	-	-
Rineloricaria pentamaculata (Langeani and de Araujo, 1994)	Native	Constant	39.59	31.01	-
SYNBRANCHIFORMES					
Synbranchidae					
Synbranchus marmoratus Bloch, 1975	Native	Constant	360.01	-	68.83

*Frequency of occurence: Constant when C≥50%, not relevant when 50%> C≥20% and accidental when C <20%. N/ha^{-1}: Number of species per hectare.

were recorded among the Characiformes with 13 (38%) species collected, followed by Siluriformes with 12 (35%) species, while the highest abundance values were recorded for the Gymnotiformes, accounting for 37% of the collected specimens, followed by Siluriformes (28%) and the Characiformes (26%). Among those families, the most representative species collected were Gymnotidae (34%), followed by Loricariidae (18%) and Characidae (16%).

Abundance and frequency of fish species occurrence

The total abundance, both in number and weight, was not significantly different among sampling sites (ANOVA; p>0.05). Evident changes were observed in the abundance distribution of the 10 most abundant species among the sampling sites (Figure 2). The most abundant species at headwater, both in number (4,018 ind. ha^{-1}) and weight (83.00 kg. ha^{-1}), was *G. inaequilabiatus* (Figure 2A). In addition, at middle site, *O. niloticus* and *A.* aff. *albifrons* were important contributors in weight (7.28 and 6.80 kg. ha^{-1}, respectively; Figure 2B).

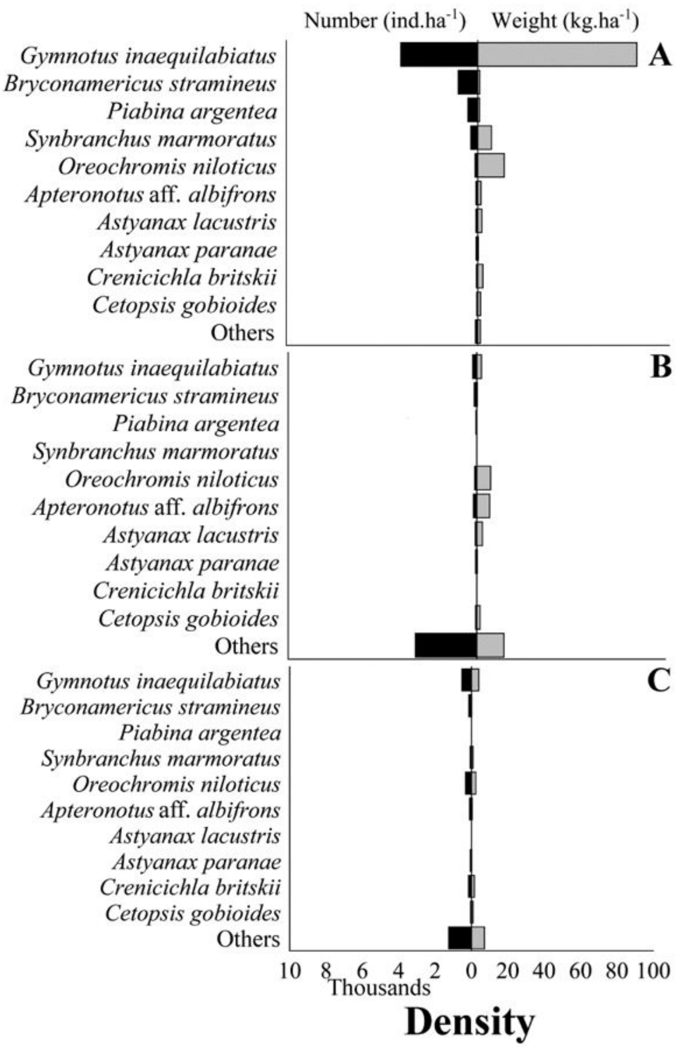

Figure 2. Density in number (ind. ha^{-1}) and weight (kg.ha^{-1}) of the 10 most abundant species captured at headwater (A). The same species are shown for the middle (B) and mouth (C) sites that were sampled in the Azul River microbasin, Piquiri river basin, Brazil, from February to November 2014.

In the set of collected species, eight non-native species were recorded: one was classified as accessory and seven were classified as constants, while *G. inaequilabiatus* and *O. niloticus* were recorded in all samplings.

The non-native species *G. inaequilabiatus* and *O. niloticus* had the 1st and 5th largest abundances, respectively (Figure 3), accounting for 35% of all samples. *G. inaequilabiatus* was collected and was dominant throughout the three sites sampled in the microbasin, while *O. niloticus* was dominant at the headwater and mouth sites.

Assemblage attributes

Species richness was higher on average at the middle site (Figure 4A). However, significant differences of this attribute were not observed among sites (p>0.05; Figure 4). In contrast, significant differences were observed in the evenness ($F_{2;9}$=15.74; p<0.01) and Shannon diversity index ($F_{2;9}$=4.47; p<0.05) among the sites (Figure 4B and C). Higher mean evenness values were observed at the mouth ($\bar{E}$=0.93; s.d.=0.03) and the middle ($\bar{E}$=0.84; s.d.=0.12) sites and were significantly different from those values at the headwater site (Tukey's test; p<0.05; Figure 4B). For the Shannon diversity index, a higher mean value was observed at the middle site ($\bar{H}$=1.80; s.d.=0.27) and differed from that at the headwater site (Tukey's test; p<0.05; Figure 4C).

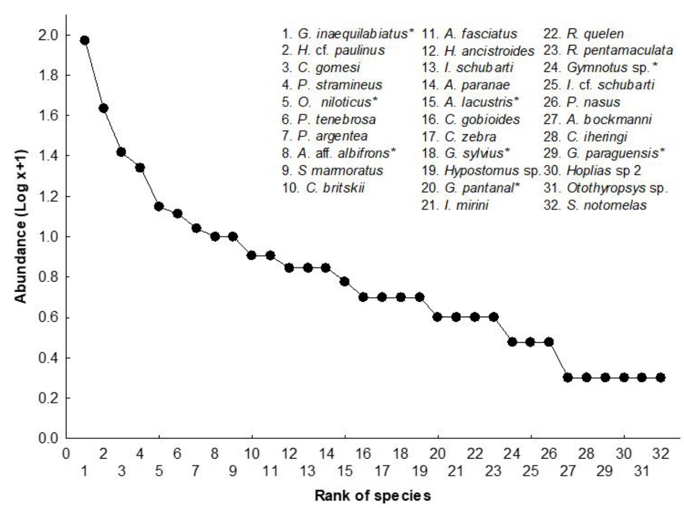

Figure 3. Species abundance curve of specimens collected in the Azul River microbasin, Piquiri River basin, Brazil, from February to November 2014, in decreasing order of abundance. (*) Non-native species that were collected.

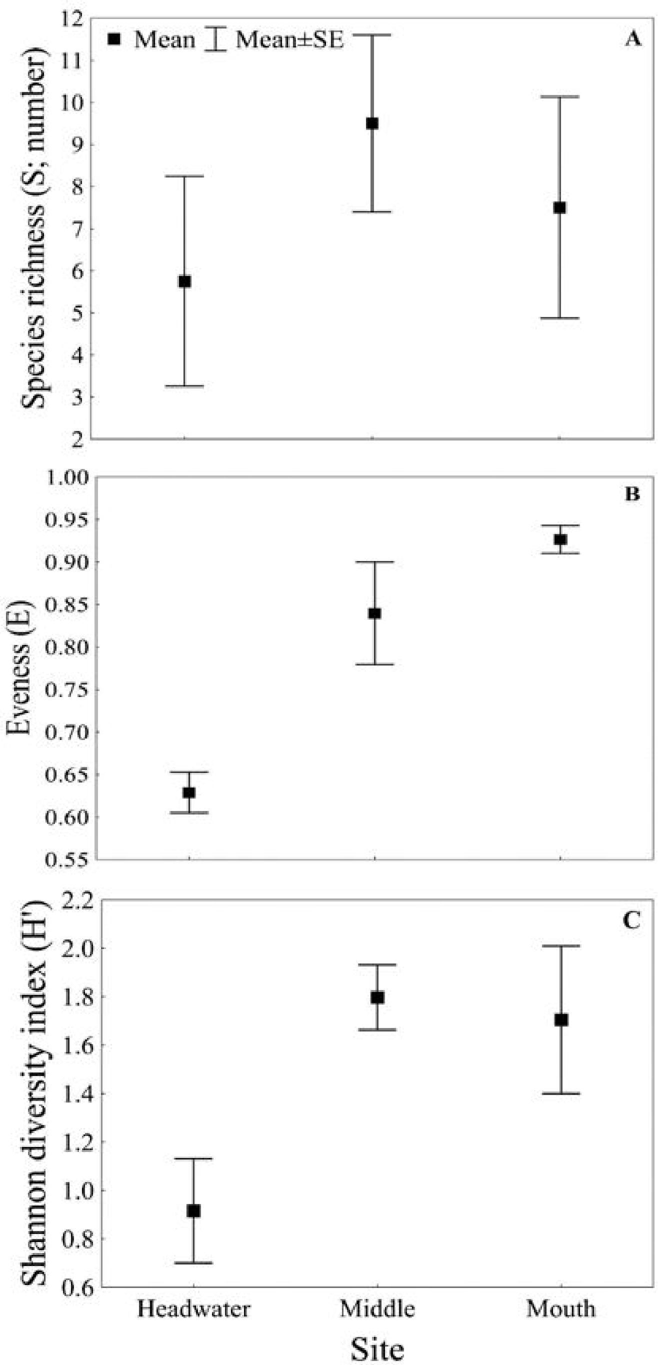

Figure 4. Mean and standard error (SE) values for the community richness attributes (S), Evenness (E) and Shannon-Wiener diversity index (H) of the fish assemblage in the Azul River microbasin, Piquiri River basin, Brazil, from February to November 2014.

Variations in composition and structure of fish assemblage

Principal coordinate analysis summarized the composition and structure of the fish assemblage and separated the sites considered in this study (Figure 5). The proportion of the variance

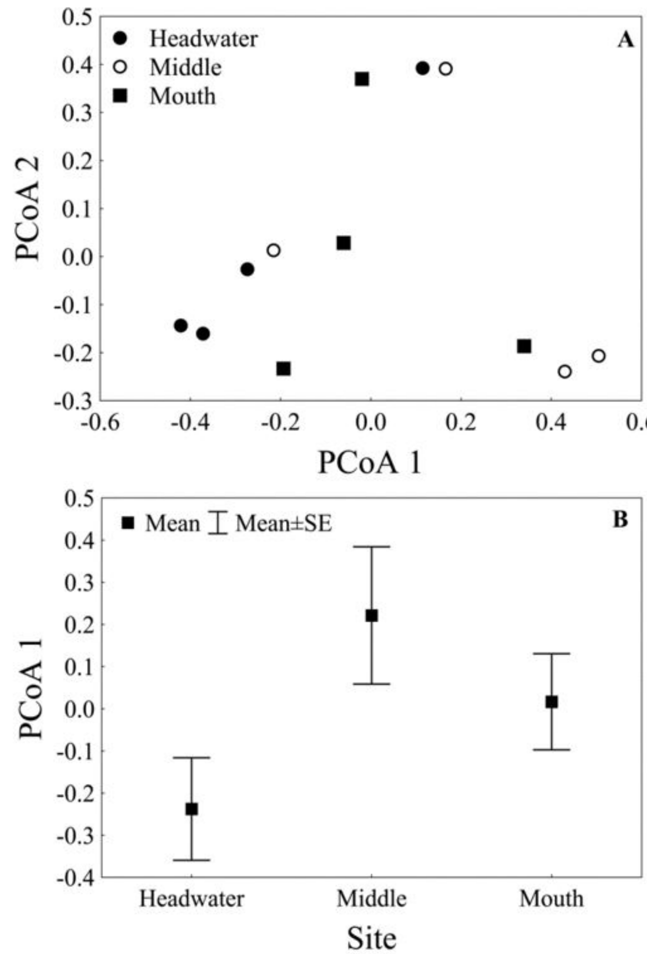

Figure 5. Principal coordinate ordination (PCoA) of the fish assemblage (A) and the variation of mean scores axis (B) among sampled sites in the Azul River microbasin, Piquiri River basin, Brazil, from February to November 2014. SE = standard error.

represented by each axis was 22% for axis 1 and 21% for axis 2, for a total of 43%.

Significant differences in the composition and structure of the fish assemblage among sites were observed (PERMANOVA; pseudo-F=2.88; Pperm<0.01), and the headwater site was distinct from the other sites (Pairwise comparisons; $p<0.05$; Figure 5B). The species that contributed the most to the differentiation from the headwater site to the other sites was *G. inaequilabiatus* (Simper; 26 and 33%, respectively; Table 2).

DISCUSSION

Our results showed differences among the sampled sites, as well as the presence of non-native species, possibly originating from fish farms surrounding the microbasin. The hypothesis that aquaculture activity influences the occurrence, abundance and dispersion of non-native fish species was corroborated, especially

Table 2. Summary of the discriminant analysis for the dissimilarity of the proportion in density (individuals/hectare) for the species collected in three sites in the Azul River microbasin, Piquiri River basin, Brazil, from February to November 2014.

Specie	Average dissimilarity (%)	Contribution (%)	Cumulative contribution (%)	Mean abundance	
				Headwater	**Middle**
Gymnotus inaequilabiatus	26.23	29.58	29.58	1,004.52	52.88
Characidium gomesi	10.10	11.40	40.98	0.00	223.38
Phenacorhamdia tenebrosa	7.65	8.63	49.61	0.00	112.69
Hypostomus cf. *paulinus*	7.53	8.49	58.10	9.90	247.94
Piabarchus stramineus	5.63	6.35	64.45	256.76	35.17
Characidium zebra	3.82	4.30	68.75	0.00	43.86
Hypostomus ancistroides	3.82	4.31	73.06	0.00	43.86
				Headwater	**Mouth**
Gymnotus inaequilabiatus	33.32	41.96	41.96	1,004.52	132.54
Piabarchus stramineus	6.99	8.81	50.77	256.76	35.86
Oreochromis niloticus	4.26	5.37	56.14	34.35	81.55
Synbranchus marmoratus	4.00	5.04	61.18	90.00	17.21
Astyanax fasciatus	3.74	4.71	65.89	0.00	76.93
Crenicichla britskii	3.42	4.31	70.20	16.93	40.59
Astyanax paranae	3.29	4.14	74.34	22.13	16.89
				Middle	**Mouth**
Characidium gomesi	11.49	13.21	13.21	223.38	0.00
Hypostomus cf. *paulinus*	9.16	10.54	23.75	247.94	36.61
Phenacorhamdia tenebrosa	8.61	9.90	33.65	112.69	9.88
Gymnotus inaequilabiatus	7.74	8.90	42.55	52.88	132.54
Characidium zebra	4.42	5.08	47.63	43.86	0.00
Hypostomus ancistroides	4.16	4.78	52.41	43.86	19.73
Oreochromis niloticus	3.97	4.67	56.98	26.61	81.55

as it relates to *O. niloticus*, which was the 5th most abundant species among the 32 collected in the microbasin and the main species produced by the fish farms in the area.

The ichthyofauna composition of the Azul River microbasin showed a high richness of species belonging to the Characiformes and Siluriformes orders, in accordance with the expected pattern for Neotropical freshwater environments (LOWE-MCCONNELL, 1999; REIS *et al*., 2016). However, the high representativeness of the abundance of non-native species of the Gymnotiformes and Perciformes orders highlights the potential negative impacts on the structure of the fish assemblages of the Azul River due to species introductions. The species *G. inaequilabiatus* and *O. niloticus* had important influences on the structure of the fish assemblage along the longitudinal gradient and had high contributions in relation to the abundance in both number and weight. Moreover, the constancy of occurrence in the samplings indicated the establishment of the species in the environment.

Previous studies have reported the introduction of the *Gymnotus* genus in several Brazilian basins (ROTTA, 2004; GRAÇA and PAVANELLI, 2007; BAUMGARTNER *et al*., 2012), especially because of its wide use as live bait by fishermen, thus facilitating accidental translocation (JÚLIO JUNIOR *et al*., 2009). FROTA *et al.* (2014) reported that *G. inaequilabiatus* specimens captured in the Paraguai River are usually sold and used as live bait along the Paraná River basin. This fact, combined with the unique biological characteristics of the species and the habitat changes resulting from human impacts such as the reduction of riparian vegetation area, facilitate their establishment and dominance.

The representatives of the Gymnotiformes order have specific characteristics, such as the capacity to emit electrical pulses used in communication, foraging, navigation and orientation in relation to the substrate (CRAMPTON and ALBERT, 2006). These characteristics facilitate the establishment of the species in environments with high turbidity and the presence of aquatic vegetation (REZENDE *et al*., 2009), as observed at the sites sampled in this study. The Azul River microbasin, which is widely exploited by agriculture and livestock activities, is characterized by a reduced area of riparian vegetation, causing high turbidity of the water and the development of dense marginal aquatic vegetation, which enables the successful colonization by the representatives of the Gymnotiformes order.

Likewise, the intense aquaculture activity in the Azul River microbasin follows the trend of Brazilian aquaculture. The production in the Azul River microbasin is based on non-native species (89% of storage), which enables the introduction of species such as the Nile tilapia (*O. niloticus*) that is listed to have the fifth largest density in the Azul River microbasin and occurred in all samplings in this study. FORNECK *et al*. (2016) conducted research in the São Camilo River, which is a tributary along the left margin of the Piquiri River and is under the influence of aquaculture activity.

The study observed similar abundance patterns, with *O. niloticus* ranked as the 2nd most abundant among the 31 species collected.

Tilapia is the most-cited example of the negative impacts of aquaculture (DIANA, 2009; ATTAYDE *et al*., 2007; OVENDEN *et al*., 2014; GU *et al*., 2015) and is largely related to a high risk of biological invasion (BRITTON and ORSI, 2012; FORNECK *et al*., 2016). Studies have indicated that tilapia can reduce native fish stocks since it competes for resources and spawning sites (ATTAYDE *et al*., 2007) and promotes predation of eggs and larvae (ARTHINGTON *et al*., 1994), hybridization with native species, introduction of pathogens and parasites, as well as changes in water quality (CANONICO *et al*., 2005). DEINES *et al*. (2016) conducted a systematic literature review to address the introduction of tilapia worldwide and verified that a high proportion of the introductions were associated with environmental impacts.

The constant occurrence of *O. niloticus* in the Azul River microbasin suggests that escapes from aquaculture ponds are frequent and inevitable (ORSI and AGOSTINHO, 1999; DIANA, 2009; AZEVEDO-SANTOS *et al*., 2011). Escapes can occur at all stages of fish production (AZEVEDO-SANTOS *et al*., 2011) because no efficient control system exists to avoid them. Fish of all sizes can escape with the effluent water, when ponds are drained for harvesting, or through other cultural practices (DIANA, 2009), amplifying the frequency of the release of propagules over time (LOCKWOOD *et al*., 2009). Several studies have suggested that propagule pressure (size and number) is, in general, the most important factor in determining establishment success (LOCKWOOD *et al*., 2007; SIMBERLOFF, 2009; BLACKBURN *et al*., 2015) and fish farming represents a constant source of propagules for species introduction.

Some non-native fish species that are produced in the microbasin were not recorded in the river (e.g., *C. carpio*, *B. amazonicus, M. macrocephalus* and *C. gariepinus*), potentially due to behavioral peculiarities of the species, selectivity of the fishing gear, and a low stocking density of these species (low propagule pressure). However, the risks for conservation biology cannot be neglected due to the high invasive potential of these species (FORNECK, *et al*., 2016; BRITTON and ORSI, 2012). For example, *Clarias gariepinus* has wide environmental tolerance and physiological plasticity, which has favored the escape and subsequent establishment in many countries, and the species is considered to be an emerging invader (ORSI and AGOSTINHO, 1999; VITULE *et al*., 2006; WEYL *et al*., 2016). At the river basin scale, escape from aquaculture facilities accounted for 66% of the introductions into the wild in Brazil, and there is evidence of individuals dispersing rapidly after escape, surviving and reproducing at multiple sites over a wide range of habitats (WEYL *et al*., 2016).

Issues concerning introductions are serious in Brazil because although the impacts of introduced fish species are a fact, the species continue to be introduced indiscriminately (VITULE, 2009; DAGA *et al*., 2016; RIBEIRO *et al*., 2017; ASSIS *et al*., 2017; MAGALHÃES and JACOBI, 2017). A common practice of the Brazilian aquaculture sector is the lack of planning and proper management of the activity, which, combined with the inattentiveness to the ecological knowledge, leads to negative consequences for biodiversity conservation (AZEVEDO-SANTOS *et al*., 2011; LIMA *et al*., 2016, PELICICE *et al*., 2017). According to AZEVEDO-SANTOS *et al*. (2015), training in aquaculture courses focuses on production and trade, and little or no attention is given to environmental issues, thereby contributing to inadequate environmental management practices.

CONCLUSION

Increased occurrence, abundance and propagation of non-native fish species were observed, possibly due to anthropogenic activities such as aquaculture. There is a special concern about the constancy of the occurrence of *Oreochromis niloticus* in the microbasin once the aquaculture activity has been fully expanded and *O. niloticus* is the main species produced. This would increase the propagule pressure and increase the biological invasion risk with consequential loss of biodiversity.

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