Oecologia Australis
25(1):142-153, 2021
https://doi.org/10.4257/oeco.2021.2501.13

ICHTHYOFAUNA OF TRIBUTARY STREAMS OF THE CINZAS RIVER BASIN, PARANAPANEMA RIVER, BRAZIL

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Abstract: Streams are environments that are very affected by human activities such as pollution, deforestation of riparian forests and introduction of exotic species. In this context, it is important to know its biodiversity for monitoring and conservation. The present study inventoried the fish fauna of three tributary streams of the Jacarezinho River (Cinzas River basin, Paranapanema River) with different environmental characteristics: Água dos Anjos, Monjolinho and Ubá streams. Quarterly collections were performed in the period between October 2012 and July 2013 at the headwaters, in the middle and at the mouth of each stream, using electrofishing. We analyzed the parameters of species richness, total abundance, relative abundance, capture constancy, rarefaction curves of Coleman, and richness estimators ACE and ICE. We captured a total of 7102 individuals, distributed in six orders, 12 families and 33 species. The most abundant order was Characiformes (76.15 %), while the most abundant species were: *Bryconamericus iheringii* (39.5 %), *Astyanax bockmanni* (19.36 %), and *Geophagus brasiliensis* (7.52 %). The highest species richness was found in Água dos Anjos stream (26), followed by Monjolinho stream (25), and the Ubá stream (15). We recorded the occurrence of four non-native species (*Bryconamericus exodon, Gymnotus inaequilabiatus, Poecilia reticulata* and *Oreochromis niloticus*), which represent a risk to the biodiversity of the studied streams. The Ubá stream was considered the most conserved, but the presence of *P. reticulata* shows that it has also suffered from anthropic impacts. This non-native species is constantly associated to degraded environments, mainly when it occurs in high abundance. Our results suggest that the fish assemblages studied present different structures, probably due to the interaction among historical, abiotic, biotic, and anthropic factors. This study has been carried out for the first time in the Cinzas River basin and can help provide theoretical input for the elaboration of monitoring and conservation plans.

Key words: Anthropic interferences; neotropical fish; richness; species introduction; stream ecology.

INTRODUCTION

Despite the extensive hydrographic network represented by streams in Brazilian water basins, few studies have been carried out involving its ichthyofauna, compared to those of large rivers and reservoirs. Over 50 % of stream fish species are medium to small size, normally up to 150 mm standard length, with little or no commercial value for consumption, compared with dam and river fish (Castro *et al.* 2003). However, knowledge about the composition and distribution of stream fish species is useful in assessing the integrity of aquatic ecosystems. Understanding how biological communities are structured in the natural environment makes it possible to implement management and actions that minimize the degradation of these ecosystems, aiming at their maintenance and conservation (Olden *et al.* 2010).

The impacts of anthropogenic activities have resulted in severe damage to Neotropical aquatic ecosystems, significantly affecting hydrology and local biological diversity (Lobo *et al.* 2015, Gonçalves *et al.* 2019). The sequence in the streams' structure can be broken due to anthropic interferences, capable of generating discontinuities in diversity patterns (Walker *et al.* 2019). The Paraná River basin is the second in terms of the drainage area of Brazil (Stevaux *et al.* 1997) and is set in a vast region with large urban centers, industrial and agricultural, being the most intensively explored region of the country (Agostinho & Júlio Jr 1999). The Cinzas River basin is located in the Paranapanema River, inserted in the upper Paraná River system, and is the main river of the North Pioneer mesoregion of Paraná, which covers 46 municipalities located in the Second and Third Plateau of the Paraná State, in Brazil.

Studies that contribute to the knowledge of the biological diversity of the south of Brazil bring complementary information about the richness and abundance of endemic, threatened, or largely distributed species. These studies help to ground future research that could contribute to the conservation of fish biodiversity in Neotropical streams (Cavalheiro & Fialho 2020). There are several studies with the ichthyofauna from streams of the upper Paraná River system (e.g. Casatti 2004, Aquino *et al.* 2009, Dias & Tejerina-Garro 2010, Felipe & Súarez 2010, Araújo *et al.* 2011, Cetra *et al.* 2012). However, only one focuses on the ichthyofauna from Cinzas River basin (Costa *et al.* 2013), making it clear that there is still a large gap in the knowledge of the ichthyofauna from this region. Therefore, the objective of this study was to inventory the ichthyofauna from second and third order tributary streams of Jacarezinho River, Cinzas River basin, upper Paraná River system; and to analyze the community structure from the species richness, abundance, relative frequency of occurrence and capture constancy of species.

MATERIAL AND METHODS

Study area

The Cinzas River basin is inserted in the Paranapanema River basin, which is located in the upper Paraná River system. The total area of the Cinzas River basin is 9,612.8 km² (about 0.01 % of upper Paraná River area) and its length is 240 km. Its headwaters are in the Serra de Furnas, in the Piraí do Sul city (24°31'33" S, 49°56'56" W), and its mouth is in the Paranapanema River, on the border of Santa Mariana and Itambaracá cities (22°56'11" S, 50°31'35" W), Paraná, Brazil (Ipardes 2004, Sema/PR 2013). The use of the soil of the basin is mainly related to areas of multiple use, with grazing, grasslands and reforestation, while the industries in the region are mainly of the agro-industrial sector, such as sugar plants, alcohol distilleries, refrigerators and milk products (Sema/PR 2013). The Jacarezinho River empties into the right margin of the Cinzas River (23°8'6.05" S, 50°14'23.61" W) in Barra do Jacaré city, State of Paraná. It has an area of 661.25 km², with its headwaters in the municipality of Siqueira Campos, Paraná (Sema/PR 2013).

The ichthyofauna was sampled in three streams tributary of Jacarezinho River: Água dos Anjos (2nd order), Monjolinho (3rd order) and Ubá (2nd order). The first two streams empty on the right margin and the last on the left margin of the Jacarezinho River. Each stream was sampled in three sections: headwater, middle and mouth (Figure 1; Supplementary Material, Figure S1). The environmental characteristics of each stream are shown in Table 1.

Fish collection

Each stretch was sampled quarterly from October 2012 to July 2013, totaling four field samples. Fish were collected in accordance with a license from Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/IBAMA - Number: 30357-3; Authentication code: 76974589; Certificate number 09/2012 of the Animal Ethics Commission

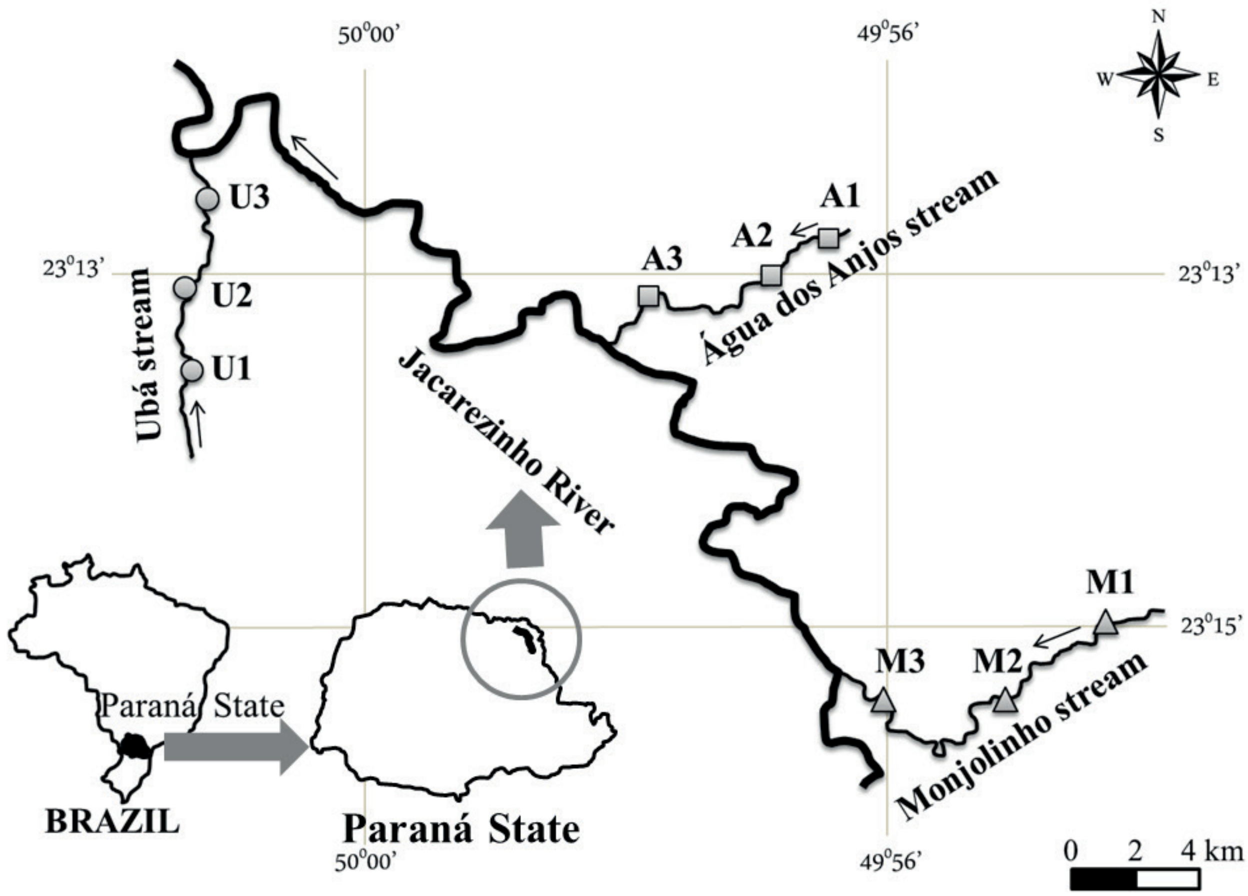

Figure 1. Sampling units in the tributary streams of the Jacarezinho River, Cinzas River basin, Paranapanema River: Ubá stream (U1, U2 and U3), Água dos Anjos stream (A1, A2 and A3) and Monjolinho stream (M1, M2 and M3). The arrows indicate the direction of water flow.

of the Universidade Estadual do Norte do Paraná) using electrofishing with alternating current of 127 volts and 6 amps. Three successive passes of dip nets were performed on each sampling unit at the downstream-upstream direction in a length of 50 meters previously blocked upstream and downstream by trawls with three mm meshes.

Captured individuals were anesthetized and sacrificed by immersion in eugenol (active ingredient: phenolic eugenol, 4-Allyl-2-methoxyphenol-$C_{10}H_{12}O_2$, derived from stems, flowers and leaves of *Eugenia caryophyllata* and *Eugenia aromatica* trees; Griffiths 2000). Posteriorly, the specimens were fixed in 10 % formalin solution and then preserved in 70 % ethanol.

The identification of species was carried out according to Oyakawa *et al.* (2006), Ota *et al.* (2018), and Malabarba & Malabarba (2020). Representative specimens of ichthyofauna were deposited at the fish collection of the Museu de Ictiologia do Núcleo de Pesquisas em Limnologia Ictiologia e Aquicultura, of the Universidade Estadual de Maringá (Nupélia/UEM).

Data analysis

The species richness, total abundance, relative abundance, and capture constancy were analyzed (sensu Magurran 2011). Regarding capture constancy, species with a frequency of occurrence in up to 25 % were classified as accidental, between 25 % and 50 % as accessory, and above 50 % as constant. In addition, it was carried out rarefaction curves of Coleman and richness estimators ACE (Abundance Coverage-based Estimator) and ICE (Incidence Coverage-based Estimator; Chazdon *et al.* 1998) independently for each stream, and for all the streams together through EstimateS 9 software (Colwell, 2013). The equation of ACE corresponds to: $ACE = S_{abund} + (S_{rare}/C_{ace}) + (F_1/C_{ace}) \cdot \gamma_{ace}^2$, where S_{abund} = number of abundant species, S_{rare} = number of rare species, C_{ace} = abundance of sample coverage estimator, F_1

Table 1. Environmental characteristics of the tributary streams of the Jacarezinho River, Cinzas River basin, Paranapanema River: Ubá stream (U1, U2 and U3), Água dos Anjos stream (A1, A2 and A3) and Monjolinho stream (M1, M2 and M3).

Streams and sampling units	Substrate	Riparian vegetation	Average depth (cm)	Average width (m)
Água dos Anjos A1: 23°13'15,80" S, 49°57'12,46" W A2: 23°13'24,80" S, 49°57'26,19" W A3: 23°13'39,60" S, 49°58'32,11" W	Abundant pebbles and gravels and lots of sand, with an unstable and not very heterogeneous bottom	Restricted or absent, due to human activities mainly related to the predominance of pasture on the banks	58.08	4.50
Monjolinho M1: 23°15'56.35" S, 49°54'57,53" W M2: 23°16'28,10" S, 49°55'46,46" W M3: 23°16'25,46" S, 49°56'42,01" W	Some sections with sand, pebbles and gravel, and others with stable rock bottom	Stretches with broad shrubs and trees and stretches with anthropic interference by pasture	56.55	6.77
Ubá U1: 23°14'15,00" S, 50°2'13,00" W U2: 23°13'43,82" S, 50°2'15,25" W U3: 23°12'59,76" S, 50°2'8,85" W	Abundant rock, pebbles, and gravel, in addition to aquatic vegetation, leaves and submerged trunks	Composed of a large number of preserved trees and shrubs, with little anthropic influence	52.97	5.22

= frequency of singletons and γ_{ace}^2 = F_1 variation coefficient estimated for rare species. In turn, for ICE: ICE = S_{freq}. + S_{rare}/C_{ice} + Q_i/C_{ice} . γ_{ice}^2, in which S_{freq}. = number of frequent species, S_{rare} = number of rare species, C_{ice} = incidence of the sample coverage estimator, Q_i = species richness occurring in i samples, and γ_{ice}^2 = Q_i variation coefficient estimated for rare species.

RESULTS

A total of 7,102 individuals were captured, distributed in six orders, 12 families and 33 species (Supplementary Material, Figures S2 and S3). The highest species richness was found in Água dos Anjos stream (26 species), followed by Monjolinho stream (25 species), and, finally, the Ubá stream (15 species) (Table 2). The accumulation, rarefaction and extrapolation of species curves showed that the richness of estimated species for all streams were 34.65 (ACE) and 35.71 (ICE). Considering each stream separately, two additional species could be registered for Água dos Anjos stream (ACE: 28.11; ICE: 27.96), three or four for Monjolinho stream (ACE: 28.33; ICE: 28.83) and two to four for Ubá stream (ACE: 16.71; ICE: 18.98). The asymptote of accumulation of species curve has not been achieved in any of the streams (Figure 2).

Among the 33 species that were caught, nine were shared among the three streams and nine occurred in only one of them. The most abundant order was Characiformes with relative abundance of 76.15 %, followed by Siluriformes, with 10.98 % and Cichliformes, with 8.46 %. Regarding to richness, Characiformes and Siluriformes obtained the same representativeness, each with 36.36 % of the 33 species collected, followed by Cichliformes, with 15.15 %, Cyprinodontiformes, Gymnotiformes and Synbranchiformes, which together amounted to 12.13 %. The most abundant families were Characidae (65.94 %), Cichlidae (8.46 %) and Loricariidae (6.83 %); and the most abundant species were *Bryconamericus iheringii* (39.5 %), *Astyanax bockmanni* (19.36 %) and *Geophagus brasiliensis* (7.52 %). We found four non-native species of the Paranapanema River, where

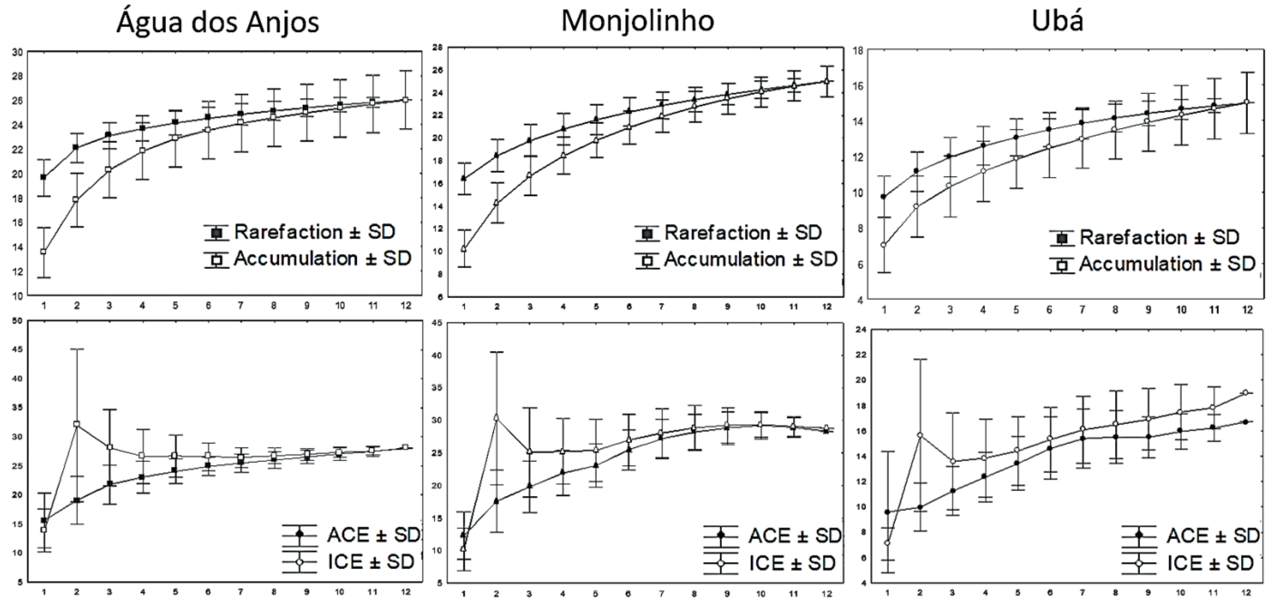

Figure 2. Species rarefaction, accumulation, and estimation in the tributary streams of the Jacarezinho River, Cinzas River basin, Paranapanema River: Água dos Anjos stream, Monjolinho stream and Ubá stream. SD = Standard Deviation.

Table 2. Taxonomic position and abundance of species of fish from Água dos Anjos stream, Monjolinho stream and Ubá stream. Capture constancy (co - constant, ace - accessory and aci – accidental) and voucher number of species of fish from Cinzas River basin, Paranapanema River. Species marked with asterisk are non-native of the streams studied.

ORDER / Family / Species	Água dos Anjos stream	Monjolinho stream	Ubá stream	Capture constancy	Voucher
CHARACIFORMES					
Parodontidae					
Apareiodon ibitiensis Amaral Campos, 1944	11	103	0	ace	NUP 14789
Apareiodon piracicabae (Eigenmann, 1907)	26	305	0	ace	NUP 14785
Crenuchidae					
Characidium zebra Eigenmann, 1909	119	147	0	co	NUP 14797
Characidae					
Astyanax lacustris (Lütken, 1875)	58	14	0	ace	NUP 14802
Astyanax bockmanni Vari & Castro, 2007	706	632	37	co	NUP 14786
Astyanax paranae Eigenmann, 1914	7	126	99	ace	NUP 14776
Astyanax sp.	9	22	19	ace	NUP 14774
Bryconamericus iheringii (Boulenger, 1887)	1415	1200	190	co	NUP 14787
Bryconamericus exodon Eigenmann, 1907*	31	55	0	ace	NUP 14788

Table 2. Continues on next page...

Table 2. ...continued

ORDER / Family / Species	Água dos Anjos stream	Monjolinho stream	Ubá stream	Capture constancy	Voucher
Oligosarcus paranensis Menezes & Géry, 1983	26	6	0	ace	NUP 14790
Serrapinnus notomelas (Eigenmann, 1915)	30	0	1	aci	NUP 14798
Erythrinidae					
Hoplias cf. *malabaricus* (Bloch 1794)	13	1	0	aci	NUP 14793
SILURIFORMES					
Trichomycteridae					
Trichomycterus diabolus Bockmann, Casatti & de Pinna, 2004	0	2	53	ace	NUP 14772
Callichthyidae					
Corydoras aeneus (Gill, 1858)	16	0	0	aci	NUP 14791
Loricariidae					
Hypostomus ancistroides (Ihering, 1911)	121	131	75	co	NUP 14782
Hypostomus hermanni (Ihering, 1905)	1	29	1	ace	NUP 14778
Hypostomus nigromaculatus (Schubart, 1964)	0	5	0	aci	NUP 14781
Hypostomus paulinus (Ihering, 1905)	0	43	0	aci	NUP 14780
Hypostomus strigaticeps (Regan, 1908)	0	70	0	aci	NUP 14783
Hypostomus sp.	0	1	0	aci	NUP 14777
Otothyropsis biamnicus Calegari, Lehmann & Reis, 2013	0	1	7	aci	NUP 14771
Heptapteridae					
Imparfinis borodini Mees & Cala, 1989	2	1	0	aci	NUP 14796
Imparfinis schubarti (Gomes, 1956)	34	2	65	co	NUP 14773
Rhamdia quelen (Quoy & Gaimard, 1824)	22	72	26	co	NUP 14784
GYMNOTIFORMES					
Gymnotidae					
Gymnotus inaequilabiatus (Valenciennes, 1842)*	24	0	0	aci	NUP 14794
CYPRINODONTIFORMES					
Poeciliidae					
Phalloceros harpagos Lucinda, 2008	89	61	0	aci	NUP 14792
Poecilia reticulata Peters, 1859*	98	0	21	ace	NUP 14803

Table 2. Continues on next page...

Table 2. ...continued

ORDER / Family / Species	Água dos Anjos stream	Monjolinho stream	Ubá stream	Capture constancy	Voucher
SYNBRANCHIFORMES					
Synbranchidae					
Synbranchus aff. *marmoratus* Bloch, 1795	17	0	3	ace	NUP 14801
CICHLIFORMES					
Cichlid					
Cichlasoma paranaense Kullander, 1983	1	0	0	aci	NUP 14800
Crenicichla britskii Kullander, 1982	59	3	0	ace	NUP 14795
Crenicichla haroldoi Luengo & Britski, 1974	2	0	0	aci	NUP 14799
Geophagus brasiliensis (Quoy & Gaimard, 1824)	526	5	3	ace	NUP 14804
Oreochromis niloticus (Linnaeus, 1758)*	0	0	2	aci	NUP 14775
Total Abundance:	3463	3037	602		

the Cinzas River basin is inserted: *Bryconamericus exodon, Gymnotus inaequilabiatus, Poecilia reticulata* and *Oreochromis niloticus* (Table 2).

DISCUSSION

The greatest species richness and abundance of Characiformes and Siluriformes reflected a common pattern to streams of the Paranapanema River (Jarduli *et al.* 2020), upper Paraná River system (Abes & Agostinho 2001, Casatti *et al.* 2001, Castro *et al.* 2003, Viana *et al.* 2013, Fagundes *et al.* 2015) and Neotropics (Lowe-McConnell 1999). Another pattern observed in this basin was the high diversity of characids and loricariids (Langeani *et al.* 2005, Araújo *et al.* 2011). However, the high abundance of the Cichlidae family is not common for streams from the upper Paraná River system, and, in this study, it was the second most abundant family. This result was mainly influenced by the high abundance of *Geophagus brasiliensis* in the Água dos Anjos stream, which is the most impacted among the three streams, mainly due to activities of pasture in the adjacent areas. Two of the most abundant species in this work, *B. iheringii* and *G. brasiliensis*, are considered generalist species, spreading out in different environmental characteristics, according to Caetano *et al.* (2016), which proves its tolerance to anthropic impacts.

The species richness found in this study (33) was similar to that obtained by Costa *et al.* (2013), which held collections in a tributary of Laranjinha River (Cinzas River basin). However, only twenty species (65 %) were shared between this study and Costa *et al.* (2013), which is a relatively low number of shared species, considering that both studies were developed in the same watershed. This great segregation of species in the same watershed may indicate the presence of different environmental characteristics in their lotic systems, which would select different species from the main watershed (Garutti 1988).

The prospect of greater potential richness for the three streams was verified by ACE and ICE estimators. This result was expected according to Colwell *et al.* (2012), because it is difficult to capture all species from their area or habitat. Some species can be rare and difficult to record, which often requires a greater sampling effort. However, over 90 % of the richness estimated at ACE and ICE was recorded in this study, showing a significant representation of the studied streams. However, the number of accidental species detected in Monjolinho stream probably leaded this stream to have the greatest potential for recording more species. Regarding the capture constancy, the highest number of constant species in Ubá stream during the sampling period may indicate a greater presence of habitats favoring permanent species. In Monjolinho stream, more accidental species were found; hypothetically, they join to the resident ones for some short time to feed or to reproduce (Lowe-McConnell 1999). The capture constancy reflects the ability of species to exploit at any given time the resources available in the environment (Lemes & Garutti 2002). Thus, there are indications that the environmental characteristics of Monjolinho stream suffered more physical and hydrological changes over time than the Ubá stream, which may have favored accidental species. Accidental species can present characteristics of generalist species, which can reveal high phenotypic variability, allowing adaptation of populations to habitats that are more heterogeneous (in space and/or time). Therefore, generalists may occur when conditions are more variable or tend to survive across a wider range of habitats (Vanderpham *et al.* 2013).

The sampling revealed the occurrence of some non-native species, such *Bryconamericus exodon*, *Gymnotus inaequilabiatus*, *Poecilia reticulata* and *Oreochromis niloticus*. *Bryconamericus exodon*, captured in the Água dos Anjos and Monjolinho streams, originated of Paraguai River basin, reached this basin after the construction of the Itaipu Reservoir (Serra & Langeani, 2006). *Gymnotus inaequilabiatus*, captured only in the Água dos Anjos stream, has an unknown origin. *Poecilia reticulata*, checked only in the Ubá stream, was introduced to control malaria mosquitoes and it was supposedly released by hobbyists for production purposes in fish farming systems (Langeani *et al.* 2007).

Although the Ubá stream is considered the most conserved among the three, the presence of *P. reticulata* showed that it is not immune to human impacts, as this species is constantly related to degraded environments (Caetano *et al.* 2016). *Poecilia* reticulata presents high reproduction rate and r-strategist behavior (Gomiero & Braga, 2007), phenotypic plasticity (Mise *et al.* 2015) and tolerance to low oxygenation (Kramer & Mehegan 1981, Gomiero & Braga 2006), characteristics that contribute to its wide distribution (Ota *et al.* 2018).

The non-native *O. niloticus* was captured only in the middle region of Ubá stream (U2), probably due to the escape of fish farming ponds during the rainy season, because there were three tanks in operation in less than 150 meters from this stream section. The presence of *O. niloticus* in this ecosystem is worrisome, because it has high reproductive success in several Neotropical systems (Orsi & Agostinho 1999). The species *O. niloticus* is characterized as r-strategist and extremely opportunist (Beveridge & Baird 2000), due to its high reproduction, growth, and adaptability rates over a wide range of environmental conditions. Therefore, *O. niloticus* can promote severe competition for resources with other native species.

Considering the occurrence of these non-native species, it is not known exactly in which of the streams they are most successful, as well as which species becomes invasive. Several theories about biological invasions have shown that the success of invasive species depends on biotic (Elton 1958) and abiotic factors (Davis *et al.* 2000), or an interaction between the two (Lockwood *et al.* 2005). According to Garcia *et al.* (2019), there is not a set of resources capable of predicting which species of fish can be successful in the invasion; however, both the biological characteristics and the degree of impact of the invaded area can facilitate the establishment and dispersion.

A total of 60 non-native species were recorded in the Paranapanema River basin, introduced through factors such as restocking projects, aquarium trade, crop leakage, use of live baits and biological control (Garcia *et al.* 2018, Jarduli *et al.* 2020). The introduction of species is one of the main threats to the biodiversity of freshwater ecosystems in the world (Olden *et al.* 2010; Seebens *et al.* 2017), being influenced by anthropogenic activities, such as the modification of rivers and streams by agricultural and urban activities (Wilson *et al.* 2009, Liermann *et al.* 2012). Thus, the Água dos Anjos stream may be the most affected by the impact of exotic species, as it is the one with the most anthropic impacts. This stream presented three of the four captured exotic species: *Bryconamericus exodon*, *Gymnotus inaequilabiatus* and *Poecilia reticulata*.

Our results suggest that the fish assemblages of the three streams of Cinzas River basin present different structures, probably due to the interaction among historical, abiotic, biotic, and anthropic factors. The study detected high proportion of species that do not co-occur in other streams of the basin, indicating the variability of environmental characteristics in their lotic systems. Currently, the Cinzas River basin is scarcely sampled. Therefore, this study broadens the knowledge of the fish species from this watershed, helping to provide theoretical subsidies to possible plans of monitoring and conservation of these basins, which are constantly under agricultural and urban pressure.

ACKNOWLEDGEMENTS

The authors acknowledge: the anonymous reviewers, who contributed to the improvement of the manuscript; the Grupo de Estudos em Recursos Hídricos e Ecologia Aplicada (Geprhea) of the Universidade Estadual do Norte do Paraná, for the technical and logistical support; the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) for identifying the species of fish collected; DLFC thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Programa de Pós-Graduação em Biologia Evolutiva of Universidade Estadual do Centro-Oeste, for financial support and for the scholarship granted to the first author. EFO is grateful to DIRPPG/UTFPR/*Campus* Londrina for financial support. CHZ thanks the Conselho de Desenvolvimento Científico e Tecnológico (CNPq) process 313623/2018-0.

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Supplementary Material:

Figure S1. Sampling units in the tributary streams of the Jacarezinho River, Cinzas River basin, upper Paraná River system: Ubá stream (U1, U2 and U3), Água dos Anjos stream (A1, A2 and A3) and Monjolinho stream (M1, M2 and M3).

Figure S2. Specimens of fish species from Água dos Anjos stream, Monjolinho stream and Ubá stream, Cinzas River basin, upper Paraná River.

Figure S3. Specimens of fish species from Água dos Anjos stream, Monjolinho stream and Ubá stream, Cinzas River basin, upper Paraná River.

Submitted: 20 April 2020
Accepted: 16 November 2020
Published on line: 16 december 2020
Associate Editor: José Luís Novaes