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Inventory

Ichthyofauna of Santa Helena Relevant Ecological Interest Area (REIA), Paraná, Brazil

Heleno Brandão[1*], Denise Lange[1], Daniel Rodrigues Blanco[1],

Lucas Emilio Perin Kampfert[1] & Igor Paiva Ramos[2,3]

[1]Universidade Tecnológica Federal do Paraná, Campus Santa Helena, Prolongamento da Rua Cerejeira, São Luiz, 85892-000, Santa Helena, PR, Brasil.
[2]Universidade Estadual Paulista, Instituto de Biociências, Programa de Pós-graduação em Ciências Biológicas (Zoologia), Rua Prof. Dr. Antônio Celso Wagner Zanin, 250, Distrito de Rubião Junior, 18618-689, Botucatu, SP, Brasil.
[3]Universidade Estadual Paulista, Faculdade de Engenharia de Ilha Solteira, Laboratório de Ecologia de Peixes, Departamento de Biologia e Zootecnia, Rua Monção, 226, Zona Norte, 15385-000, Ilha Solteira, SP, Brasil.
**Corresponding author: helenob@utfpr.edu.br*

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Abstract: The Relevant Ecological Interest Area (REIA), popularly known as “Refúgio Biológico de Santa Helena”, is part of the Atlantic Forest Biome and one of the 78 ecoregions mapped by IBAMA as basic unit for planning priorities focused on national biodiversity conservation. Quarterly collections were carried out from November 2017 to November 2019 to inventory the ichthyofauna of this Conservation Unit. Specimens were captured with the aid of gillnets, fishing sieve and seine. In total, 3,919 specimens belonging to two class, eight orders, 27 families and 74 species were sampled. Characiformes and Siluriformes presented the highest species richness; they accounted for 40 (54%) and 17 (23%) species, respectively. *Geophagus sveni* (181 individuals = 17%), *Trachelyopterus galeatus* (109 individuals = 10%) and *Schizodon borellii* (105 individuals = 10%) were the most abundant fish species captured with gillnet. *Moenkhausia* was the most abundant genus captured with seine and fishing sieve, with emphasis on species *Moenkhausia bonita* (930 individuals = 33%) and *Moenkhausia gracilima* (845 individuals = 30%). Moreover, two “endangered” species (*Brycon orbignyanus* and *Pseudoplatystoma corruscans*) were registered. Therefore, we present an updated inventory of species belonging to the ichthyofauna of REIA, and it may contribute to future management plans focused on this Conservation Unit.
Keywords: *Ichthyofaunistic Inventory; Conservation Unit; Endangered Species.*

Ictiofauna da Área de Relevante Interesse Ecológico (ARIE) de Santa Helena, Paraná, Brasil

Resumo: A Área de Relevante Interesse Ecológico (ARIE), popularmente conhecida como “Refúgio Biológico de Santa Helena”, faz parte do Bioma Mata Atlântica, uma das 78 ecorregiões mapeadas pelo IBAMA como unidade básica de planejamento e prioridades para a conservação da biodiversidade nacional. Foram realizadas coletas trimestrais de novembro de 2017 a novembro de 2019 para inventariar a ictiofauna desta Unidade de Conservação. Os espécimes foram capturados com o auxílio de redes de emalhar, peneira e rede de arrasto. No total, foram amostrados 3.919 exemplares pertencentes a duas classes, oito ordens, 27 famílias e 74 espécies. Characiformes e Siluriformes apresentaram a maior riqueza de espécies; somando um total de 40 (54%) e 17 (23%) espécies, respectivamente. *Geophagus sveni* (181 indivíduos = 17%), *Trachelyopterus galeatus* (109 indivíduos = 10%) e *Schizodon borellii* (105 indivíduos = 10%) foram as espécies de peixes mais abundantes capturadas com rede de espera. *Moenkhausia* foi o gênero mais abundante capturado com rede de arrasto e peneira, com destaque para as espécies *Moenkhausia bonita* (930 indivíduos = 33%) e *Moenkhausia gracilima* (845 indivíduos = 30%). Além disso, duas espécies “ameaçadas” (*Brycon orbignyanus* e *Pseudoplatystoma corruscans*) foram registradas. Assim, apresentamos um inventário atualizado das espécies pertencentes à ictiofauna da ARIE, podendo contribuir para futuros planos de manejo voltados para esta Unidade de Conservação.
Palavras-chave: *Inventário Ictiofaunístico; Unidade de Conservação; Espécies ameaçadas.*

Introduction

The Atlantic Forest biome is one of the main biodiversity hotspots worldwide (Rezende et al. 2018). This biome comprises 17 Brazilian states and originally covered approximately 1.3 million km^2 (Hirota & Ponzoni 2019). However, most of its native forest remnants were subjected to anthropic actions that have severely fragmented and degraded it; consequently, nowadays, it only covers 12% of its original area (Pires et al. 2018, Kasecker et al. 2018). Nevertheless, it is considered a biodiversity *hotspot* that plays prominent role in conservation biology; thus, protecting this biome can help stopping the endangerment of several species (Norman 2003).

The Atlantic Forest provides essential ecosystem services such as water supply, climate regulation, agriculture, fishing, electric power, and tourism (Varjabedian 2010, SOS Mata Atlântica 2022). However, it is under severe threat due to anthropic actions that lead to its degradation and continuous shrinking (SOS Mata Atlântica 2020) as well as affect fish biodiversity at different ecological levels (Bezerra et al. 2019); thus, it is urgent and necessary adopting conservation measures. Conservation Units are one of the ways to protect this biome and its biota since they help protecting the remaining fauna and flora in Brazil and abroad. However, the effective protection provided by these areas, mainly for both freshwater ecosystems and their biodiversity, remains insufficient (Azevedo-Santos et al. 2019).

Conserving aquatic habitats – and, most specifically, South American fish – is a growing challenge due to the fast anthropogenic changes taking place in the 21st century; thus, conservationists and public policy-makers (Reis et al. 2016) should pay greater attention to this topic, since the conservation of South American Freshwater fish in the so-called "Anthropocene" faces increasing challenges due to the significant number of human activities leading to large-scale environmental degradation (Pelicice et al. 2021).

The South American freshwater fish fauna is one of the most diverse on the planet; it accounts for approximately 5,160 species, although estimates point towards final diversity ranging from 8,000 to 9,000 species for continental fresh waters and nearshore marine waters combined (Reis et al. 2016). Unfortunately, all fish species in this geographic region are exposed to some endangerment level, mainly due to habitat loss and degradation processes. This context justifies the importance of taking priority actions based on scientific information to promote freshwater ecosystems' preservation and restoration, as well as to preserve natural flow regimes, connectivity, river and riparian environments and critical habitats (Pelicice et al. 2021).

Public protection policies developed for Conservation Units (CUs), with emphasis on protecting aquatic environments, are strongly recommended; ichthyofaunistic inventories are one of the ways to help developing these policies and management plans focused on freshwater fauna conservation (Azevedo-Santos et al. 2021). Species inventories help identifying watershed regions that need to be better inventoried (Jarduli et al. 2020); they are considered useful ecological indicators, since they help improving the knowledge about taxonomic groupings, featuring species' functional diversity, understanding the social value of different regions and the composition of migratory species, as well as identifying endangered species (Poff et al. 2010).

Environments presenting endangered species – which are classified as "Critically Endangered – CR", "Endangered – EN" or "Vulnerable – VU", based on the International Union for the Conservation of Nature and Natural Resources (IUCN) – should be prioritized in conservation and preservation programs (IUCN 2021), and subjected to permanent monitoring and inspections (Cavalli et al. 2018). Paraná State has 110 municipal protected areas, 14 Environmental Protection Areas (EPAs), 78 Municipal Parks, eight Municipal Forests, two Ecological Stations, two Forest Gardens, one Protected Forest, one Natural Monument, one Botanical Garden, two Ecological Reserves, and one Relevant Ecological Interest Area (REIA), which is known as "Refúgio Biológico de Santa Helena" (IAT 2020). These protected areas cover 2,878.76 km^2, which only correspond to 0.001% of total Paraná State's area (IBGE 2020).

REIA, also known as "Refúgio Biológico de Santa Helena" (RBSH) is a peninsula located within Itaipu Reservoir, in Santa Helena County, Paraná State, Brazil. It is located approximately 100 km away from Foz do Iguaçu County, in Paraná Hydrographic Basin 3, where Itaipu Binacional dam is located in (25°24'19.51"S 54°35'7.05"W). It is a private conservation unit belonging to Itaipu Binacional. RBSH area accounts for 1,482 ha of reforested native and non-native vegetation and it shelters fauna rescued during the reservoir filling season (Kliver 2010).

According to these data, CUs are scarce; therefore, it is necessary encouraging the implementation of new conservation areas and rigorous inspection procedures to ensure the preservation and conservation of natural resources, as recommended by the Sustainable Development Goals of the 2030 Agenda (ONU 2021). In addition, urgent fieldwork and collaborative collections must be carried out, while there is still time, due to imminent risk of species endangerment (Bailly et al. 2021, Engel et al. 2021), a fact that turns fish fauna inventories into important tools to help better understanding and preserving the aquatic fauna (Frota et al. 2021, Pereira et al. 2021).

We carried out an updated inventory of the ichthyofauna belonging to Santa Helena Relevant Ecological Interest Area to aid the sustainable management of this conservation unit based on knowledge about the diversity of fish species distributed.

Materials and Methods

1. *Study area*

The Conservation Unit (CU), known as "Refúgio Biológico de Santa Helena" (RBSH), was launched in 1984 to help sheltering and protecting animals that had lost their habitats due to Itaipu Binacional Reservoir formation, in October 1982. It presents strategic location, since it is part of the biodiversity corridor area covered by Paraná Biodiversity Program. In addition, it is connected to Itaipu Reservoir protection strip (Kliver 2010), a fact that further justifies the importance of maintaining and conserving this environment. This Conservation Unit is located right to the South of the former mouth of São Francisco Falso River, in Paraná River basin, which covers approximately 4,695 km and is formed by the confluence between Grande and Paranaíba rivers (Carolsfeld et al. 2003).

2. *Collection data*

Ichthyofauna sampling was carried out quarterly from November 2017 to November 2019. Fish were collected at six different sites: RB1 (24°51'15.12" S 54°21'21.12" W); RB2 (24°48'30.50" S 54°21'5.33" W); RB3 (24°49'39.97" S 54°21'27.63" W) with the aid of gill nets,

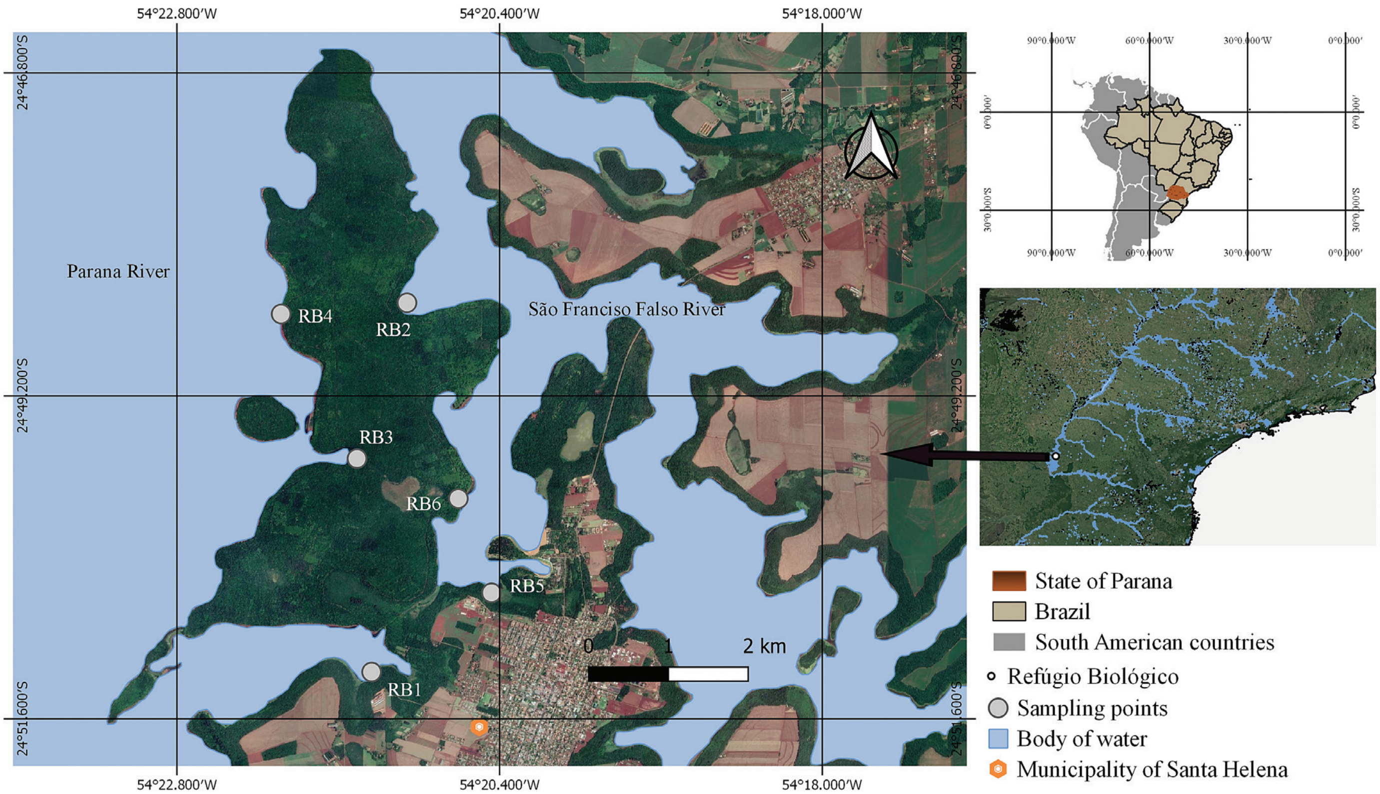

Figure 1. Brazilian map highlighting "Refúgio Biológico de Santa Helena", Itaipu Reservoir, Upper Paraná River, Brazil (arrow), and the location of the six sampling points (QGIS Geographic Information System. Open-Source Geospatial Foundation Project. http://qgis.org"; Google Earth website. http://earth.google.com/, 2020).

fishing sieve and seine; and RB4 (24°48'35.5" S 54°22'01.5" W); RB5 (24°50'39.8" S 54°20'27.6" W) and RB6 (24°49'57.8" S 54°20'42.4" W) with the aid of fishing sieve and seine (Figures 1 and 2). Sampling areas were selected to assure the highest environmental heterogeneity level to increase the likelihood of sampling the maximum number of species that occur in the ichthygeographic complex, which was defined in the current scientific research (Table 1).

The area of the gill nets used in the current study, was equal to 482 m^2 installed around the conservation unit. These gillnets were set in the water at dusk and removed at dawn (12-h exposure). A sieve (1.0 × 0.6 m) was used to sample fish from the coastal zone; whereas a seine (10 m, in length; and 2.4 mm mesh opening) was used whenever the environment presented ideal conditions for it. After the sampling points were selected, the effort was standardized in 10 minutes. The current research has the following authorizations: ICMBIO via SISBIO: n. 57181; Animal Use Ethics Committee (CEUA) 2016-031. It was registered at the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the following code: A3242E0.

Information about the conservation status of fish species was provided based on criteria set by the Red List of Threatened Species and by the International Union for Conservation of Nature (IUCN 2021). Meristic and morphometric data used in the species identification process were based on Graça & Pavanelli, (2007). Species identification was mainly performed based on Ota et al. (2018) and confirmed through specialized literature about the respective taxon. The taxonomic status classification was based on Fricke, R., Eschmeyer, W. N. & R. Van der Laan (eds) (2021), whereas endemism classification was based on Langeani et al. (2007) and Ota et al. (2018).

Fish Orders and Families were named and classified based on Betancur-R. et al. (2017), Oliveira et al. (2011), Thomaz et al. (2015) and Mirande (2019). Migratory species were classified following Agostinho et al. (2007) and Carolsfeld et al. (2003).

Specimens were deposited in the Ichthyological Collection of Federal Technological University of Paraná UTFPR, Santa Helena *Campus* (CISH), as well as in the Ichthyological Collection of Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura, NUPÉLIA (NUP) (vouchers numbers Table 2).

For the percentage calculations, absolute numbers were used, considering the effort separately (gill nets and sieve/trawlers).

Species accumulation curve based on sampling effort (Figure S1), in association with the bootstrap method (Smith & Van Belle 1984), was used to evaluate sampling efficiency. Standard error was calculated by using the function 'specaccum' in the 'vegan' package (Oksanen et al. 2014) of R 4.0 software (R Development Core Team 2019).

Results

The freshwater ichthyofauna of RBSH comprises 74 species distributed in two classes, eight orders and 27 families (Table 2, Figure 3 and Figure 4). Characiformes was the most representative order (40 species), it was followed by Siluriformes (15 species) and Cichliformes (9 species). Characidae recorded the largest number of

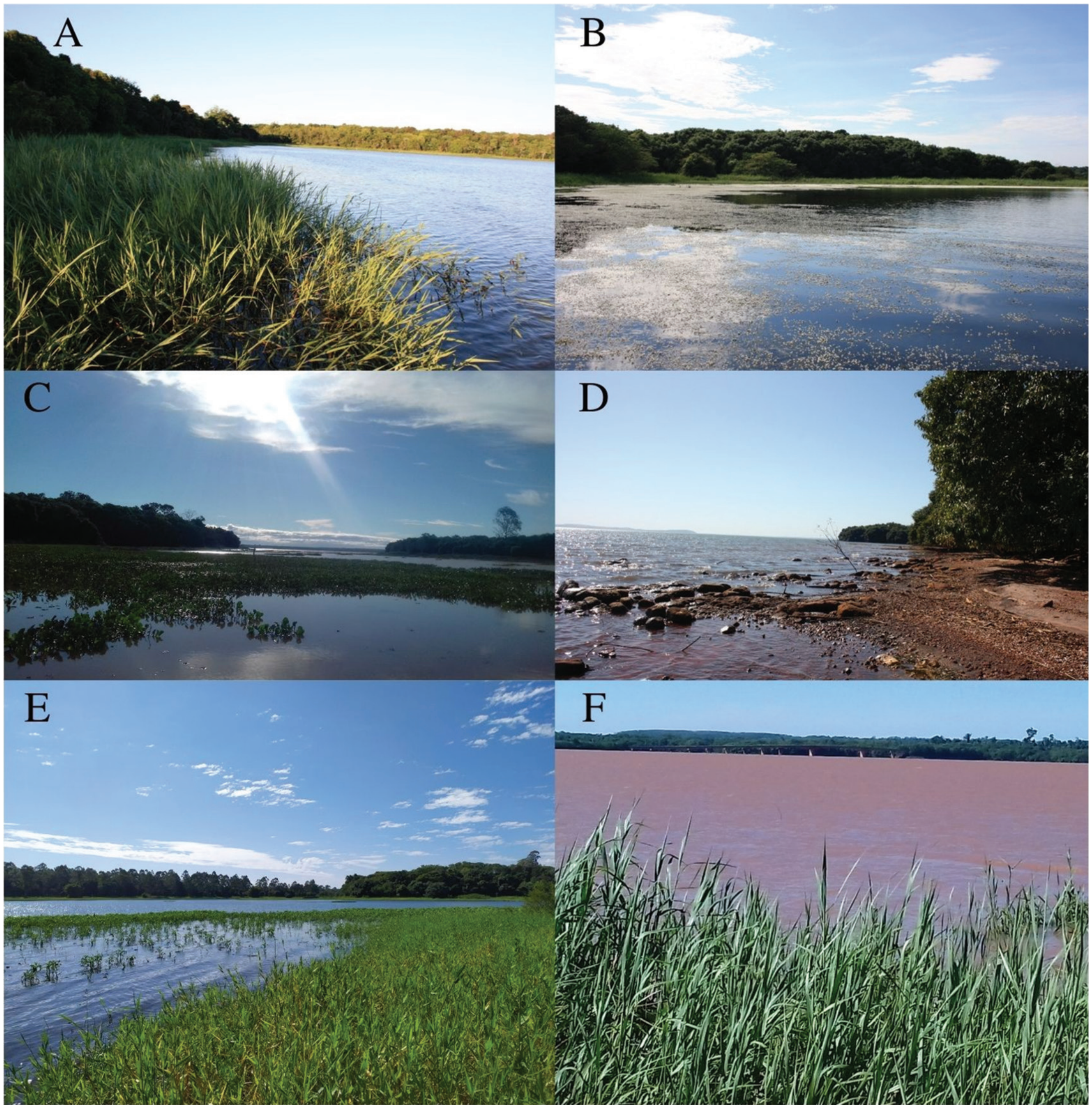

Figure 2. Sampling sites at "Refúgio Biológico de Santa Helena", Itaipu Reservoir, Upper Paraná River, Brazil. RB1 has the tip of the peninsula on one side and Itaipu protection strip on the other side; they form a bay whose environment is covered by *Brachiaria* grass and floating macrophytes (A); RB2 has access to the mouth of São Francisco Falso River, which is the main tributary of Itaipu Lake (B); RB3 has direct connection to Paraná River and forms a bay with calm waters, whose main feature lies on its extensive aquatic macrophyte banks (C); RB4 presents the formation of sand and gravel banks on Paraná River banks (D); RB5 bank is covered by Poaceae and floating macrophytes (E); and the RB6 environment is located 1.2 km from Santa Helena balneary's front side, PR (F).

Table 1. Features of sampling points around "Refúgio Biológico de Santa Helena", Itaipu Reservoir, Upper Paraná River, Brazil.

Sites	Floating macrophytes	Coastal vegetation	Forest fragment	Environment
RB1	Presence of *Eichhornia* sp., and *Elodea* sp.	Grass formation extending into the water	Itaipu protection strip	Semi lentic
RB2	Presence of *Eichhornia* sp., and *Elodea* sp.	Formation of grasses that extend into the water	Riparian vegetation	Semi lentic
RB3	High amounts of *Eichhornia* sp., *Salvinia* sp., *Pistia* sp., and *Elodea* sp.	Grass formation extending into the water	Riparian vegetation	Lentic
RB4	Absent	Absent	Riparian vegetation	Lotic
RB5	High amounts of *Eichhornia* sp., *Salvinia* sp., and *Elodea* sp.	Grass formation extending into the water	Itaipu protection strip	Lentic
RB6	Presence of *Eichhornia* sp., and *Elodea* sp.	Grass formation extending into the water	Itaipu protection strip	Semi lentic

Table 2. List of fish species' incidence and total abundance of sampled fish around "Refúgio Biológico de Santa Helena", Itaipu Reservoir, Upper Paraná River, Brazil RB = Refúgio Biológico [Biological Refuge]; *Fish captured only based on using sieve; (**) Fish captured by using both sieve and gill nets; [LDM] = long-distance migration; [(+)] conservation *status*: Endangered (EN) A2cd (ICMBio, 2018); [(−)] conservation *status*: [VU] = Vulnerable and [CR] = Critically Endangered MMA Ordinance Nº. 148, of June 7, 2022 and IUCN red list categories and criteria: [(DD)] = Data Deficient and [(LC)] = Least Concern.

Taxa	RB1	RB2	RB3	RB4	RB5	RB6	Abundance	Voucher	Ota et al. 2018
CHONDRICHTHYES									
MYLIOBATIFORMES									
Potamotrygonidae									
Potamotrygon sp.	X						2	NUP 23069	Non-native
Potamotrygon amandae Loboda & Carvalho, 2013[DD]	X		X				4	NUP 23072	Non-native
ACTINOPTERYGII									
CHARACIFORMES									
Acestrorhynchidae									
Acestrorhynchus lacustris (Lütken, 1875)**	X		X		X		5	NUP 23100	Native
Cynodontidae									
Rhaphiodon vulpinus Spix & Agassiz, 1829[LDM]	X	X	X				25	NUP 23105	Native
Characidae									
Stethaprioninae									
Astyanax lacustris (Lütken, 1875)*	X	X	X		X	X	14	NUP 23085	Native
Psalidodon aff. fasciatus (Cuvier, 1819)*		X					7	NUP 23043	Native
Hemigrammus ora Zarske, Le Bail & Géry, 2006*	X	X	X	X	X	X	133	NUP 23029	Non-native
Hyphessobrycon eques (Steindachner, 1882)*	X	X	X		X		128	NUP 23094	Possibly non-native
Hyphessobrycon moniliger Moreira, Lima & Costa 2002*					X		1	NUP 23063	Non-native
Moenkhausia gracilima Eigenmann, 1908*	X	X	X	X	X	X	845	NUP 23080	Native
Moenkhausia bonita Benine, Castro & Sabino, 2004*	X	X	X	X	X		930	NUP 23083	Native
Moenkhausia forestii Benine, Mariguela & Oliveira, 2009*	X		X		X		12	NUP 23076	Non-native
Psellogrammus kennedyi (Eigenmann, 1903)*	X	X	X		X		18	NUP 23074	Non-native
Characinae									
Galeocharax gulo (Cope, 1870)		X	X				2	NUP 23056	Native
Roeboides descalvadensis Fowler, 1932*	X	X	X	X	X		82	NUP 23075	Non-native
Stevardiinae									
Piabarchus stramineus (Eigenmann, 1908)*		X		X			54	NUP 23087	Native
Knodus moenkhausii (Eigenmann & Kennedy, 1903)*	X						1	NUP 23040	Possibly non-native
Diapoma guarani (Mahnert & Géry, 1987)*		X	X		X		65	NUP 23093	Native
Aphyocharacinae									
Aphyocharax anisitsi Eigenmann & Kennedy, 1903*	X	X	X		X		18	NUP 23117	Native
Aphyocharax sp.*	X	X	X	X	X		33	NUP 23070	Native
Cheirodontinae									
Serrapinnus kriegi (Schindler, 1937)*					X		8	NUP 23064	First record
Serrapinnus notomelas (Eigenmann, 1915)*	X	X	X	X	X		81	NUP 23084	Native

Continue...

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Taxa	RB1	RB2	RB3	RB4	RB5	RB6	Abundance	Voucher	Ota et al. 2018
Bryconidae									
Brycon orbignyanus (Valenciennes, 1850)[LDM (+) (–)CR]			X				1	CISH 191/O	Native
Crenuchidae									
Characidium aff. *zebra* Eigenmann, 1909*	X			X			2	NUP 23101	Native
Erythrinidae									
Hoplias aff. *malabaricus* (Bloch, 1794)	X	X	X	X	X	X	64	NUP 23044	unvalued
Hoplias intermedius (Günther, 1864)*					X		3	NUP 23026	Native
Hoplerythrinus unitaeniatus (Agassiz, 1829)*	X						1	NUP 23047	Non-native
Hemiodontidae									
Hemiodus orthonops Eigenmann & Kennedy, 1903[LDM]	X	X	X				58	NUP 23027	Non-native
Parodontidae									
Apareiodon affinis (Steindachner, 1879)*		X	X	X	X	X	103	NUP 23097	Native
Prochilodontidae									
Prochilodus lineatus (Valenciennes, 1836)[LDM]		X	X				4	NUP 23045	Native
Anostomidae									
*Leporinus friderici (*Bloch, 1794)[LDM **]	X	X	X				43	NUP 23033	Native
Leporinus lacustris Campos, 1945	X		X				14	NUP 23034	Native
Leporinus cf. *tigrinus* Borodin, 1929*	X						5	NUP 23102	Non-native
Megaleporinus macrocephalus (Garavello & Britski, 1988)[LDM]	X	X	X				20	NUP 23109	Non-native
Megaleporinus obtusidens (Valenciennes, 1836)[LDM – LC]	X	X	X				4	NUP 23036	Native
Schizodon borellii (Boulenger, 1900)[LDM]	X	X	X				105	NUP 23037	Non-native
Schizodon nasutus Kner, 1858[LDM]	X						1	NUP 23038	Native
Serrasalminae									
Metynnis lippincottianus (Cuvier, 1818)**	X	X	X		X		120	NUP 23022	Non-native
Piaractus mesopotamicus (Holmberg, 1887)[LDM]	1		1				2	NUP 23106	Native
Serrasalmus marginatus Valenciennes, 1837**	X	X	X		X		106	NUP 23028	Non-native
Serrasalmus maculatus Kner, 1858**	X	X	X				43	NUP 23030	Native
Lebiasinidae									
Pyrrhulina australis Eigenmann & Kennedy, 1903*					X		4	CISH 159/O	Native
SILURIFORMES									
Auchenipteridae									
Ageneiosus inermis (Linnaeus, 1766)[LDM]		X	X				2	NUP 23025	Non-native
Ageneiosus ucayalensis Castelnau, 1855	X	X					3	NUP 23032	Non-native
Auchenipterus osteomystax (Miranda Ribeiro, 1918)	X		X				8	NUP 23115	Non-native
Trachelyopterus galeatus (Linnaeus, 1766)**	X	X	X				110	NUP 23107	Native
Callichthyidae									
Hoplosternum littorale (Hancock, 1828)		X	X				3	NUP 23031	Native

Continue...

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Taxa	RB1	RB2	RB3	RB4	RB5	RB6	Abundance	Voucher	Ota et al. 2018
Doradidae									
Pterodoras granulosus (Valenciennes, 1821)	X	X	X				22	NUP 23057	Non-native
Loricariidae									
Hypostominae									
Hypostomus strigaticeps (Regan, 1908)	X						1	NUP 23048	Native
Pterygoplichthys ambrosettii (Holmberg, 1893)**	X	X	X		X		46	NUP 23061	Non-native
Loricariinae									
Loricariichthys platymetopon Isbrücker & Nijssen, 1979		X	X				3	NUP 23055	Non-native
Loricariichthys rostratus Reis & Pereira, 2000	X	X	X				6	NUP 23060	Non-native
Pimelodidae									
Pimelodus mysteriosus Azpelicueta, 1998	X	X	X				3	NUP 23059	Non-native
Sorubim lima (Bloch & Schneider, 1801)[LDM]	X						1	NUP 23088	Non-native
Iheringichthys labrosus (Lütken, 1874)			X				1	NUP 23071	Non-native
Pinirampus pirinampus (Agassiz, 1829)[LDM]	X		X				6	NUP 23058	Native
Pseudoplatystoma corruscans (Spix & Agassiz, 1829)[LDM (–)VU]			X				2	CISH 42TB	Native
GYMNOTIFORMES									
Gymnotidae									
Gymnotus sylvius Albert & Fernando-Matioli, 1999*			X		X		5	NUP 23081	Native
Hypopomidae									
Brachyhypopomus gauderio Giora & Malabarba, 2009*			X		X		4	NUP 23023	Non-native
Sternopygidae									
Eigenmannia trilineata López & Castello, 1966*			X				5	NUP 23095	Native
Rhamphichthyidae									
Rhamphichthys hahni (Meinken, 1937)**			X				2	CISH 118TB	Non-native
CICHLIFORMES									
Cichlidae									
Astronotus crassipinnis (Heckel, 1840)**	X	X	X				21	NUP 23096	Non-native
Cichlasoma paranaense Kullander, 1983*	X	X					4	NUP 23077	Native
Apistogramma commbrae (Regan, 1906)*			X		X		7	NUP 23103	Non-native
Geophagus sveni Lucinda, Lucena & Assis, 2010**	X	X	X			X	183	NUP 23116	Non-native
Geophagus iporangensis Haseman 1911		X					1	NUP 23039	Possibly Native
Satanoperca setepele Ota, Deprá, Kullander, Graça & Pavanelli, 2021**	X	X	X	X			69	NUP 23092	Non-native
Crenicichla britskii Kullander, 1982*	X	X	X		X		11	NUP 23078	Native
Cichla kelberi Kullander & Fereira, 2006**	X	X	X		X	X	34	NUP 23108	Non-native
Laetacara araguaiae Ottoni & Costa, 2009*	X		X		X		29	NUP 23079	Non-native
INCERTAE SEDIS									
Sciaenidae									
Plagioscion squamosissimus (Heckel, 1840)[LC]	X	X	X				88	CISH 148TB	Non-native

Continue...

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Taxa	RB1	RB2	RB3	RB4	RB5	RB6	Abundance	Voucher	Ota et al. 2018
SYNBRANCHIFORMES									
Synbranchidae									
Synbranchus marmoratus Bloch, 1785*[LC]			X		X		6	CISH 178/A	Native
PLEURONECTIFORMES									
Achiridae									
Catathyridium jenynsii (Günther, 1862)		X	X				8	NUP-23024	Non-native
CYPRINODONTIFORMES									
Poeciliidae									
Pamphorichthys hollandi (Henn, 1916)*	X		X	X	X	X	54	NUP-23082	Native
Total species = 74							**3,919**		

species (18), it was followed by Cichlidae (9) Anostomidae (7) and Pimelodidae (5). In total, 3,919 individuals were collected (Figure 5).

With respect to the abundance of individuals sampled with gill nets, *Geophagus sveni* Lucinda, Lucena & Assis, 2010 was the most abundant species (181 specimens); it accounted for 16.7% of all collected specimens. Among specimens collected with fishing sieve, *Moenkhausia bonita* Benine, Castro, Sabino, 2004 was the most representative species with 930 collected individuals (32.8% of the total sample).

Total species richness varied among sampled sites; RB3 recorded the largest number of species (57); it was followed by RB1 and RB2 (51 and 44 species, respectively). Sampling sites exclusively using fishing sieve presented the following species richness: RB5 (30 species), RB4 (13 species) and RB6 (9 species), as shown in Table 2.

RB3 recorded the highest species richness (35 species) in sampling sites where fish collection was only based on gill nets; *G. sveni* was the most abundant species (76 individuals) in these sites, and it was followed by *Schizodon borellii* (Boulenger, 1900), which accounted for 66 individuals. RB1 recorded 31 species; *G. sveni* and *Trachelyopterus galeatus* (Linnaeus, 1766) were the most abundant species found in it – they accounted for 62 and 40 individuals, respectively. RB2 recorded 28 species; *G. sveni* and *T. galeatus* were the most representative ones (43 and 37 individuals, respectively).

RB5 recorded the highest species richness (30 species) in sampling sites where fish collection was based on fishing sieve; *M. bonita* (127 individuals) and *Hemigrammus ora* Zarske, Le Bail, Géry, 2006 (39 individuals) were the most abundant species found in it. RB1 presented 26 species; *Moenkhausia gracilima* Eigenmann, 1908 (190 individuals) and *M. bonita* (185 individuals) were the most abundant species in it. RB2 recorded 22 species; *M. gracilima* (431 individuals) and *M. bonita* (420 individuals) were the most representative ones. RB3 presented 31 species; *M. gracilima* was the most representative one (207 individuals) and it was followed by *M. bonita* (87 individuals). RB4 has shown 11 species; *M. bonita* and *Piabarchus stramineus* (Eigenmann, 1908) were the most representative species collected in this site (111 and 34 individuals, respectively). RB6 was the site presenting the smallest number of species (7); *Apareiodon affinis* (Steindachner, 1879) was the most representative species (66 individuals).

We have registered at least 14 migratory species in the surroundings of this Conservation Unit. RB3 was the sampling site showing the highest migratory species richness (12); it also presented two endangered species, *Brycon orbignyanus* (Valenciennes, 1850) and *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829). Eleven (11) migratory species were recorded at RB1. RB2 recorded the lowest migratory species richness; it presented eight migratory species; among them, one finds, *Megaleporinus obtusidens* (Valenciennes 1836), *Prochilodus lineatus* (Valenciennes 1836) and *Rhaphiodon vulpinus* Spix & Agassiz, 1829.

It is worth emphasizing that we recorded three different reproduction patterns in habitats located around the Conservation Unit, namely: (i) internal fertilization and internal development – represented by species *Pamphorichthys hollandi* (Henn, 1916), which was recorded at RB1, and by family Potamotrygonidae, which was recorded at RB1 and RB3; (ii) fertilization and external development, although with internal gametic association (insemination – see: Fukakusa et al. (2020) – represented by family Auchenipteridae, which was recorded at RB1, RB2 and RB3; and (iii) fertilization and external development (without insemination) with other species.

We registered the presence of 33 non-native species in the upper Paraná River basin (Ota et al. 2018). We emphasize that the four most abundant species collected with gillnets are non-native, which indicates their ability to adjust in this ecosystem. Among the recorded species, *Brycon orbignyanus* (Valenciennes, 1850), was considered Endangered (EN) by the Akama et al. (2018) and Critically Endangered (CR) by the Official List of Extinct Brazilian Fauna Species (MMA Ordinance No. June 7, 2022) and *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) Vulnerable (VU) also by the MMA Ordinance No. June 7, 2022.

Discussion

Results have shown that the Relevant Ecological Interest Area (REIA) – "Refúgio Biológico de Santa Helena" – provides habitat for at least 74 fish species, including on long-distance migrant and endangered species. Species accumulation curve did not reach the asymptote; this outcome suggested the incidence of an even richer fish fauna composition in this environment.

Agostinho et al. (2007), recorded species such as *Steindachnerina insculpta* (Fernández-Yépez, 1948), *Crenicichla nierdeleinii* (Holmberg, 1891), *Leporellus vittatus* (Valenciennes, 1850), *Hypophthalmus*

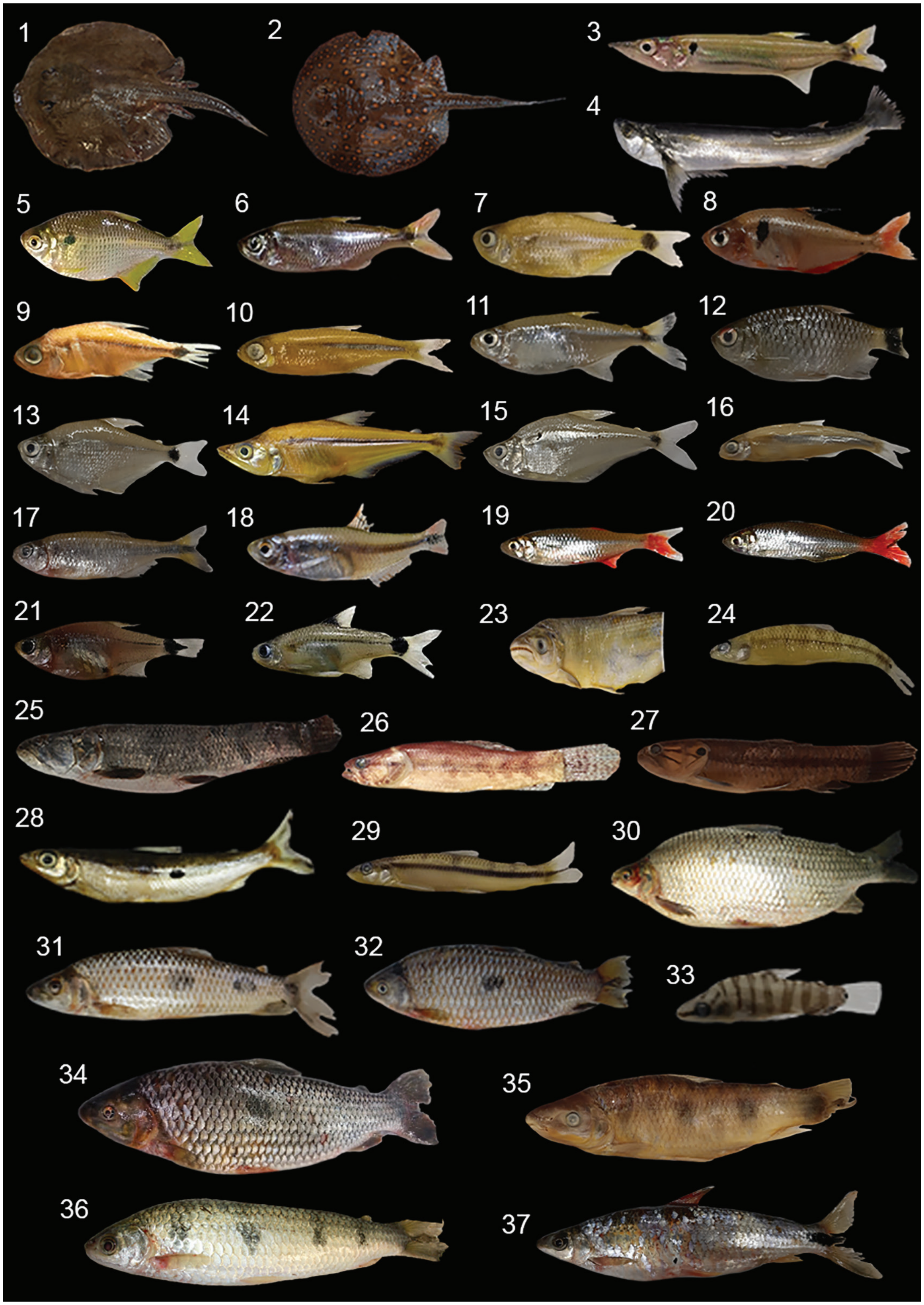

Figure 3. Sample representation of the main fish species collected in the surroundings of "Refúgio Biológico de Santa Helena" – images out of scale. 1) *Potamotrygon* sp. 210 mm; 2) *Potamotrygon amandae* 195 mm; 3) *Acestrorhynchus lacustris* 153.42 mm; 4) *Rhaphiodon vulpinus* 360 mm; 5) *Astyanax lacustris* 45 mm 6) *Psalidodon* aff. *fasciatus* 35 mm; 7) *Hemigrammus ora* 26 mm; 8) *Hyphessobrycon eques* 18 mm; 9) *Hyphessobrycon moniliger* 27.2 mm; 10) *Moenkhausia* gracilima 17.77 mm; 11) *Moenkhausia bonita* 18.55 mm; 12) *Moenkhausia forestii* 23.48 mm; 13) *Psellogrammus kennedyi* 25.38 mm; 14) *Galeocharax gulo* 141.2 mm; 15) *Roeboides descalvadensis* 27.97 mm; 16) *Piabarchus stramineus* 18.59 mm; 17) *Knodus moenkhausii* 28.4 mm; 18) *Diapoma guarani* 24.37 mm; 19) *Aphyocharax anisitsi* 30.59 mm; 20) *Aphyocharax* sp. 28.76 mm; 21) *Serrapinnus kriegi* 25.35 mm; 22) *Serrapinnus notomelas* 17.58 mm; 23) *Brycon orbignyanus* (damaged in the gill net – approximate value 188 mm); 24) *Characidium* aff. *zebra* 28.78 mm; 25) *Hoplias* aff. *malabaricus* 240 mm; 26) *Hoplias intermedius* 32.69 mm; 27) *Hoplerythrinus unitaeniatus* 166 mm; 28) *Hemiodus orthonops* 152.24 mm; 29) *Apareiodon affinis* 31.15 mm; 30) *Prochilodus lineatus* 458 mm; 31) *Leporinus friderici* 197.24 mm; 32) *Leporinus lacustris* 155 mm; 33) *Leporinus* cf. *tigrinus* 15.38 mm; 34) *Megaleporinus macrocephalus* 175 mm; 35) *Megaleporinus obtusidens* 235 mm; 36) *Schizodon borellii* 180 mm; 37) *Schizodon nasutus* 215 mm.

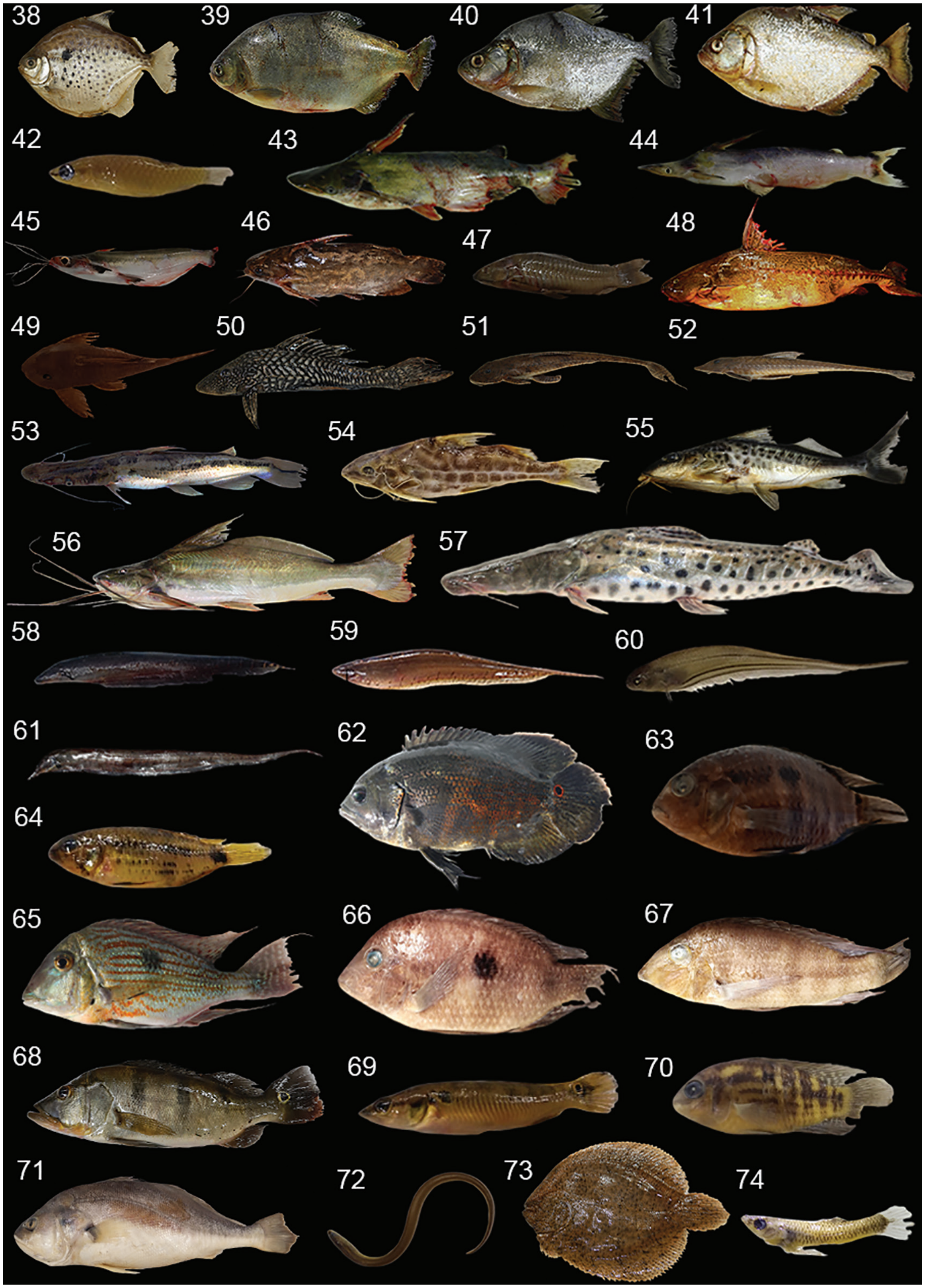

Figure 4. Sample representation of the main fish species collected in the surroundings of "Refúgio Biológico de Santa Helena" – images out of scale. 38) *Metynnis lippincottianus* 18.24 mm; 39) *Piaractus mesopotamicus* 295 mm; 40) *Serrasalmus marginatus* 150 mm; 41) *Serrasalmus maculatus* 160 mm; 42) *Pyrrhulina australis* 47.12 mm; 43) *Ageneiosus inermis* 360 mm; 44) *Ageneiosus ucayalensis* 220 mm; 45) *Auchenipterus osteomystax* 210 mm; 46) *Trachelyopterus galeatus* 99.98 mm; 47) *Hoplosternum littorale* 156 mm; 48) *Pterodoras granulosus* 248 mm; 49) *Hypostomus strigaticeps* 139 mm; 50) *Pterygoplichthys ambrosettii* 293 mm; 51) *Loricariichthys platymetopon* 254 mm; 52) *Loricariichthys rostratus* 235 mm; 53) *Sorubim lima* 286 mm; 54) *Pimelodus mysteriosus* 220 mm; 55) *Iheringichthys labrosus* 138.72 mm; 56) *Pinirampus pirinampus* 379 mm; 57) *Pseudoplatystoma corruscans* 570 mm; 58) *Gymnotus sylvius* 168.5 mm; 59) *Brachyhypopomus gauderio* 67.78 mm; 60) *Eigenmannia trilineata* 198 mm; 61) *Rhamphichthys hahni* 740 mm; 62) *Astronotus crassipinnis* 233 mm; 63) *Cichlasoma paranaense* 50.73 mm; 64) *Apistogramma commbrae* 33.56 mm; 65) *Geophagus sveni* 135 mm; 66) *Geophagus iporangensis* 148.51 mm; 67) *Satanoperca setepele* 129.07 mm; 68) *Crenicichla britskii* 33.72 mm; 69) *Cichla kelberi* 272 mm; 70) *Laetacara araguaiae* 35.9 mm; 71) *Plagioscion squamosissimus* 430 mm; 72) *Synbranchus marmoratus* 595 mm; 73) *Catathyridium jenynsii* 194 mm; 74) *Pamphorichthys hollandi* 19.79 mm.

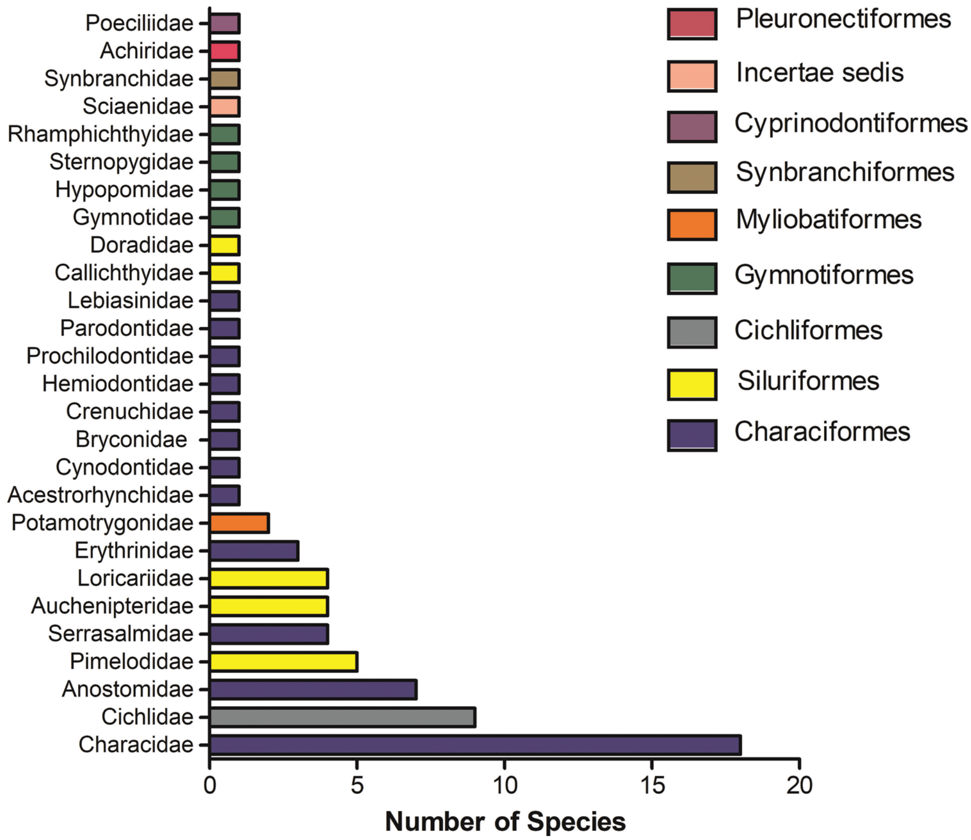

Figure 5. Species richness recorded for each family and order found in "Refúgio Biológico de Santa Helena", Itaipu Reservoir, Upper Paraná River, Brazil. Families within each order are represented by the same color. (purple = Characiformes; pink = incertae sedis to Sciaenidae; yellow = Siluriformes; light gray = Cichliformes; green = Gymnotiformes; orange = Myliobatiformes; brown = Synbranchiformes; lilac = Cyprinodontiformes; dark pink = Pleuronectiformes).

edentatus Spix & Agassiz, 1829 and *Salminus brasiliensis* (Cuvier, 1816) in Santa Helena Balneary, Itaipu Reservoir, in 1987. The RBSH Management Plan carried out in 2010 presented the record of *H. edentatus* Spix & Agassiz, 1829. The non-registration of the six species listed above does not mean that they disappeared from the study site, since differences among capture methods and the effects of fishing equipment selectivity can influence sampling results.

Geophagus sveni was the most abundant species recorded with the passive collection effort (gill nets); this outcome has shown the important role played by Cichliformes in the investigated ichthyogeographic region. Specie *G. sveni* is native to the middle portion of the Tocantins Rivers drainage and its incidence in Paraná River basin can be associated with aquaculture or with its trade as ornamental fish (Langeani et al. 2007, Lucinda et al. 2010, Soares et al. 2017).

Moenkausia bonita, *M. gracilima* and *H. ora* were the most abundant species collected through the active capture (sieve) method. The numerical representation of these species can indicate that they close their life cycles in RBSH coastal zone habitats that function play an important role in filtering the functional characteristics of fish (Quirino et al. 2021). Small species often present high food plasticity and the ability to colonize different waterbodies, mainly lentic environments, although they essentially occupy coastal zones (Casatti et al. 2003, Vidotto & Carvalho 2007). The record of 32 species captured in shallow areas based on the sieve technique reinforces the importance of preserving these coastal areas, which have important environmental maintenance functions, such as the structural protection of habitats, food resources and reproduction (Cassatti et al. 2003).

The diversity of neotropical freshwater fish species mainly comprises three ostariophysan (Characiformes, Siluriformes and Gymnotiformes) and two Acanthomorpha (Cichliformes, Cyprinodontiformes) fish orders (Tagliacollo et al. 2021) – all the orders were recorded in the current study. Characiformes and Siluriformes presented the highest species richness. Characidae was the most representative family, a fact that may be linked to the wide geographic distribution of its species in continental waters covering Southwestern Texas, Mexico, and Central and South America (Nelson et al. 2016). RB3 was the sampling point presenting the highest species richness (57 species, in total). This richness may be associated with the physical features of this environment, which is formed by a calm-water bay with extensive underwater macrophyte banks that can provide places for the reproduction, refuge and feeding.

The Upper Paraná River floodplain (about 230 km) above the Itaipu reservoir, represents the last free-flowing section of the upper Paraná River and serves as a nursery habitat for many migratory species, which are very important for artisanal fisheries in tropical river systems, in terms of economic value and ecological sustainability (Hoeinghus et al. 2009). Long-distance migratory species, captured in low numbers in this study, as *B. orbignyanus, P. lineatus, Leporinus friderici* (Bloch, 1794)*, Megaleporinus obtusidens, Piaractus mesopotamicus* (Holmberg, 1887)*, Pinirampus pirinampus* (Agassiz, 1829) and *P. corruscans*, often show low abundance in dammed, as reported by

Agostinho et al. (2007), who evaluated 77 reservoirs in South America and observed that more than 50% of the analyzed environments did not have migratory species as components of their dominant fauna, as well as that few reservoirs presented more than two migratory species among the prevalent ones. One of the main impacts on this group of species lies on the interruption of their natural migration routes resulting from the construction of artificial dams (Carolsfeld et al. 2003, Agostinho et al. 2016, Azevedo-Santos et al. 2019), a fact that significantly changes ecosystem function, sediment balance flood pulse and thermal regime (Reis et al. 2016), and that can even decrease migratory species' body size (Lopes et al. 2020).

Therefore, protecting species showing reduced natural stocks and/or endangered species, such as *B. orbignyanus* (EN/CR) and *P. corruscans* (Spix & Agassiz, 1829) (VU) is an important service provided by Conservation Units (Akama et al. 2018). Maintaining tributaries without artificial dams, where fish can complete their life cycle, is the most viable and effective alternative for species conservation (Marques et al. 2018, Lopes et al. 2021). São Francisco Falso River, which is the main tributary near "Refúgio Biológico de Santa Helena", is an example of free-dam river it that can help protect the fish fauna of the region.

According to Bailly et al. (2021), the Paraná-Paraguay basin hosts approximately 23 long-distance migratory fish species; "Refúgio Biológico de Santa Helena" a recorded 10 of these species, which corresponded to 43.5% of species recorded in their research. Among the herein recorded fish species, one finds the natives *B. orbignyanus, M. obtusidens, P. mesopotamicus, P. pirinampu, P. lineatus, P. corruscans, R. vulpinus* and the non-natives *Megaleporinus macrocephalus* (Garavello, Britski, 1988), *Pterodoras granulosus* (Valenciennes, 1821), and *Sorubim lima* (Bloch & Schneider, 1801). In addition, the current study has also recorded the following migratory species: the native *L. friderici* (Bloch, 1794) and non-natives *Ageneiosus inermis* (Linnaeus, 1766), *Hemiodus orthonops* Eigenmann & Kennedy, 1903, and *S. borellii* (Agostinho et al. 2007).

The record of migratory species in the present study may be associated with habitats forming the Conservation Unit. In addition to *B. orbignyanus*, it is worth emphasizing the incidence of the *P. corruscans*, which directly depend on upstream migration to complete their reproductive cycle (Carolsfeld et al. 2003). It is noteworthy that most of these species have economic importance, to a higher or lesser degree of acceptance (Bailly et al. 2021). Thus, the current research strongly encourages restoring and maintaining environments to help maintaining long-distance migratory and endangered species populations.

Migratory species play key role in aquatic ecosystem conservation processes. *Piaractus mesopotamicus*, for example, plays important role in seeds' (Muniz et al. 2014) cout spread due to its feeding behavior as herbivore; catfish species *P. pirinampu* and *P. corruscans* have high commercial value, whereas all other registered species also play fundamental role in ecosystem maintenance through ecological processes, besides having commercial value (Carolsfeld et al. 2003).

Fish communities are subjected to increasing global-scale anthropogenic pressures capable of changing their biodiversity and threatening ecosystem services (Villéger et al. 2017). If on take into consideration that practically all environments in the Paraná River watershed have suffered one, or more, environmental impacts, the conservation of native migratory species can be a promising alternative for ecosystem protection purposes. The fact that they require dam-free river stretches free and coastlines preserved for reproduction purposes, gives them in the status of key species for the conservation and preservation of these ecosystems. Thus, such a protection can help conserving several habitats and identifying areas with endemic or endangered species, as well as areas with high biological diversity (Agostinho 2007a, Azevedo-Santos et al. 2019). Thus, results in the current research may represent a step towards identifying and protecting fish species that occur in the vicinity of Conservation Units, by encouraging the implementation of conservation actions in continental water environments.

Among the anthropic impacts, one finds species introduction, as well as artificial impoundments, which have significant negative impacts on freshwater ecosystems (Muniz et al. 2021). The submersion of the geographic barrier "Sete Quedas" [Guairá Falls], in association with other anthropic actions such as fishkeeping and fish farms, are the likely explanation for the incidence of non-native species in the investigated area. *Plagioscion squamosissimus* (Heckel, 1840) stood out among these species. It was introduced in the environment before the damming process took place (Cecilio et al. 1997) and recorded high abundance level in Itaipu reservoir in 2000, based on Benedito-Cecilio & Agostinho (2000). This Amazon-native species (Casatti 2005) was introduced by São Paulo Energy Company (CESP) in dams located in Northeastern Brazil in the 1950s; it reached Paraná River in the 1970s (Braga 1997). Its introduction may also be associated with its trading potential (Ota et al. 2018). This species is one of the most frequent non-native species in Brazil, together with species *Poecilia reticulata* Peters, 1859 and with species belonging to genera *Cichla* Bloch & Schneider 1801 and *Astronotus* Swainson 1839 (Latini et al. 2016).

Species Kullander & Ferreira, 2006 and *Astronotus crassipinnis* (Heckel, 1840) were recorded around RBSH; they were the first to evidence declining species richness, as well as biomass and ecosystem functions associated with them (Leal et al. 2021). However, among the Cichlids, *G. Sveni* was the most representative in the study area of this research and its occurrence can be associated with the aquarium trade (Ota et al. 2018).

Species *Potamotrygon amandae* is widely distributed in Paraná-Paraguay basin (Loboda & Carvalho, 2013); this species can also be mentioned as example of occupation in upper Paraná River basin, due to the submergence of "Sete Quedas". *Potamotrygon* Garman, 1877 has significant medical importance since its sting is dangerous to human health (Haddad Júnior 2003, Moreira & Vidal 2022). We emphasize that it was not possible to identify an individual of *Potamotrygon* at the species level, due to an atypical color pattern and overlapping characteristics between the species recognized by Ota et al. (2018) for the study area. For this reason, we maintain the identification as *Potamotrygon* sp. and further efforts and comparative material are needed to elucidate the taxonomic identity of this individual.

The species popularly known in the region as "piranhas" are another example of non-native species establishment. Although *Serrasalmus marginatus* Valenciennes, 1837 and its congener, *Serrasalmus maculatus* Kner, 1858, compete to each other, *Serrasalmus marginatus* Valenciennes, 1837 recorded higher abundance in the investigated site, likely because it is more aggressive than its congener (Agostinho et al. 2007, Agostinho & Júlio Jr. 2002). Rodrigues et al. (2018) argue that *S. maculatus* behaves as a competitor, deviating from its preferences for food and reproductive resources of non-native species, which allows its population to persist in the upper Paraná floodplain. Thus, *S. marginatus* remained more abundant (71.1%) than *S. maculatus* after 12 years, at least in the region sampled in the current study.

This pattern of decreasing a native species to the detriment of a non-native species was also suggested by Ganassin et al. (2021) between the non-native migratory *S. borellii* and its congeneric *Schizodon altoparanae* Garavello & Britski, 1990.

Our record of *Serrapinnus kriegi* (Schindler, 1937) demonstrates that even a well sampled area can open new records and should be monitored. This record represents the first record for the Itaipu reservoir, and the second record in the Upper Paraná Basin (Vicentin et al. 2019). This species was described for the Paraguay River basin, but are currently also recognized for the basins of Lower Paraná and Uruguay Rivers (Miquelarena et al. 2008, Mantinian 2011, Carvajal-Vallejos et al. 2014, Bertaco et al. 2016, Serra et al. 2018). Its origin in the Upper Paraná River basin is still uncertain.

Although the Upper Paraná fish fauna is well-documented (Langeani et al. 2007, Ota et al. 2018), studies focusing on Conservation Units remain scarce. Most studies conducted in Brazilian protected areas address terrestrial ecosystems, a fact that limits freshwater biodiversity protection since, overall, they only cover small stretches of river systems (Azevedo-Santos et al. 2019).

One of the actions capable of helping to preserve sensitive areas lies on strengthening inspections conducted in buffer zones, based on Law n. 9,985, from July 18th, 2000, and on the general objective of Decree n. 4,339/2002, which highlights the importance of promoting the conservation, *in situ* and *ex situ*, of biodiversity components, such as genetic, species and ecosystem variability, as well as ecosystem services maintained by biodiversity. Furthermore, the current study can be used as reference to help updating the ichthyofauna in the management plan developed for “Refúgio Biológico de Santa Helena”, Brazil.

Supplementary Material

The following online material is available for this article:

Figure S1 – Species accumulation curve, based on the methodologies adopted to collect fish on the banks of “Refúgio Biológico de Santa Helena”, Itaipu Reservoir, Upper Paraná River Basin, Brazil.

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Author Contributions

Heleno Brandão: provided substantial contribution to all research stages.

Daniel Rodrigues Blanco: provided substantial contribution to all research stages.

Lucas Emilio Perin Kamfert: provided substantial contribution to all research stages.

Denise Lange: have contributed to data analysis and interpretation; to critical revision; as well as added intellectual content to the current study.

Igor Paiva Ramos: have contributed to data analysis and interpretation; to critical revision; as well as added intellectual content to the current study.

Conflicts of Interest

The author(s) declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

Animal Use Ethics Committee (CEUA), Universidade Tecnológica Federal do Paraná, Dois Vizinhos campus– protocolo nº 2016-031.

Data Availability

The data that supporting in the current this study is openly available in Dataverse (https://data.scielo.org/dataverse/brbn) at (https://doi.org/10.48331/scielodata.WG8LBJ).

Observation to Dataverse: Submitted for Review – The draft version of this dataset is currently under review prior to publication.

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