

Research Article

Evidence for the unexpected dispersal of *Leporinus tigrinus* Borodin, 1929 through hydroelectric dams in the most regulated Neotropical river basin

Rafael Cezar Cavaretto^{1,2,*}, Lenice Souza-Shibatta³, Leandro Fernandes Celestino^{1,2}, Patrícia Sarai da Silva^{1,2}, Oscar Akio Shibatta⁴, Sílvia Helena Sofia⁵, Sérgio Makrakis² and Maristela Cavicchioli Makrakis²

¹Programa de Pós-graduação em Recursos Pesqueiros e Engenharia de Pesca, Universidade Estadual do Oeste do Paraná – UNIOESTE, Campus de Toledo, Toledo, Paraná State, Brazil

²Grupo de Pesquisa em Tecnologia em Ecohidráulica e Conservação de Recursos Pesqueiros e Hídricos – GETECH, Universidade Estadual do Oeste do Paraná – UNIOESTE, Campus de Toledo, Toledo, Paraná State, Brazil

³Laboratório de Sistemática Molecular, Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina – UEL, Londrina, Paraná State, Brazil

⁴Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina – UEL, Londrina, Paraná State, Brazil

⁵Laboratório de Genética e Ecologia Animal – LAGEA, Departamento de Biologia Geral, Universidade Estadual de Londrina – UEL, Londrina, Paraná State, Brazil

*Corresponding author

E-mail: rafaelcavaretto@yahoo.com.br

Present Address: Rua Nossa Senhora do Perpétuo Socorro, 230, Jardim Panorama, Campo Grande, Mato Grosso do Sul State, Brazil. 79044-610

Citation: Cavaretto RC, Souza-Shibatta L, Celestino LF, da Silva PS, Shibatta OA, Sofia SH, Makrakis S, Makrakis MC (2020) Evidence for the unexpected dispersal of *Leporinus tigrinus* Borodin, 1929 through hydroelectric dams in the most regulated Neotropical river basin. *Aquatic Invasions* 15(3): 497–513, <https://doi.org/10.3391/ai.2020.15.3.09>

Received: 26 November 2019

Accepted: 9 April 2020

Published: 21 May 2020

Handling editor: Amy Deacon

Thematic editor: Elena Tricarico

Copyright: © Cavaretto et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS

Abstract

The Upper Paraná River Basin (UPRB) is harmed by several human activities, among which species introduction is particularly intense, making it the Neotropical ecoregion with the highest number of non-native fish species. *Leporinus tigrinus* Borodin, 1929 is a native species of the Tocantins-Araguaia River Basin and was recorded for the first time in the UPRB in 1999. Since then, the species was progressively recorded in lower portions of the UPRB (downstream Corumbá River). Therefore, the aim of this study was to provide evidence of possible introduction, establishment and dispersion of the species in that region. For this purpose, an inventory of its occurrence was performed by sampling ichthyoplankton, juveniles and adults of fishes in tributaries of the Porto Primavera Reservoir, as well as by compiling data from scientific collections considering all the UPRB. Diet and reproductive aspects were also analyzed. Overall, 23 specimens of *L. tigrinus* (10 adults and 13 juveniles) and five eggs (identified by DNA Barcoding) were sampled, evidencing the occurrence, reproduction, and recruitment of this species in the basin. The results of this study support the conclusion that *L. tigrinus* is an introduced species in the lower portion of the UPRB, capable of dispersing over long distances by passing through several reservoirs and dams (from upstream to downstream), possibly via turbines, spillways, locks, and fishways. Its dispersal pathway differs from the patterns observed for most invasive fish species in this basin, and suggests a substantial downstream dispersion through dams, even in the absence of fishways.

Key words: species introduction, establishment, dispersion, connectivity

Introduction

Non-native species are among the main drivers of recent extinctions (Bellard et al. 2016a) and have been leading to the impoverishment and

homogenization of ecosystems worldwide (Olden et al. 2004; Daga et al. 2015; Toussaint et al. 2016). Most of the immigrant organisms perish en route or soon after their arrival in new locations (Mack et al. 2000). Nevertheless, some of them can become invaders, especially when they find suitable environments and dispersion corridors which favor their persistence, proliferation and spreading in the recipient ecosystem (Hulme 2009; Bellard et al. 2016b; Braasch et al. 2019).

Fluvial ecosystems are particularly susceptible to invasions as a result of being interconnected in their multidimensionality (Ward 1989). However, the current boom in global dam construction is limiting this connectivity (Nilsson et al. 2005; Zarfl et al. 2015; Makrakis et al. 2019) and restricting long free-flowing rivers to remote regions of the world (Grill et al. 2019). Dams can enable invasions by causing disturbances that favor the establishment of non-native species (Havel et al. 2005; Johnson et al. 2008). Also, a highly-regulated river basin may inhibit or even prevent the movement and dispersion of fish (Pelicice et al. 2015; Reis et al. 2016), potentially limiting the range expansion of introduced species.

In South America, the Upper Paraná River Basin (UPRB) is the most regulated river basin (Agostinho et al. 2008) – its main river and largest tributaries were converted into a reservoir cascade (Makrakis et al. 2019). Initially, it encompassed the stretches of the Paraná River Basin located upstream the Sete Quedas Falls, a natural barrier capable of preventing the dispersal of most fish species between the Upper and the Lower Paraná River basins (Vitule et al. 2012), two distinct ichthyofaunistic provinces (Abell et al. 2008). Following the construction of the Itaipu Dam and the subsequent flooding of the Sete Quedas Falls in 1982, there was massive colonization of the Upper Paraná River Basin by endemic species from the Lower Paraná River Basin (Júlio Júnior et al. 2009; Vitule et al. 2012). The dispersal barrier was relocated roughly 150 km downstream, being then represented by the dam. In 2002, a fishway known as “Canal da Piracema” was implemented in this dam, enabling some species of the Lower Paraná River to have access to the Upper Paraná River (Makrakis et al. 2007, 2011), which has resulted in new biological invasions over time (Agostinho et al. 2015; Ota et al. 2018).

Currently, the UPRB presents the highest number of non-native fish species out of all Neotropical ecoregions (105 species; Gubiani et al. 2018). It is possible to identify at least two distinct patterns of biological invasion in this basin. The first pattern was observed for the highest number of invasive fish (around 60 species), which migrated from the Lower Paraná River (Gubiani et al. 2018; Ota et al. 2018). These species have limited upstream dispersion due to dams, which restrict their range to the lowest reaches of the basin (Júlio Júnior et al. 2009; Casimiro et al. 2017). The other pattern is presented by species of human interest, such as those originating from fish farming, fish stocking, and aquarium trade, as well as

species sought for sport fishing and live bait (Gubiani et al. 2018). These species have had multiple introduction events and foci, and as a result are more widely diffused in the basin (Daga et al. 2015; Garcia et al. 2018).

A third pattern, however, may be occurring in the UPRB, involving the downstream dispersal of invasive species, despite the fragmentation caused by dams. This phenomenon was potentially observed for *Leporinus tigrinus* Borodin, 1929, a fish species native to the Tocantins-Araguaia River Basin (Britski and Garavello 2007) with recent records for the UPRB. The first capture of this species in the UPRB dates from 1999, from a broad collection of fishes in the Corumbá River, an uppermost drainage of this basin (Pavanelli et al. 2007). On that occasion, the authors considered *L. tigrinus* as native to that drainage, and, by extension, the species was considered native to the UPRB (Langeani et al. 2007). *L. tigrinus* had never been recorded until that date in the lower portions of the UPRB (downstream Corumbá River), which have a well-documented ichthyofauna. For this reason, the species was considered introduced (Froehlich et al. 2017), or possibly introduced (Santos et al. 2013; Ota et al. 2018) in the region. Even when considered to be an alien species, the reasons behind its introduction continue to be ignored (Froehlich et al. 2017).

This study aimed to clarify the origin of *L. tigrinus* in the lower portion of the Upper Paraná River Basin (i.e., native vs. non-native), by providing evidence of its possible introduction, establishment and unusual dispersal across an intensely fragmented environment, and to test the hypothesis that dams of hydroelectric plants are not efficient to prevent its dispersal.

Materials and methods

Study area

With a drainage area of 2,966,900 km² and extending through five countries (Brazil, Argentina, Bolivia, Paraguay, and Uruguay), the Paraná River Basin (also known as La Plata River Basin) is the fifth-largest in the world, and the second-largest in South America (Wolf et al. 1999). Its upper stretch, the Upper Paraná River Basin (UPRB), currently extends to the Itaipu Dam (Figure 1) and is considered an ecoregion of high ichthyofaunal diversity (Langeani et al. 2007).

This basin drains great urban centers as well as important agricultural and industrial areas of Brazil. It is subject to various anthropogenic impacts, such as deforestation, pollution, habitat loss, the construction of hydroelectric dams (which affect all of its main watercourses), and the introduction of alien species (Agostinho et al. 2008; Pelicice et al. 2017).

The largest reservoir in the UPRB is formed by the Engenheiro Sergio Motta Hydropower Plant (also known as Porto Primavera). Some tributaries of this reservoir still preserve natural characteristics of the hydrological regime, such as the Aguapeí River (a dam-free watercourse), and the Pardo

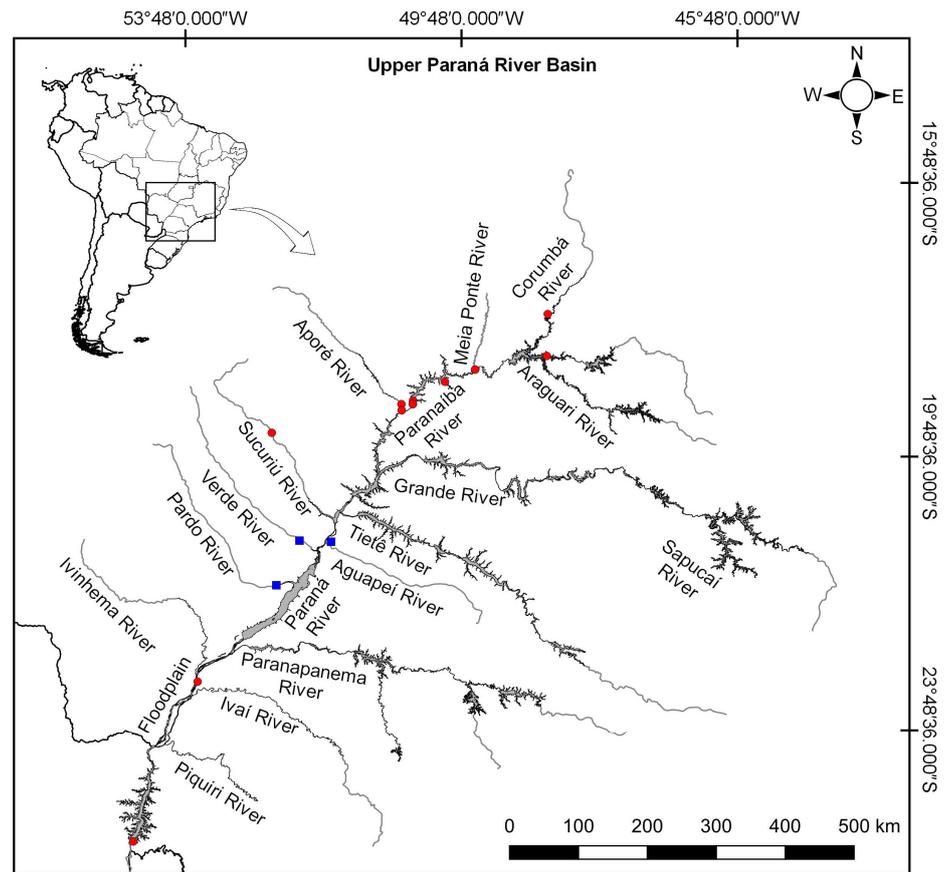


Figure 1. Study area (Upper Paraná River Basin) and records of *Leporinus tigrinus* in the basin. The red circles represent museum records, and the blue squares represent the locations in which the species was recorded in the present study: Aguapeí River (−21.083967, −51,712953); Pardo River (−21.689089, −52.481667); Verde River (−21.036889, −52.120333).

and Verde rivers (lightly regulated only in upper stretches). Along with the Paraná River floodplain, located downstream from the Porto Primavera Dam, these three tributaries are acknowledged as critical reproductive sites for many fish species (Da Silva et al. 2015, 2019). They contribute to the fish diversity maintenance in the Porto Primavera Reservoir, by acting as source environments (Marques et al. 2018).

Occurrence data

To determine the current range of *L. tigrinus* in the UPRB, we (i) compiled occurrence records from scientific collections available in an online database (SpeciesLink 2019), and (ii) performed field sampling.

Fish sampling was carried out from 2011 to 2018, in both breeding and non-breeding seasons, in three tributaries of the Porto Primavera Reservoir (Aguapeí, Pardo, and Verde rivers), where *L. tigrinus* was historically absent (Marques et al. 2018). In all, 141 samples (47 per tributary) were collected using two sets of 10-m-long gillnets of different mesh sizes (2.5; 3; 4; 5; and 6 cm), installed at 5 p.m. in the river channels and marginal lagoons. In the river channels, gillnets were installed close to the banks, in locations of low water flow, while in the lagoons they were distributed

along the banks, as well as in limnetic zones. The time of exposure was approximately four hours, and the sampled fish were preserved in ice until they arrived in the laboratory, where specimens of *L. tigrinus* were identified according to Santos and Jégu (1989) and Ota et al. (2018).

All collections were conducted following the Ethics Committee on the Use of Animals of the Universidade Estadual do Oeste do Paraná (Protocol Number 59/09), and the System of Authorization and Information on Biodiversity (SISBIO) issued the authorization for the collections (no. 31137–5).

A spatio-temporal distribution map was made to check if *L. tigrinus* is spreading in the UPRB. This map considered the year and spatial position of the first record, and the following records which expanded the distribution of the species in the basin (records from both scientific collections and field samplings were considered). Finally, a dispersion graph, followed by a Spearman correlation between the spatial position and year of record, was constructed, using the map data. This analysis was performed through software SAS University Edition 9.4, and the significance level was 5%.

Reproduction and recruitment

To detect the reproduction and recruitment of *L. tigrinus* in the basin, the sex and gonadal maturation stage of collected fish were identified by the macroscopic analysis, according to Vazzoler (1996). Juvenile fish (with immature gonads) were considered to be an indication of recruitment.

Ichthyoplankton were also studied to detect the spawning of *L. tigrinus*. Sampling was carried out monthly from November 2017 to April 2018, at the same three tributaries of the Porto Primavera Reservoir. The collections were made through horizontal tows lasting 10 minutes along the surface of the water (at 20 cm depth), between 9 and 11 p.m., using a conical-cylindrical plankton net (0.5 mm mesh). The samples were preserved in 90% ethanol and were refrigerated until stored in a freezer at -20°C . In the laboratory, fish eggs and larvae were separated from other organisms and detritus.

Molecular identification of ichthyoplankton (DNA barcoding)

Since the morphological identification of eggs and larvae is challenging and the early-development stages of *L. tigrinus* have not yet been described (Nakatani et al. 2001), the presence of the species in the ichthyoplankton was identified by DNA barcoding. For that, total genomic DNA of the sampled eggs and larvae was extracted in a buffer containing Chelex 100 and proteinase K. The cytochrome *c* oxidase subunit I gene (COI, 648 bp) was amplified using the primers described by Ward et al. (2005). The amplicons were sequenced and analyzed in ABI-PRISM 3500 XL automated sequencer (Applied Biosystems). The detailed protocol of the extraction, amplification, and sequencing of the COI gene is described in Frantine-Silva et al. (2015).

The nucleotide sequences were edited and aligned using Clustal W (Thompson et al. 1994), a program incorporated into MEGA7 software (Kumar et al. 2016). Then, these sequences were compared to the sequences in GenBank database through BLAST (Basic Local Alignment Search Tool), which searches for regions of similarity between sequences and is mostly used for species identification (Kelly et al. 2011; Stoeckle et al. 2011; Abdullah and Rehbein 2016). Ichthyoplankton sequences with similarity higher than 98% to *L. tigrinus* sequences in Genbank were assigned to this species. This threshold was adopted because, in the UPRB, the maximum intraspecific genetic divergence observed for species of the genus *Leporinus* was 0.9%, and the minimum interspecific genetic divergence was 3.3% (Pereira et al. 2013).

Diet composition

Specimens of *L. tigrinus* collected in the field samplings also had their diet analyzed, after being preserved in 10% formaldehyde. Stomachs were removed through an abdominal incision, and only those which were full, or partially full, were considered.

The stomach contents were identified in the following categories using a stereomicroscope: organic detritus (particulate organic matter in different stages of decomposition), sediment (mineral particles), terrestrial plants (fruit, seeds and leaves of vascular plants), aquatic plants (bryophytes), filamentous algae, insects and macroinvertebrates (Arachnida, Bivalvia, Gastropoda, Annelida, and Diplopoda). Food items were quantified according to the volumetric method (Hyslop 1980), estimated using graduated test tubes and a glass counting plate (Hellawell and Abel 1971).

Results

Occurrence and range expansion

The first record of *L. tigrinus* in the Upper Paraná River Basin (UPRB) occurred in 1999, in the Corumbá Reservoir, state of Goiás, Brazil (Pavanelli et al. 2007), which still corresponds with the uppermost limit of the species distribution in the basin. Over time, the species was recorded sequentially in lower reaches of the basin, in the Paranaíba and Paraná rivers. *Leporinus tigrinus* was also recorded in some tributaries of these rivers, in stretches without dispersal barriers between them and the main streams (Table 1, Figure 1, Figure 2A). The cascade of eight reservoirs where the species currently occurs is formed by the Corumbá, Itumbiara, Cachoeira Dourada, São Simão, Ilha Solteira, Jupia, Porto Primavera, and Itaipu dams (Table 2). Of these dams, only Porto Primavera and Itaipu have fishways (a fish ladder in the former and a lateral system in the latter).

The current southernmost limit of the species' range is the Canal da Piracema inlet, in the Itaipu Dam (Figure 2A). The range expansion of

Table 1. Museum records of *Leporinus tigrinus* in the Upper Paraná River Basin, in ascending chronological order (<http://www.splink.org.br>). Only the specimens with geographical coordinates of their collection sites were considered. (*) first record in the basin. (**) downstream expansion of the species' range. Records in **bold** were those obtained by the present study. Nupélia - UEM = Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá. MCT - PUCRS = Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul. LIRP - USP = Laboratório de Ictiologia de Ribeirão Preto da Universidade de São Paulo. DZSJRP - UNESP = Departamento de Zoologia de São José do Rio Preto da Universidade Estadual Paulista. MZUEL = Museu de Zoologia da Universidade Estadual de Londrina.

Date (dd/mm/yyyy)	River	Location	Latitude	Longitude	Institution	Voucher Number
09/04/1999*	Corumbá	Caldas Novas/GO	-17.726944	-48.548333	Nupélia - UEM	NUP 1093
20/03/2001**	Meia Ponte	Cachoeira Dourada/GO	-18.537222	-49.600833	MCT - PUCRS	MCP-Peixes 42747
23/03/2001	Meia Ponte	Cachoeira Dourada/GO	-18.537500	-49.600833	MCT - PUCRS	MCP-Peixes 33962
09/10/2008**	Claro	Cacu/GO	-19.0425	-50.669444	Nupélia - UEM	NUP 8874
17/02/2009	Claro	Cacu/GO	-19.133055	-50.665555	Nupélia - UEM	NUP 8875
26/07/2009	Claro	Cacu/GO	-19.133055	-50.665555	Nupélia - UEM	NUP 8876
30/07/2009	Paranaíba	São Simão/GO	-19.0425	-50.503888	LIRP - USP	LIRP 7308
01/01/2010	Paranaíba	São Simão/GO	-18.994444	-50.50222	LIRP - USP	LIRP 7811
01/01/2010	Paranaíba	São Simão/GO	-19.0425	-50.503888	LIRP - USP	LIRP 7829
01/01/2011	Paranaíba	São Simão/GO	-19.042580	-50.50388	LIRP - USP	LIRP 8387
01/04/2011	Paranaíba	São Simão/GO	-19.0425	-50.503888	LIRP - USP	LIRP 8635
01/11/2011	Paranaíba	São Simão/GO	-19.0425	-50.503888	LIRP - USP	LIRP 8578
09/03/2012	Paranaíba	Araguari/MG	-18.33833	-48.56194	DZSJRP - UNESP	DZSJRP-Pisces 15775
24/07/2012**	Sucuriú	Cassilândia/MS	-19.461666	-52.548055	Nupélia - UEM	NUP 14407
21/01/2013**	Pardo	Bataguassu/MS	-21.689089	-52.481667	MZUEL	MZUEL 19912
22/04/2013	Paranaíba	Santa Vitória/MG	-18.716388	-50.038888	Nupélia - UEM	NUP 16872
25/04/2013	Pardo	Bataguassu/MS	-21.689089	-52.481667	MZUEL	MZUEL 19913
16/01/2014	Pardo	Bataguassu/MS	-21.689089	-52.481667	MZUEL	MZUEL 19915
04/04/2015**	Paraná	Querência do Norte/PR	-23.097777	-53.623055	Nupélia - UEM	NUP 17488
08/06/2016	Verde	Brasilândia/MS	-21.036889	-52.120333	MZUEL	MZUEL 19916
17/01/2017**	Paraná (Canal da Piracema)	Foz do Iguaçu/PR	-25.438888	-54.555833	Nupélia - UEM	NUP 19638
11/01/2018	Verde	Brasilândia/MS	-21.036889	-52.120333	MZUEL	MZUEL 19890

L. tigrinus in the UPRB occurred in a descending direction (Spearman correlation: $\rho = 0.97$; $p < 0.01$) (Figure 2B), covering approximately 1,300 km of river over 18 years ($72.2 \text{ km year}^{-1}$).

The field sampling carried out in the present study broadened the known occurrence area of the species as a result of new records in the three tributaries of the Porto Primavera Reservoir since 2013 (Figure 1; Table 1). Through the sampling period, 23 specimens of *L. tigrinus* were collected, of which six were deposited in the ichthyological collection of the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL19890, 19912, 19913, 19915, 19916). Since the first capture of *L. tigrinus* in the tributaries, its abundance corresponded to 0.27% of all collected fish ($n = 8,613$).

Reproduction and recruitment

The reproductive activity of *L. tigrinus* was detected in the three tributaries of the Porto Primavera Reservoir. Of the ten caught adults (mean standard length: $160.7 \text{ mm} \pm 23.4$), six were evaluated. Two female specimens captured in December 2013, in the Pardo and Aguapeí rivers, were in reproduction (mature ovaries). The other four specimens were captured during the non-breeding season (April and June) and presented resting ovaries.

Among the 793 eggs and 689 fish larvae sampled in the spawning season of 2017–2018, 132 eggs and 215 larvae were identified through DNA Barcoding

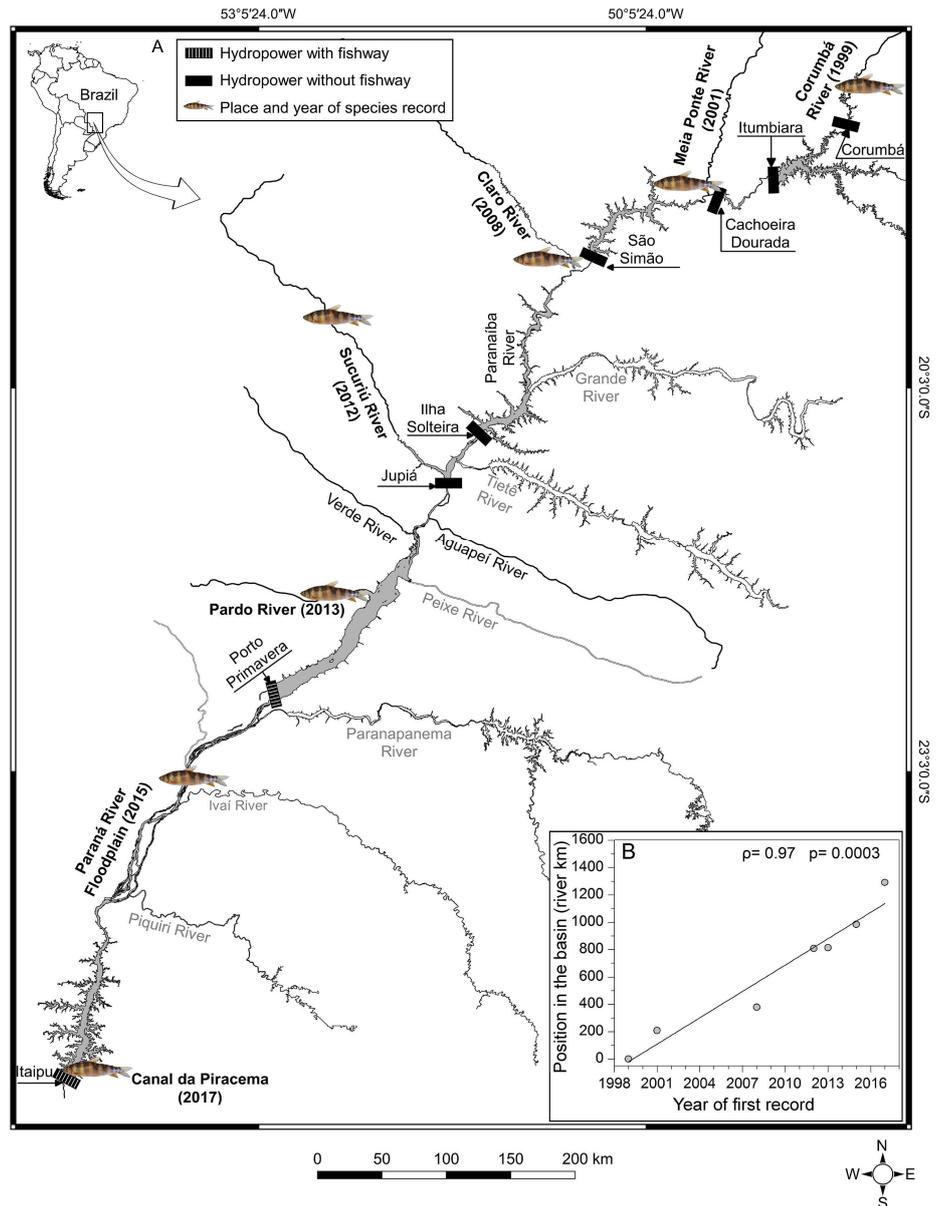


Figure 2. Spatio-temporal dispersion map, showing the range expansion of *Leporinus tigrinus* over time in the Upper Paraná River Basin (A), and Spearman correlation between record position in the Upper Paraná River Basin and year of the first record (B).

Table 2. Characteristics of the hydroelectric dams in the range of *Leporinus tigrinus* in the Upper Paraná River Basin, and possible dispersal pathways from upstream to downstream of the dams. K = Kaplan turbines; F = Francis turbines; T = Turbines; S = Spillways; FW = Fishway; L = Locks.

Hydroelectric Dam	Total reservoir volume (km ³)	Quantity and type of turbine	Dispersal pathways
Corumbá	1.5	3 F	T and S
Itumbiara	17	6 F	T and S
Cachoeira Dourada	0.5	7 K and 3 F	T and S
São Simão	12.5	6 F	T and S
Ilha Solteira	15.5	20 F	T, S, and L
Jupia	2.4	14 K	T, S, and L
Porto Primavera	15.7	14 K	T, S, L, and FW
Itaipu	29	20 F	T, S, and FW



Figure 3. Egg of *Leporinus tigrinus* in cleavage-stage, with 2.54 mm in diameter, sampled in the Verde River in December 2017 (A). Juvenile of *L. tigrinus* collected in a marginal lagoon of the Pardo River, on January 16, 2014 (MZUEL 19915; standard length: 89 mm) (B). Photo by Rafael C. Cavaretto.

techniques. Five eggs collected in the Verde River were assigned to *L. tigrinus* since they presented 100% similarity with the sequence of this species in the GenBank database (accession number: KF568997.1, from a specimen collected in the Araguaia River; Ramirez et al. 2016). New sequences generated in this study were submitted to GenBank (MN186394 to MN186398).

The sampled *L. tigrinus* eggs were pelagic, non-adherent, and spherical. One egg in embryonic development of cleavage-stage, with 2.54 mm in diameter and large perivitellinic space (0.89 mm), was photographically recorded (Figure 3A).

Juvenile specimens of *L. tigrinus* were also recorded, which comprised 56.5% ($n = 13$) of the 23 collected specimens of this species, and 7.8% of all juveniles collected ($n = 167$) in the tributaries since the first record of *L. tigrinus* for this stretch of the basin (Figure 3B). Two juveniles were collected in January 2013, three in January 2014, two in January 2015, and six in November 2018.

Diet composition

Stomachs were removed from 11 collected specimens, of which seven presented stomach contents. The analysis revealed a predominantly detritivorous diet, and the food items were, in order of importance in the diet, organic detritus (79.3% of total volume), aquatic plants (10.1%), terrestrial plants (5.4%), insects (2.8%), sediment (1.0%), filamentous algae (0.9%), and macroinvertebrates (0.4%).

Discussion

The present study gathered new evidence that supports the hypothesis that *L. tigrinus* is an introduced species to lower portions of the UPRB. Pavanelli et al. (2007) and Langeani et al. (2007) considered *L. tigrinus* as native to the UPRB, based on a single record of the species in the Corumbá Reservoir in 1999 (NUP 1093; Table 1). Before that year, *L. tigrinus* has never been recorded in the basin, despite the high number of inventories

made downstream of Corumbá River (e.g. Agostinho et al. 2007; Graça and Pavanelli 2007).

Data compiled in scientific collections along with those obtained in the present study revealed that the first record of *L. tigrinus* in the UPRB was followed by new records sequentially located in lower sections of the basin (Figure 2A). In the sampled section of our study, located approximately 750 km downstream from the area of the first record, the species had only begun to be captured in 2013, despite intensive collection efforts dating back to 1999 (Marques et al. 2018). The correlation between the chronology and the spatial position of subsequent records (Figure 2B) suggests the dispersal of *L. tigrinus* to new reaches of the basin, in an upstream-downstream direction.

Currently, the range of *L. tigrinus* in the UPRB spans the longitudinal stretch from the Corumbá Reservoir to the Canal da Piracema inlet (approximately 1,300 km), encompassing segments of the Corumbá, Paranaíba and Paraná rivers - a cascade of eight reservoirs. The species was also recorded in stretches of some tributaries without dispersion barriers with the main stream. Conversely, *L. tigrinus* was not recorded in the largest tributaries of the Upper Paraná River (Grande, Tietê and Paranapanema rivers), possibly because the upstream dispersal to these watercourses was prevented by dams. Thus, *L. tigrinus* seems to have expanded its distribution in a descending direction, by crossing dams and reservoirs, and laterally to dam-free stretches of tributaries. Such results suggest that dams are permeable to downstream but insurmountable to upstream movements of this species, in the absence of fishways.

Until now, it was possible to identify at least two distinct patterns of biological invasion in the UPRB, involving different vectors and dispersal pathways. The first one, described by Júlio Júnior et al. (2009), refers to the species that accessed the Upper Paraná River after the flooding of the Sete Quedas Falls and the implementation of the Itaipu Dam fishway (Canal da Piracema), which constitutes a continuous source of propagules (Makrakis et al. 2007, 2011). These species originating from the Lower Paraná River have their upstream dispersal limited by dams, although some species have reached upper stretches of the basin by utilizing fishways and ship locks (Júlio Júnior et al. 2009; Casimiro et al. 2017). Thus, in this pattern of invasion, stretches without barriers (natural or human-made) constitute an open route for dispersal of invasive species, whereas dams act as filters. Although dams are usually insurmountable, the passage of some species is possible when fishways are available (ladders, locks, elevators).

The other pattern encompasses multiple vectors (fish farming, fish stocking, aquarium trade, sport fishing, and live bait) with several and widespread introduction foci. The result is the presence of invaders in disseminated but not necessarily connected reaches of the basin (Daga et

al. 2015; Garcia et al. 2018; Gubiani et al. 2018). Many of these species (such as cichlids and small characids) are non-migratory, generalists in terms of resource use, tolerant of environmental variations, and able to complete their entire life cycle in reservoirs, tending to remain in these locations (Agostinho et al. 2008). Others, such as migratory species originating from stocking (Garcia et al. 2018) or those escaping from fish farms (Forneck et al. 2016), display rheophilic behavior and present vast home range. However, the high human interest in these species makes it challenging to predict whether its presence in a given stretch of the basin is due to the existence of a local introduction focus or the dispersal from previously introduced sites.

The dispersal of *L. tigrinus* in the UPRB differs from these patterns, and the fact that this is the most-studied river basin in Brazil (Graça and Pavanelli 2007; Langeani et al. 2007; Marques et al. 2018; Ota et al. 2018) allowed the detection of what may be a pathway for future biological invasions. Since *L. tigrinus* is not a species of particular human interest, a scenario involving multiple widespread introductions would not be realistic. As a result, the most plausible *L. tigrinus* invasion dynamics would require its introduction in an upper region of the basin (near or at Corumbá Reservoir), followed by its dispersion to lower reaches, despite intense fragmentation. Even though the vector of this possible introduction is unknown (Froehlich et al. 2017), species of the genus *Leporinus* are occasionally used as live bait, and *L. tigrinus* has potential for use in the aquarium trade. It is essential to highlight that the Upper Paraná and Tocantins-Araguaia river basins are contiguous, which facilitates the transport of species between them by human interaction.

The question of whether *L. tigrinus* is native or introduced to the Corumbá River Basin remains controversial. It is possible to speculate that the species is native to that river since that region drained north until the Miocene, with subsequent capture of rivers by orogenetic events and draining to the south (Beurlen 1970). Although there is a lack of dating of the origin of *L. tigrinus*, the Miocene was a period of significant diversification for Neotropical fishes (Albert and Reis 2011). In contrast, the lack of records of this species downstream of the Corumbá River Basin is strongly supported by scientific collections and long-term surveys that failed to collect the species in the region (e.g. from Britski 1972 to Agostinho et al. 1997). This absence is highly unlikely to be due to species misidentification, since *Leporinus octofasciatus* Steindachner, 1915, another barred species that occurs in this basin, has a color pattern and pelvic-fin ray counts that readily distinguish it from *L. tigrinus* (see Ota et al. 2018). The Corumbá River was considered an area of fish endemism isolated by rapids and canyons (Pavanelli and Britski 1999). The formation of the Corumbá Reservoir may have created conditions for *L. tigrinus* to

disperse downstream, which allowed it to achieve the current distribution in the UPRB gradually.

Dams and their reservoirs constitute barriers to upstream and downstream movements of fish, trapping populations between dams (Agostinho et al. 2008). Otherwise, the results of our study provide evidence that *L. tigrinus* was able to cross seven hydroelectric dams and their reservoirs, possibly through turbines and spillways (only the Porto Primavera Dam provides a fishway; Table 2). The use of these routes in the passage through dams had already been suggested by Makrakis et al. (2012) and Antonio et al. (2007), when studying migratory fish patterns in the Paraná River Basin. In North America, mark-recapture data in the Missouri River indicated that turbines and spillways could establish substantial downstream connectivity, even for species of large body size, despite the potential mortality (Pracheil et al. 2015).

Studies related to the spatial distribution of ichthyofauna in the Peixe Angical Reservoir, situated in the Upper Tocantins River, demonstrated that *L. tigrinus* preferably occupies bathypelagic (deep) waters in the lacustrine zone (Freitas et al. 2008). The strong preference towards deeper and inner regions of the reservoir could facilitate the entry of *L. tigrinus* specimens into turbines and spillways. It may provide indication as to how this species was able to cross seven dams in the UPRB, of which only one possess a fishway. Furthermore, an evaluation of the reproductive activity of fish species before and after the formation of a reservoir in the Tocantins River demonstrated that *L. tigrinus* is a species that is positively affected by reservoirs, showing a considerable increase in the percentage of spawning-capable females (from 30.4% to 83.3%) after the reservoir formation (Angulo-Valencia et al. 2016). This characteristic may have contributed to the species' success in the reservoir cascade of the UPRB.

An alien species is considered established when it constitutes persistent and self-sustainable populations in the new environment through reproduction and recruitment (Vermeij 1996). Although preliminarily, the present study indicates that *L. tigrinus* is possibly established in the sampled area, since we detected two females in reproduction, five fertilized eggs, and 13 juveniles of this species, which may be an indication of recruitment. Regardless of its presumed preference for lentic habitats (Freitas et al. 2008), our data suggest that *L. tigrinus* relies on lotic environments for its reproduction, since its pelagic eggs were collected drifting passively in a free-flowing stretch of the Verde River. The necessity to move in search of biotopes suitable for each life phase may also explain such a wide dispersal within the new area.

Considerable intraspecific divergence in the COI gene is observed in fish species with distributions ranging over multiple hydrographic basins (Ramirez et al. 2017; Silva-Santos et al. 2018; Bignotto et al. 2020). However, in the present study, the COI gene sequences from eggs collected in the

UPRB (Verde River) showed 100% similarity to the sequence of *L. tigrinus* from the Tocantins-Araguaia River Basin. The high similarity would be due to a short period of isolation, since some level of genetic differentiation is expected between geographically isolated populations (Hebert et al. 2003). Thus, we can suggest that populations of *L. tigrinus* were separated in two different basins recently, reinforcing the hypothesis of introduction in the UPRB. However, research with additional genetic markers and a larger sample size is desirable to support this hypothesis.

The study of ecological and life-history traits provides essential information in understanding how species thrive in novel environments (Agostinho et al. 2015; Liu et al. 2017), and plasticity is undoubtedly of central importance (Sol and Weis 2019). Regarding *L. tigrinus*, Monteiro et al. (2008) detected some versatility in its feeding habits, since it changed from piscivore to omnivore after the construction of a dam in the Tocantins River. This apparent trophic plasticity is reinforced by the present study, which revealed a third feeding habit for the species, predominantly detritivore. Organic detritus is also dominant in the diet of *Hemiodus orthonops* Eigenmann & Kennedy, 1903, a non-native fish of high abundance in the Upper Paraná (Agostinho et al. 2015). Bezerra et al. (2019) observed a tendency toward the dominance of bottom-feeding fish species in ecosystems altered by damming and species introduction, such as UPRB.

Our results support the view that *L. tigrinus* is non-native to the lower portion of Upper Paraná River Basin. Also, it is a candidate invasive species to this region, if considering the broader concept whereby a species is considered invasive when it spreads in a new area (Richardson et al. 2000; Heger et al. 2013). Furthermore, the knowledge of its unique dispersal pathway denotes substantial upstream to downstream connectivity in dams, even in the absence of fishways, and may explain the success of other invasive species in the occupation of reservoir cascades. Lastly, our findings indicate that *L. tigrinus* might continue its dispersal to lower reaches of the Paraná (La Plata) River Basin, since dams seem to be a permeable barrier to downstream movements of this species.

Acknowledgements

The authors thank Companhia Energética de São Paulo - CESP for the logistic and financial support. Additional thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Productivity Grant in Technological Development and Innovative Extension – DT (SM), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the Doctoral scholarship (LFC and PSS). We are grateful to Suelen Pini and Adriani Cristina Monteiro for assistance in the diet analysis. Special thanks to the reviewers and the Handling Editor for helpful comments.

References

Abdullah A, Rehbein H (2016) DNA barcoding for the species identification of commercially important fishery products in Indonesian markets. *International Journal of Food Science & Technology* 52: 266–274, <https://doi.org/10.1111/ijfs.13278>

- Abell R, Thieme ML, Revenga C, Bryer M, Kottelat M, Bogutskaya N, Coad B, Mandrak N, Balderas SC, Bussing W, Stiassny MLJ, Skelton P, Allen GR, Unmack P, Naseka A, Ng R, Sindorf N, Robertson J, Armijo E, Higgins JV, Heibel TJ, Wikramanayake E, Olson D, López HL, Reis RE, Lundberg JG, Pérez MHS, Petry P (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience* 58: 403–414, <https://doi.org/10.1641/B580507>
- Agostinho AA, Júlio Jr HF, Gomes LC, Bini LM, Agostinho CS (1997) Composição, abundância e distribuição espaço-temporal da ictiofauna. In: Vazzoler AEM, Agostinho AA, Hahan NS (eds), A planície de inundação do alto rio Paraná: aspectos físicos, biológicos e socioeconômicos. EDUEM, Maringá, Brazil, pp 179–208
- Agostinho AA, Gomes LC, Pelicice FM (2007) Ecologia e Manejo de Recursos Pesqueiros em Reservatórios do Brasil. EDUEM, Maringá, Brazil, 501 pp
- Agostinho AA, Pelicice FM, Gomes LC (2008) Dams and the fish fauna of the Neotropical region: impacts and management related to diversity and fisheries. *Brazilian Journal of Biology* 68: 1119–1132, <https://doi.org/10.1590/S1519-69842008000500019>
- Agostinho AA, Suzuki HI, Fugi R, Alves DC, Tonella LH, Espindola LA (2015) Ecological and life history traits of *Hemiodus orthonops* in the invasion process: looking for clues at home. *Hydrobiologia* 746: 415–430, <https://doi.org/10.1007/s10750-014-2030-2>
- Albert JS, Reis RE (2011) Introduction to Neotropical freshwaters. In: Albert JS, Reis RE (eds), Historical biogeography of Neotropical freshwater fishes. University of California Press, Berkeley and Los Angeles, USA, pp 3–10, <https://doi.org/10.1525/california/9780520268685.001.0001>
- Angulo-Valencia MA, Agostinho AA, Suzuki HI, Luz-Agostinho KDG, Agostinho CS (2016) Impoundments affect fish reproduction regardless of reproductive strategy. *Lakes and Reservoirs: Research and Management* 21: 362–374, <https://doi.org/10.1111/lre.12151>
- Antonio RR, Agostinho AA, Pelicice FM, Bailly D, Okada EK, Dias JHP (2007) Blockage of migration routes by dam construction: can migratory fish find alternative routes? *Neotropical Ichthyology* 5: 177–184, <https://doi.org/10.1590/S1679-62252007000200012>
- Bellard C, Cassey P, Blackburn TM (2016a) Alien species as a driver of recent extinctions. *Biology Letters* 12: 1–4, <https://doi.org/10.1098/rsbl.2015.0623>
- Bellard C, Leroy B, Thuiller W, Rysman JF, Courchamp F (2016b) Major drivers of invasion risks throughout the world. *Ecosphere* 7: 1–14, <https://doi.org/10.1002/ecs2.1241>
- Bezerra LAV, Ribeiro VM, Freitas MO, Kaufman L, Padiál AA, Vitule JRS (2019) Benthification, biotic homogenization behind the trophic downgrading in altered ecosystems. *Ecosphere* 10: e02757, <https://doi.org/10.1002/ecs2.2757>
- Beurlen K (1970) Geologie von Brasilien. Gebrüder Borntraeger, Berlin, Germany, 452 pp
- Bignotto TS, Maniglia TC, Gomes VN, Oliveira IJ, Agostinho CS, Prioli SMAP, Prioli AJ (2020) Genetic evidence for a species complex within the piranha *Serrasalmus maculatus* (Characiformes, Serrasalminidae) from three Neotropical river basins based on mitochondrial DNA sequences. *Genetics and Molecular Biology* 43: e20190131, <https://doi.org/10.1590/1678-4685-gmb-2018-0131>
- Braasch J, Barker BS, Dlugosch KM (2019) Expansion history and environmental suitability shape effective population size in a plant invasion. *Molecular Ecology* 28: 2546–2558, <https://doi.org/10.1111/mec.15104>
- Britski HA (1972) Peixes de água doce do Estado de São Paulo: Sistemática. In: Branco SM (ed), Poluição e piscicultura. Faculdade de Saúde Pública da USP e Instituto de Pesca, São Paulo, Brazil, pp 79–108
- Britski HA, Garavello JC (2007) Família Anostomidae. In: Buckup PA, Menezes NA, Ghazzi MS (eds), Catálogo das Espécies de Peixes de Água Doce do Brasil. Museu Nacional - UFRJ, Rio de Janeiro, Brazil, pp 23–27
- Casimiro ACR, Garcia DAZ, Costa ADA, Britton JR, Orsi ML (2017) Impoundments facilitate a biological invasion: dispersal and establishment of non-native armoured catfish *Loricariichthys platymetopon* (Isbrückler & Nijssen, 1979) in a Neotropical river. *Limnologia* 62: 34–37, <https://doi.org/10.1016/j.limno.2016.11.001>
- Da Silva PS, Makrakis MC, Miranda LE, Makrakis S, Assumpção L, Paula S, Dias JHP, Marques H (2015) Importance of reservoir tributaries to spawning of migratory fish in the Upper Paraná river. *River Research and Applications* 31: 313–322, <https://doi.org/10.1002/rra.2755>
- Da Silva PS, Miranda LE, Makrakis S, Assumpção L, Dias JHP, Makrakis MC (2019) Tributaries as biodiversity preserves: an ichthyoplankton perspective from the severely impounded Upper Paraná River. *Aquatic Conservation: Marine and Freshwater Ecosystems* 29: 258–269, <https://doi.org/10.1002/aqc.3037>
- Daga VS, Skóra F, Padiál AA, Abilhoa V, Gubiani EA, Vitule JRS (2015) Homogenization dynamics of the fish assemblages in Neotropical reservoirs: comparing the roles of introduced species and their vectors. *Hydrobiologia* 746: 327–347, <https://doi.org/10.1007/s10750-014-2032-0>
- Forneck SC, Dutra FM, Zacarkim CE, Cunico AM (2016) Invasion risks by non-native freshwater fishes due to aquaculture activity in a Neotropical stream. *Hydrobiologia* 773: 193–205, <https://doi.org/10.1007/s10750-016-2699-5>

- Frantine-Silva W, Sofia SH, Orsi ML, Almeida FS (2015) DNA barcoding of freshwater ichthyoplankton in the Neotropics as a tool for ecological monitoring. *Molecular Ecology Resources* 15: 1226–1237, <https://doi.org/10.1111/1755-0998.12385>
- Freitas IS, Lucinda PHF, Soares AB, Pelicice FM, Akama A (2008) Variações espaciais na estrutura da ictiofauna entre os ambientes do reservatório de Peixe Angical. In: Agostinho CS, Pelicice FM, Marques EE (eds), Reservatório de Peixe Angical: Bases ecológicas para o manejo da ictiofauna. UFT, Porto Nacional, Brazil, pp 39–48
- Froehlich O, Cavallaro M, Sabino J, Suárez YR, Vilela MJA (2017) Checklist da ictiofauna do Estado de Mato Grosso do Sul, Brasil. *Iheringia, Série Zoologia* 107: e2017151, <https://doi.org/10.1590/1678-4766e2017151>
- Garcia DAZ, Britton JR, Vidotto-Magnoni AP, Orsi ML (2018) Introductions of non-native fishes into a heavily modified river: rates, patterns and management issues in the Paranapanema River (Upper Paraná ecoregion, Brazil). *Biological Invasions* 20: 1229–1241, <https://doi.org/10.1007/s10530-017-1623-x>
- Graça WJ, Pavanelli CS (2007) Peixes da planície de inundação do alto rio Paraná e áreas adjacentes. EDUEM, Maringá, Brazil, 241 pp
- Grill G, Lehner B, Thieme M, Geenen B, Tickner D, Antonelli F, Babu S, Borrelli P, Cheng L, Crochetiere H, Macedo HE, Filgueiras R, Goichot M, Higgins J, Hogan Z, Lip B, McClain ME, Meng J, Mulligan M, Nilsson C, Olden JD, Opperman JJ, Petry P, Liermann CR, Sáenz L, Salinas-Rodríguez S, Schelle P, Schmitt RJP, Snider J, Tan F, Tockner K, Valdujo PH, van Soesbergen A, Zarfl C (2019) Mapping the world's free-flowing rivers. *Nature* 569: 215–221, <https://doi.org/10.1038/s41586-019-1111-9>
- Gubiani ÉA, Ruaro R, Ribeiro VR, Eichelberger ACA, Bogoni RF, Lira AD, Cavalli D, Piana PA, Graça WJ (2018) Non-native fish species in Neotropical freshwaters: how did they arrive, and where did they come from? *Hydrobiologia* 817: 57–69, <https://doi.org/10.1007/s10750-018-3617-9>
- Havel JE, Lee CE, Vander Zanden JM (2005) Do reservoirs facilitate invasions into landscapes? *BioScience* 55: 518–525, [https://doi.org/10.1641/0006-3568\(2005\)055\[0518:DRFILL\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0518:DRFILL]2.0.CO;2)
- Hebert PDN, Ratnasingham S, DeWaard JR (2003) Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* 270: S96–S99, <https://doi.org/10.1098/rsbl.2003.0025>
- Heger T, Saul WC, Trepl L (2013) What biological invasions “are” is a matter of perspective. *Journal of Nature Conservation* 21: 93–96, <https://doi.org/10.1016/j.jnc.2012.11.002>
- Hellawell JM, Abel R (1971) A rapid volumetric method for the analysis of the food of fishes. *Journal of Fish Biology* 3: 29–37, <https://doi.org/10.1111/j.1095-8649.1971.tb05903.x>
- Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10–18, <https://doi.org/10.1111/j.1365-2664.2008.01600.x>
- Hyslop EJ (1980) Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology* 17: 411–429, <https://doi.org/10.1111/j.1095-8649.1980.tb02775.x>
- Johnson PTJ, Olden JD, Zaden MJV (2008) Dam invaders: impoundments facilitate biological invasions into freshwaters. *Frontiers in Ecology and the Environment* 6: 357–363, <https://doi.org/10.1890/070156>
- Júlio Júnior HF, Tós CD, Agostinho AA, Pavanelli CS (2009) A massive invasion of fish species after eliminating a natural barrier in the upper rio Paraná basin. *Neotropical Ichthyology* 7: 709–718, <https://doi.org/10.1590/S1679-62252009000400021>
- Kelly LJ, Hollingsworth PM, Coppins BJ, Ellis CJ, Harrold P, Tosh J, Yahr R (2011) DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. *New Phytologist* 191: 288–300, <https://doi.org/10.1111/j.1469-8137.2011.03677.x>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874, <https://doi.org/10.1093/molbev/msw054>
- Langeani F, Castro RMC, Oyakawa OT, Shibatta OA, Pavanelli CS, Casatti L (2007) Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. *Biota Neotropica* 7: 181–197, <https://doi.org/10.1590/S1676-06032007000300020>
- Liu C, Comte L, Olden JD (2017) Heads you win, tails you lose: life-history traits predict invasion and extinction risk of the world's freshwater fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems* 27: 773–779, <https://doi.org/10.1002/aqc.2740>
- Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz F (2000) Biotic Invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689–710, [https://doi.org/10.1890/1051-0761\(2000\)010\[0689:BICEGC\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2)
- Makrakis MC, Miranda LE, Makrakis S, Fontes Júnior HM, Morlis WG, Dias JHP, Garcia JO (2012) Diversity in migratory patterns among Neotropical fishes in a highly regulated river basin. *Journal of Fish Biology* 81: 866–881, <https://doi.org/10.1111/j.1095-8649.2012.03346.x>
- Makrakis S, Gomes LC, Makrakis MC, Fernandez DR, Pavanelli CS (2007) The Canal da Piracema at Itaipu Dam as a fish pass system. *Neotropical Ichthyology* 5: 185–195, <https://doi.org/10.1590/S1679-62252007000200013>

- Makrakis S, Miranda LE, Gomes LC, Makrakis MC, Fontes Júnior HM (2011) Ascent of Neotropical migratory fish in the Itaipu reservoir fish pass. *River Research and Applications* 27: 511–519, <https://doi.org/10.1002/rra.1378>
- Makrakis S, Bertão APS, Silva JFM, Makrakis MC, Sanz-Ronda FJ, Celestino LF (2019) Hydropower development and fishways: a need for connectivity in rivers of the Upper Paraná Basin. *Sustainability* 11: 1–24, <https://doi.org/10.3390/su11133749>
- Marques H, Dias JHP, Perbiche-Neves G, Kashiwaqui EAL, Ramos IP (2018) Importance of dam-free tributaries for conserving fish biodiversity in Neotropical reservoirs. *Biological Conservation* 224: 347–354, <https://doi.org/10.1016/j.biocon.2018.05.027>
- Monteiro AS, Oliveira AHM, Pelicice FM, Oliveira RJ (2008) Alterações na disponibilidade de recursos alimentares e na dieta das principais espécies de peixes. In: Agostinho CS, Pelicice FM, Marques EE (eds), Reservatório de Peixe Angical: Bases ecológicas para o manejo da ictiofauna. UFT, Porto Nacional, Brazil, pp 75–84
- Nakatani K, Agostinho AA, Bialetzki A, Baumgartner G, Sanches PV, Makrakis MC, Pavanelli C (2001) Manual de identificação de ovos e larvas de peixes brasileiros de água doce. EDUEM, Maringá, Brazil, 378 pp
- Nilsson C, Reidy CA, Dynesius M, Revenga C (2005) Fragmentation and flow regulation of the world's large river systems. *Science* 308: 405–408, <https://doi.org/10.1126/science.1107887>
- Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution* 19: 18–24, <https://doi.org/10.1016/j.tree.2003.09.010>
- Ota RR, Deprá GC, Graça WJ, Pavanelli CS (2018) Peixes da planície de inundação do alto rio Paraná e áreas adjacentes: revised, annotated and updated. *Neotropical Ichthyology* 16: e170094, <https://doi.org/10.1590/1982-0224-20170094>
- Pavanelli CS, Britski HA (1999) Description of a new species of *Steindachnerina* (Teleostei: Characiformes: Curimatidae) from the upper Rio Paraná basin, Brazil. *Ichthyological Exploration of Freshwaters* 10(3): 211–216
- Pavanelli CS, Graça WJ, Zawadzki CH, Britski HA, Vidotti AP, Avelino GS, Veríssimo S (2007) Fishes from the Corumbá Reservoir, Paranaíba River drainage, upper Paraná River basin, State of Goiás, Brazil. *Check List* 3: 58–64, <https://doi.org/10.15560/3.1.58>
- Pelicice FM, Pompeu PS, Agostinho AA (2015) Large reservoirs as ecological barriers to downstream movements of Neotropical migratory fish. *Fish and Fisheries* 16: 697–715, <https://doi.org/10.1111/faf.12089>
- Pelicice FM, Azevedo-Santos VM, Vitule JRS, Orsi ML, Lima Junior DP, Magalhães ALB, Pompeu PS, Petrere Júnior M, Agostinho AA (2017) Neotropical freshwater fishes imperiled by unsustainable policies. *Fish and Fisheries* 18: 1119–1133, <https://doi.org/10.1111/faf.12228>
- Pereira LHG, Hanner R, Foresti F, Oliveira C (2013) Can DNA Barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genetics* 14: 20, <https://doi.org/10.1186/1471-2156-14-20>
- Pracheil BM, Mestl GE, Pegg MA (2015) Movement through dams facilitates population connectivity in a large river. *River Research and Applications* 31: 517–525, <https://doi.org/10.1002/rra.2751>
- Ramirez JL, Carvalho-Costa LF, Venere PC, Carvalho DC, Troy WP, Galetti Júnior PM (2016) Testing monophyly of the freshwater fish *Leporinus* (Characiformes, Anostomidae) through molecular analysis. *Journal of Fish Biology* 88: 1204–1214, <https://doi.org/10.1111/jfb.12906>
- Ramirez JL, Birindelli JL, Carvalho DC, Affonso PRAM, Venere PC, Ortega H, Carrillo-Avila M, Rodríguez-Pulido JA, Galetti Jr PM (2017) Revealing hidden diversity of the underestimated Neotropical Ichthyofauna: DNA Barcoding in the recently described genus *Megaleporinus* (Characiformes: Anostomidae). *Frontiers in Genetics* 8: 149, <https://doi.org/10.3389/fgene.2017.00149>
- Reis RE, Albert JS, Di Dario F, Mincarone MM, Petry P, Rocha LA (2016) Fish biodiversity and conservation in South America. *Journal of Fish Biology* 89: 12–47, <https://doi.org/10.1111/jfb.13016>
- Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and Invasion of Alien Plants: Concepts and Definitions. *Diversity and Distributions* 6: 93–107, <https://doi.org/10.1046/j.1472-4642.2000.00083.x>
- Santos GM, Jégu M (1989) Inventário taxonômico e redescritção das espécies de Anostomídeos (Characiformes, Anostomidae) do Baixo Rio Tocantins, PA, Brasil. *Acta Amazonica* 19: 159–213, <https://doi.org/10.1590/1809-43921989191213>
- Santos CJ, Tencatt LFC, Ota RR, Graça WJ (2013) Second record of *Leporinus tigrinus* Borodin, 1929 (Characiformes: Anostomidae) in the Upper Paraná River basin, Brazil. *Check List* 9: 1543–1544, <https://doi.org/10.15560/9.6.1543>
- Silva-Santos R, Ramirez JL, Galetti Júnior PM, Freitas PD (2018) Molecular evidences of a hidden complex scenario in *Leporinus* cf. *friderici*. *Frontiers in Genetics* 9: 47, <https://doi.org/10.3389/fgene.2018.00047>
- Sol D, Weis JS (2019) Highlights and insights from “Biological Invasion and Animal Behaviour”. *Aquatic Invasions* 14: 551–565, <https://doi.org/10.3391/ai.2019.14.3.12>

- SpeciesLink (2019) Centro de Referência em Informação Ambiental, Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP. <http://www.splink.org.br> (accessed 15 May 2019)
- Stoeckle MY, Gamble CC, Kirpekar R, Young G, Ahmed S, Little DP (2011) Commercial teas highlight plant DNA barcode identification successes and obstacles. *Scientific Reports* 1: 42, <https://doi.org/10.1038/srep00042>
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680, <https://doi.org/10.1093/nar/22.22.4673>
- Toussaint A, Beauchard O, Oberdorff T, Brosse S, Villéger S (2016) Worldwide freshwater fish homogenization is driven by a few widespread non-native species. *Biological Invasions* 18: 1295–1304, <https://doi.org/10.1007/s10530-016-1067-8>
- Vazzoler AEAM (1996) *Biologia da reprodução de peixes teleósteos: Teoria e prática*. EDUEM, Maringá, Brazil, 169 pp
- Vermeij GJ (1996) An agenda for invasion biology. *Biological Conservation* 78: 3–9, [https://doi.org/10.1016/0006-3207\(96\)00013-4](https://doi.org/10.1016/0006-3207(96)00013-4)
- Vitule JRS, Skóra F, Abilhoa V (2012) Homogenization of freshwater fish faunas after the elimination of a natural barrier by a dam in Neotropics. *Diversity and Distributions* 18: 111–120, <https://doi.org/10.1111/j.1472-4642.2011.00821.x>
- Ward JV (1989) The four-dimensional nature of lotic ecosystems. *Journal of the North American Benthological Society* 8: 2–8, <https://doi.org/10.2307/1467397>
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1847–1857, <https://doi.org/10.1098/rstb.2005.1716>
- Wolf AT, Natharius JA, Danielson JJ, Ward BS, Pender JK (1999) International river basins of the world. *International Journal of Water Resources Development* 15: 387–427, <https://doi.org/10.1080/07900629948682>
- Zarfl C, Lumsdon AE, Berlekamp J, Tydecks L, Tockner K (2015) A global boom in hydropower dam construction. *Aquatic Sciences* 77: 161–170, <https://doi.org/10.1007/s00027-014-0377-0>