The main channel and river confluences as spawning sites for migratory fishes in the middle Uruguay River

Marlon da Luz Soares[1], Marthoni Vinicius Massaro[2], Paula Betina Hartmann[2], Samuel Elias Siveris[2], Fernando Mayer Pelicice[3] and David Augusto Reynalte-Tataje[1]

Correspondence:
Marlon da Luz Soares
marlo.bio87@gmail.com

Evidence indicates that migratory fish reproduce in the middle Uruguay River, but the location of spawning sites remains unknown. To identify spawning sites in the basin, fish eggs and larvae were sampled monthly between October 2016 and January 2017. The sampling was carried out in three sections along the middle Uruguay River, covering two environments: the main channel and the mouth of tributaries (Comandaí, Ijuí, and Piratinim rivers). A total of 11,519 eggs and 3,211 larvae were captured, belonged to ten migratory species. Eggs and larvae of migratory fishes, were widely distributed, with predominance of segmented eggs, and larvae in yolk-sac and pre-flexion stages, with higher densities near the confluence with the Piratinim River. Larvae assemblages showed spatial variation, indicating that spawning sites differ among migratory species. This study provides new information about fish reproduction in the middle Uruguay River, revealing that migratory species spawn in different localities along the main channel and tributaries. This region may function as a critical site for fish reproduction, although it is currently threatened by the risk of hydropower expansion, emphasizing the need for measures that preserve their environmental conditions, hydrological connectivity and ecological functions.

Keywords: Conservation, Ichthyoplankton, Lotic environment, Potamodromous, Spatial patterns.

Submitted September 1, 2021
Accepted June 20, 2022
by Andrea Bialetzki
Epub August 8, 2022

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 20, no. 3, Maringá 2022

1 Universidade Federal da Fronteira Sul, Programa de Pós-Graduação em Ambiente e Tecnologias Sustentáveis, Av. Jacob Reinaldo Haupenthal, 1580, 97900-000 Cerro Largo, RS, Brazil. (MLS) marlo.bio87@gmail.com (corresponding author), (DART) david.tataje@uffs.edu.br.

2 Universidade Federal da Fronteira Sul, Curso de Ciências Biológicas, Av. Jacob Reinaldo Haupenthal, 1580, 97900-000 Cerro Largo, RS, Brazil. (MVM) bio.marthonivinicius@gmail.com, (PBH) paulahartmann7@gmail.com, (SES) samuelbiologia1991@gmail.com.

3 Núcleo de Estudos Ambientais, Programa de Pós-Graduação em Biodiversidade, Ecologia e Conservação, Universidade Federal de Tocantins, Rua 3, Quadra 17, Jardim dos Ipês, 77500-000 Porto Nacional, TO, Brazil. (FMP) fmpelicice@gmail.com.

Evidências indicam que os peixes migradores se reproduzem no médio rio Uruguai, mas a localização dos locais de desova ainda permanece desconhecida. Para identificar estes locais, foram realizadas mensalmente entre outubro de 2016 e janeiro de 2017, amostragens de ovos e larvas de peixes em três seções ao longo do médio rio Uruguai, cobrindo dois ambientes: o canal principal e a foz dos afluentes (rios Comandaí, Ijuí e Piratinim). Foram capturados 11.519 ovos e 3.211 larvas, pertencentes a dez espécies migradoras. Ovos e larvas de peixes migradores, foram amplamente distribuídos, com predomínio de ovos segmentados e larvas em estágios de larval-vitelino e pré-flexão, com maiores densidades próximo à confluência com o rio Piratinim. As assembleias de larvas mostraram variação espacial, indicando que os locais de desova diferem entre as espécies migradoras. Este estudo fornece novas informações sobre a reprodução de peixes no médio rio Uruguai, revelando que espécies migradoras desovam em diferentes localidades ao longo do canal principal e afluentes. Esta região pode funcionar como um local crítico para a reprodução de peixes, embora atualmente esteja ameaçada pelo risco de expansão da energia hidrelétrica, enfatizando a necessidade de medidas que preservem suas condições ambientais, conectividade hidrológica e funções ecológicas.

Palavras-chave: Ambiente lótico, Conservação, Ictioplâncton, Modelo espacial, Potamódromo.

INTRODUCTION

Freshwater fish display a range of reproductive tactics (Nakatani *et al*., 2001), among which migratory behavior is an important feature shared by many species. The purpose of reproductive migration is to release eggs in habitats that maximize survival, and spawning sites generally differ from nursery and feeding grounds (Suzuki *et al*., 2009; Ávila-Simas *et al*., 2014). The diversity of migratory behavior is remarkable and may involve anadromous, catadromous, or potamodromous life cycles. For example, the European eel *Anguilla anguilla* (Linnaeus, 1758) migrates toward the ocean to spawn, while the Atlantic salmon *Salmo salar* Linnaeus, 1758 migrates toward continental rivers, *i.e*., homing (Hendry *et al*., 2004). Some European cyprinids migrate between lentic and lotic environments (Skov *et al*., 2008), while some Asian cyprinids migrate toward upstream reach spawning (Lucas, Baras, 2008). In the Neotropical region, which holds extraordinary fish diversity (Albert *et al*., 2020), migratory dynamics and spawning sites remain poorly understood, but a diversity of behavior has been reported (Makrakis *et al*., 2012; Duponchelle *et al*., 2021). Studies conducted in the Paraná, Amazon, and São Francisco basins indicate that most fish migrate toward the upper sections and tributaries to spawn (Vazzoler, Menezes, 1992), which may involve hundreds (Agostinho, Zalewski, 1996; Nakatani *et al*., 1997; Lopes *et al*., 2019a) to thousands of kilometers (Godoy, 1975; Barthem, Goulding, 1997; Barthem *et al*., 2017).

Recent research conducted in the Uruguay River basin has shown different patterns, as some migratory species reproduce at various sites along the river channel (Reynalte-

Tataje, Zaniboni-Filho, 2008), in addition to the confluence of tributary rivers (Zaniboni-Filho, Schultz, 2003). These studies have been conducted in the upper Uruguay River, a region characterized by waterfalls and anthropic impacts (*i.e.*, dams), which pose significant constraints to fish migration (Reynalte-Tataje, 2008; Hermes-Silva *et al*., 2009; Silva *et al*., 2012). The middle Uruguay River represents a different scenario, with a long fluvial stretch of over 800 km, with no dam or significant geographic barrier that could restrict fish movements. At the upper limit of this segment, the Salto do Yucumã a large waterfall, constitutes a partial barrier for fish movement and restricts the dispersion to upper Uruguay; however, no other barrier exists downstream until the Santo Grande Dam. The absence of significant barriers may clarify whether reproductive dynamics are similar to those observed in the upper Uruguay River, or to those described in other basins (*e.g.*, migration to upstream reaches). In this sense, it is essential to understand the reproductive dynamics and critical environments in river segments that have been subjected to limited impacts, especially if we consider that most ichthyoplankton studies in Brazil and the world have been conducted in altered environments (Sanches *et al*., 2006; Hermes-Silva *et al*., 2009; Mu *et al*., 2014).

In this study, we investigated the distribution of eggs and larvae of migratory fish to identify spawning sites in the middle Uruguay River basin. Based on patterns observed in the upper Uruguay River (Hermes-Silva *et al*., 2009; Reynalte-Tataje *et al*., 2012a), we investigated the hypothesis that fish spawn in different sites along the main channel and the confluence with tributaries, and predicted the predominance of eggs and larvae in early development stages across these environments. Particularly, this study examined (i) spatial variations in the abundance of eggs and larvae of migratory fish; (ii) the distribution of different embryonic and larvae stages; and (iii) the taxonomic composition of larvae assemblages in the main channel and tributaries.

MATERIAL AND METHODS

Study area. The Uruguay River is an important sub-system of the Río La Plata basin. The upper basin has its sources at 1,800 m elevation. The river is channelized in the valley, with steep slopes and considerable variation in water flow. The middle basin is a long unregulated segment of about 800 km. It is a lowland region that begins at 130 m of elevation, with an average slope of 0.16%. The lower basin is about 350 km long, with a difference in elevation of about 1 m (Zaniboni-Filho, Schulz, 2003). The wet period occurs between June and October, with relevant inter-annual variations (Zaniboni-Filho, Schulz, 2003). According to Reynalte-Tataje, Zaniboni-Filho (2008), the driest period occurs during the austral summer.

This study was carried out in the middle Uruguay River in a period characterized by regular climatic conditions, without the occurrence of extreme climatic events (INPE, 2021). In this stretch, the river channel is characterized by slow currents, pools, channels, backwaters, islands, and some rapids. Floodplains and wetlands are absent, but these environments are found approximately 150 km downstream. Three sections were selected along a 70 km river segment. We sampled two sites in each section, one site in the tributary river and the other in the Uruguay River, upstream from the confluence with the tributary, totaling six sites (Fig. 1).

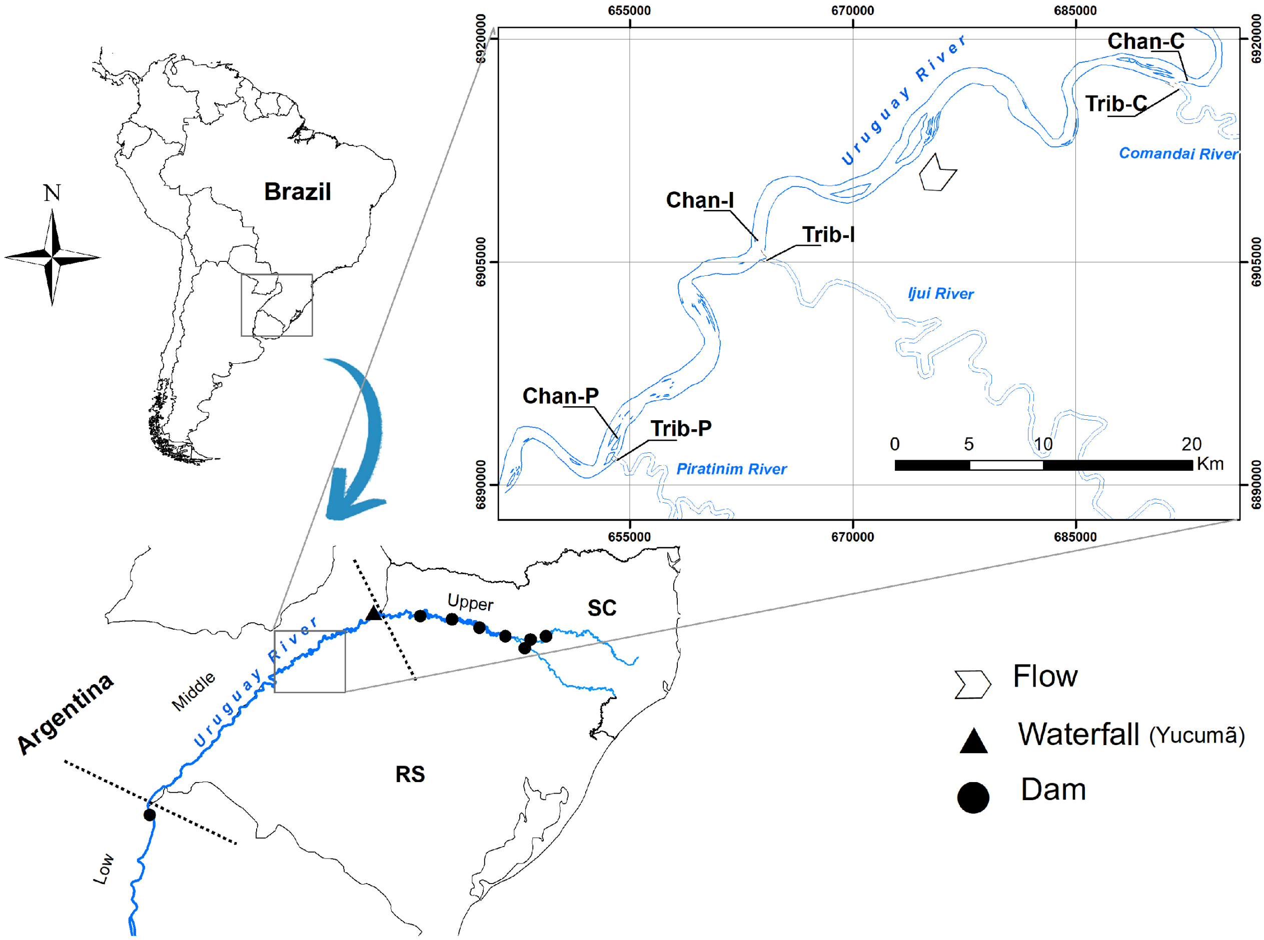

FIGURE 1 | The Uruguay River basin, the study area in the middle Uruguay River, and the six sampling sites investigated (Chan-C, Trib-C, Chan-I, Trib-I, Chan-P, and Trib-P).

The Comandaí Section (27°51'19"S 55°02'56"W) is located 240 km downstream from The Salto do Yucumã (Yucumã Falls), the limit between the upper and middle Uruguay sub-basins. The sampling site in the main channel (hereafter Chan-C) is a river segment, 1,070 m wide, with shallow areas and some interspersed deep pools. The sampling site in the tributary (hereafter Trib-C) is located on the Comandaí River, 150 m upstream from its confluence with the Uruguay River. The tributary is 45 m wide with a drainage area of 2,263 km². This tributary is not regulated by upstream dams.

The Ijuí Section (27°57'54"S 55°20'04"W) is located 42 km downstream from the Comandaí River Section. The sampling site in the main channel (hereafter Chan-I) of the Uruguay River is a river segment, 950 m wide, with shallow areas, and some deep pools. The sampling site in the tributary (hereafter Trib-I) is located on the Ijuí River, 150 m upstream from its confluence with the Uruguay River. The tributary is 140 m wide with a drainage area of 10,700 km². This tributary is regulated by two dams located upstream.

The Piratinim Section (28°04'49"S 55°25'45"W) is located 28 km downstream from the Ijuí River Section. The sampling site in the main channel (hereafter Chan-P) is a river segment, 1,300 m wide, with shallow areas, deep pools, rapids, and fluvial islands. The sampling site in the tributary (hereafter Trib-P) is located on the Piratinim River, 150 m upstream from its confluence with the Uruguay River. The tributary is 130 m wide with a drainage area of 7,500 km^2. This tributary is not regulated by upstream dams.

Sampling. Monthly samples were taken between October 2016 and January 2017, during the reproductive period of migratory species in the basin (Reynalte-Tataje *et al*., 2008a). Sampling occurred during the first two hours after dusk, following the method described by Hermes-Silva *et al*. (2009). Fish eggs and larvae were collected with conical-cylindrical plankton nets, 500 µm mesh size, 1,5 m long, and equipped with a mechanical flow meter. Each site was sampled for three days at 24-hour intervals, by positioning two nets against the water flow near the surface. Nets remained set for 10 min, a procedure that was repeated, generating four samples/day/site. Daily abundance was then calculated as the sum of the four samples divided by the total filtered volume of the four samples, totaling three monthly replicates for each sampling site. We collected 72 samples (6 sites x 4 months x 3 replies). All material was stored in polyethylene bottles and fixed in 4% formalin.

In the laboratory, eggs and larvae were sorted under a stereomicroscope with the aid of a Bogorov plate. It is not possible to identify eggs of migratory species, but these eggs have a higher perivitelline space (> 30 %) (Nakatani *et al*., 2001); then, eggs with this characteristic were assigned as belonging to migratory fishes. Eggs were separated according to their degree of embryonic development: Segmentation (S), Head-Tail (HT), and Free-Tail (FT) (Nakatani *et al*., 2001). Larvae were identified as the lowest taxonomic level following Nakatani *et al*. (2001) and Reynalte-Tataje, Zaniboni-Filho (2008). Larvae were also separated according to the notochord-flexion development of caudal fins and supporting elements, as proposed by Ahlstrom, Moser (1976) modified by Nakatani *et al*. (2001): LV = Yolk-sac larvae, PF = Preflexion, F = Flexion, and PoF = Postflexion stages.

The larvae were classified as belonging to migratory or non-migratory species following specific literature (*e.g*., Carolsfeld *et al*., 2003; Reynalte-Tataje, Zaniboni-Filho, 2008; Massaro *et al*., 2019). Neotropical migratory fishes have complex life cycles and migrate over long distances (>100 km) between feeding and spawning sites (Carolsfeld *et al*., 2003). These fish are potamodromous and migrate seasonally between river habitats; they do not have parental care and release eggs in the water current. Otherwise, non-migratory fishes include a variety of behaviors (*e.g*., sedentary, short-distance displacements, rheophilic, limnophilic, and parental care), differing substantially from patterns observed for migratory species (Winemiller, 2003; Suzuki *et al*., 2004). The vouchers was deposited in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá (NUP), Paraná, Brazil.

Data analysis. The number of eggs and larvae was converted to density (individuals/10 m^3 of filtered water), following Tanaka (1973) modified by Nakatani *et al.* (2001). In order to investigate differences in the abundance of eggs and larvae among sampling sites, we used the non-parametric Kruskal-Wallis test, followed by Dunn's comparison, because the data did not meet parametric assumptions. To test differences in taxonomic composition, stages of embryonic development, and stages of larval development between sampling sites, we used a permutational multivariate analysis of variance (one-way PERMANOVA) with 999 permutations, based on Bray-Curtis similarity applied to untransformed data (Anderson, 2005). The indicator value method (IndVal; Dufrêne, Legendre, 1997) was employed to determine species, embryonic stages, and larval stages that indicated of each sampling site. Statistical significance implied $p < 0.05$. All analyses were run in PCORD v. 5 (McCune, Mefford, 2011) or the R environment (R Development Core Team, 2018).

RESULTS

We collected 14,730 individuals, being 11,519 eggs (78.2%) and 3,211 larvae (21.8%). Migratory fish summed 2,480 eggs and 517 larvae.

Egg density. We recorded migratory and non-migratory fish eggs at all sampling sites. Eggs of migratory fish were more abundant at Chan-P and Trib-P (Fig. 2A; $p < 0.05$).

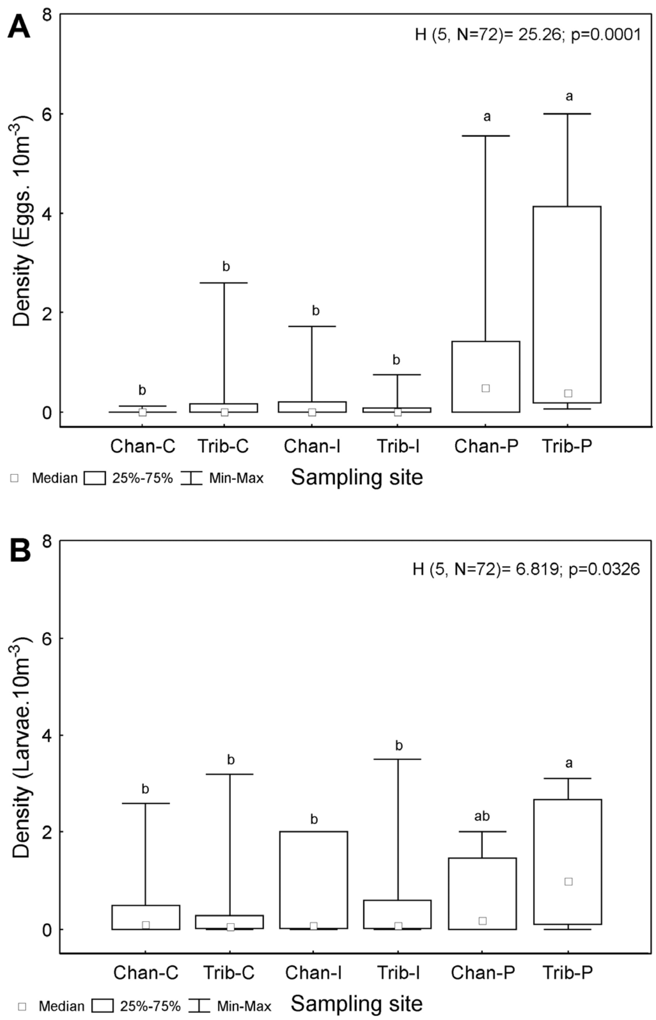

FIGURE 2 | Spatial distribution of median, first and third quartile, maximum and minimum densities of fish eggs and larvae collected in the middle Uruguay River and tributaries, between October 2016 and January 2017. Different letters within each graph indicate a statistically significant difference ($p < 0.05$). **A.** Eggs; and **B.** Larvae.

Most eggs (> 75 %) were in the segmentation stage (with 2 to 16 blastomeres) (Fig. 3). Eggs in the head-tail stage were also widely distributed, but with low frequency, while the free-tail stage was less abundant (Fig. 3). Significant differences in embryonic development stages were detected among sampling sites (PERMANOVA; pseudo-F = 2875.2, $p < 0.0001$). The highest presence of eggs in the segmentation stage was found at the mouth of the three tributary rivers (Trib-C, Trib-I, and Trib-P) and also at Chan-P (IndVal, $p < 0.05$; Tab. 1; Fig. 3).

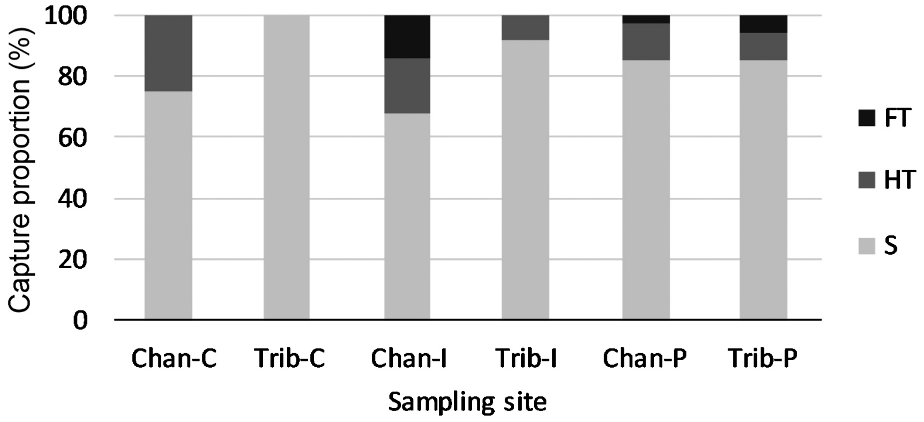

FIGURE 3 | Proportion Capture (%) of the different embryonic development stages of fishes captured in the middle Uruguay River and tributaries, between October 2016 and January 2017. Degree of embryonic development: Segmentation (S), Head-Tail (HT) and Free-Tail (FT).

TABLE 1 | Results of the indicator value (IndVal) applied to stages of embryonic and larval development of migratory fish, discriminated among sampling sites in the Middle Uruguay River, RS, Brazil. *Only sampling sites that showed embryonic stages and indicator larvae were presented. Embryonic Stages: Segmentation (S), Head-Tail (HT) and Free-Tail (FT). Larval stages: LV = Larval vitelline, PF = Preflexion, F = Flexion and PoF = Postflexion.

Sampling site	Embrionyc stage*	IndVal	p
Trib-C	S	45.3	0.001
Trib-I	S	49.1	0.023
Chan-P	S	47.8	0.019
Trib-P	S	42.4	0.007
Sampling site	**Larval stage***	**IndVal**	**p**
Chan-I	PF	24.2	0.016
Trib-I	PF	22.6	0.036
Chan-P	PF	31.3	0.009
Trib-P	LV	21.2	0.042

Larvae density and composition. Larvae belonged to four orders, 20 families, 44 genera, and 35 species were captured. Siluriformes accounted for 49.5% of the catch and Characiformes summed 43.5%. We recorded larvae of migratory and non-migratory fish in all sampling localities, however, only migratory species larvae were analyzed (representing 16.1% of all larvae captured). The largest density of migratory species larvae was registered in the Piratinim River confluence area, mainly at Trib-P (Fig. 2B; $p < 0.05$). The list of identified larvae and their distribution in the different sampling sites are shown in the Tab. **S1**.

We detected different development stages, but initial phases predominated (Yolk-sac larvae and preflexion). The distribution of larval stages differed among sampling sites (PERMANOVA; pseudo-F = 699.4, $p < 0.0001$). The highest proportion of the LV stage was found at Trib-P, and the PF stage at Trib-I, Chan-I, and Chan-P (IndVal, $p < 0.05$; Tab. 1; Fig. 4).

The composition of migratory fish larvae differed significantly among sampling sites (PERMANOVA; pseudo-F = 1834.8, $p < 0.0001$). IndVal showed that some migratory species are more present in certain sites: *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) (Chan-C), *Megaleporinus obtusidens* (Valenciennes, 1837) (Chan-I), and *Prochilodus lineatus* (Valenciennes, 1837) (Chan-P) were found mainly in the main channel of the Uruguay River, while *Pimelodus maculatus* Lacepède, 1803, and *Salminus brasiliensis* (Cuvier, 1816) were found in the tributary Piratinim River (Trib-P) (IndVal, $p < 0.05$; Tab. 2).

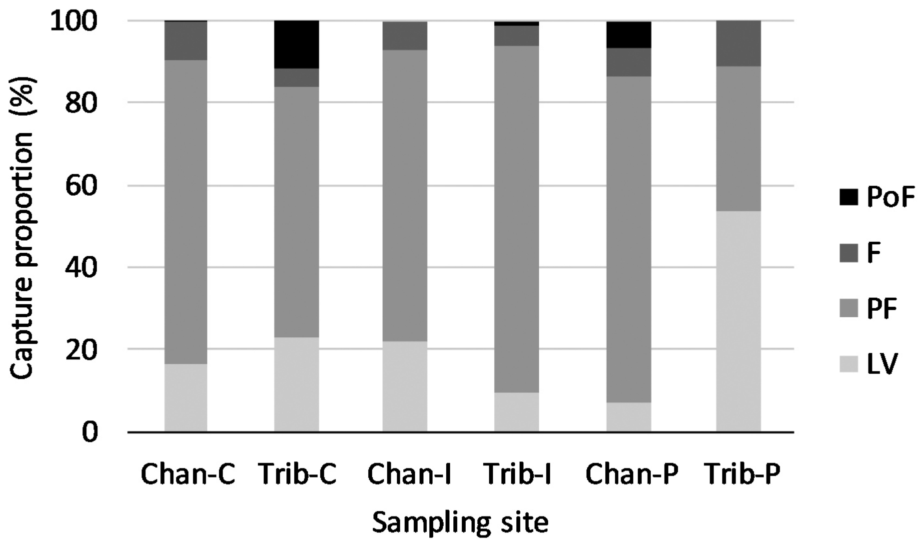

FIGURE 4 | Proportion Capture (%) of the different larval development stages of fishes captured in the middle Uruguay River and tributaries, between October 2016 and January 2017. Degree of larval stages of development: LV = Yolk-sac larvae, PF = Preflexion, F = Flexion and PoF = Postflexion.

TABLE 2 | Results of the indicator value (IndVal) applied to the taxonomic composition of migratory fish larvae captured, discriminated between sampling sites in the middle Uruguay River, RS, Brazil. *Only sampling sites that presented indicator species were presented.

Sampling site	Species*	IndVal	p
Chan-C	*Pseudoplatystoma corruscans*	21.1	0.024
Chan-I	*Megaleporinus obtusidens*	15.8	0.045
Chan-P	*Prochilodus lineatus*	16.6	0.038
Trib-P	*Pimelodus maculatus*	37.9	0.006
	Salminus brasiliensis	19.8	0.017

DISCUSSION

Our results evidence migratory fish spawns at different localities along the middle Uruguay River, with the main channel and tributaries working as spawning sites. Confirming our prediction, we found eggs and larvae in different localities, with a predominance of early development stages. It indicates that spawning is taking place close to the sampling area (approximately 10 km upstream) considering the average water velocity in the Uruguay River (<1.0 $m.s^{-1}$) (Cardini *et al*., 2004); however the occurrence of other development stages suggest also to spawning activity in more distant sites. In fact, eggs and larvae in early development were also recorded in the Uruguay River approximately 210 km upstream from the study site (Ziober *et al*., 2015), indicating that the middle section works as a macro spawning area. These findings support our hypothesis that fish spawn in different sites along this river segment, including the main channel and the confluence with tributaries. This stretch is free-flowing and surrounded by two protected areas (Parque Estadual do Turvo in Brazil, and Reserva de la Biosfera Yabotí in Argentina), creating suitable conditions for the reproduction of migratory species. Therefore, migratory fishes in the Uruguay River basin apparently have multiple spawning sites, which include the main channel and tributaries in the upper (Reynalte-Tataje *et al*., 2012a; Silva *et al*., 2015) and middle reaches of the basin (Ziober *et al*., 2015; Reynalte-Tataje *et al*., 2017). However, middle Uruguay may have substantial importance considering that upper and lower reaches are disturbed by river regulation (Fig. 5); middle Uruguay, therefore, seems to work as a relevant reproduction site for migratory fishes, with the potential to supply stocks in other locations of the basin (Zaniboni-Filho, Schulz, 2003; Fuentes *et al*., 2016; Reynalte-Tataje *et al*., 2017; Serra *et al*., 2019).

Eggs from migratory species were collected in all sampling sites, and they accounted for most individuals in the samples compared to larvea. Eggs at early development stages (segmentation) were the most abundant, showing that most spawning activities took place in areas close to our sampling sites. Studies about the embryonic development of migratory fishes indicate that segmentation starts within the first two hours after hatching (Ninhaus-Silveira *et al*., 2006; Reynalte-Tataje *et al*., 2008b; Luz *et al*., 2018; Araújo *et al*., 2020). It means that organisms would disperse downstream over a few kilometers from the spawning site in a flowing river characterized by the presence of

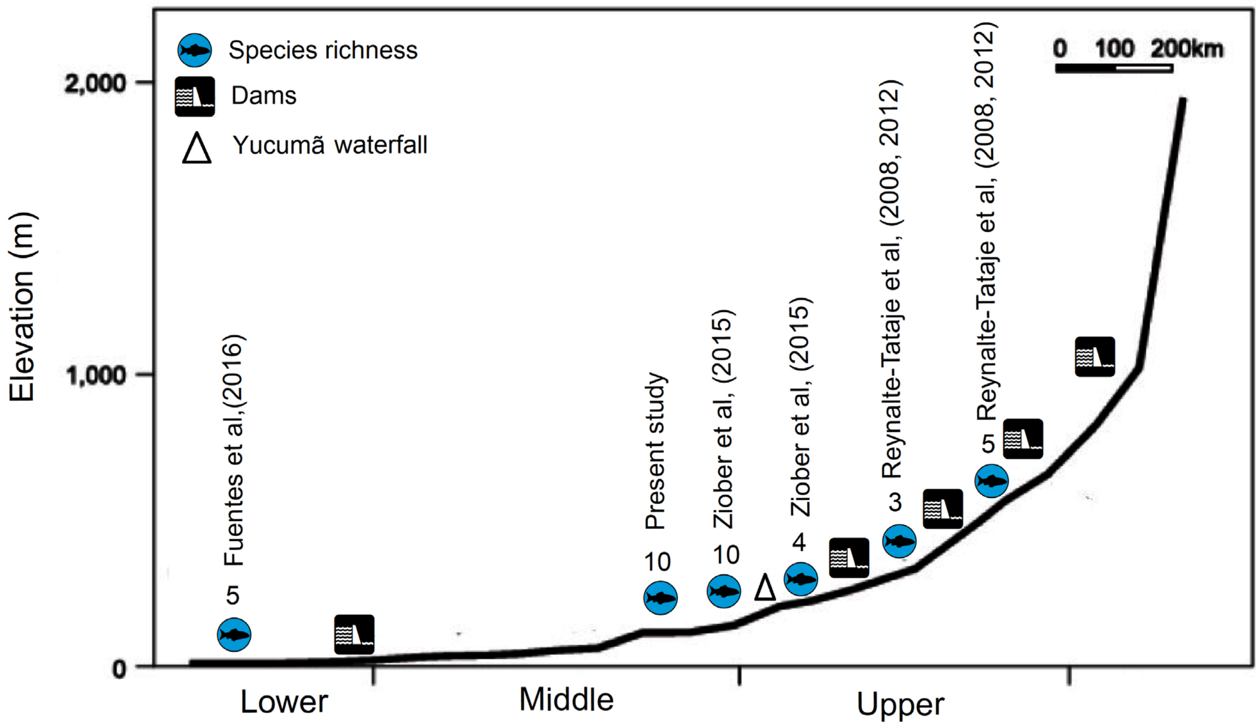

FIGURE 5 | Longitudinal profile of the Uruguay River depicting the upper, middle and lower reaches, the position of dams and the number of migratory species recorded in studies that sampled ichthyoplankton.

pools and rapids. This information is relevant as it indicates that spawning grounds are near the six sampling sites, in the main channel and tributaries. Eggs at more developed stages were captured near the confluence with the Ijuí and Piratinim rivers (Ch-I and Ch-P sites), and they may have been originated from spawning activities occurring near the confluence with the Comandaí River (Ch-C site) and/or other sites located farther upstream. In this river segment, the spawning area must be limited to the segment downstream from the Yucumã Falls, as this waterfall blocks upstream displacements. In fact, a study carried out in 24 sampling sites that included locations above and below this waterfall showed spawning activity (migratory and non-migratory fish) mainly downstream from the Falls, with almost no spawning in the upstream region (Ziober *et al*., 2015). Therefore, our study provided evidence that spawning occurs in different localities of the middle Uruguay River, especially in areas close to the confluence with the Comandaí, Ijuí, and Piratinim rivers.

Larvae were also widely distributed in the study area, although in lower densities if compared with eggs. However, the percentage of larvae in the ichthyoplankton was above 20%, which is higher than the value observed (*ca*. 5%) in upper Uruguay (Silva *et al*., 2012; Reynalte-Tataje *et al*., 2013). The predominance of larvae in early development stages (yolk-sac larvae and preflexion) also indicates that spawning sites are close to the study area, but the incidence of advanced stages, although in low densities, points to reproductive activity in the upper stretch of the Uruguay River and tributaries. Studies carried out on upper Uruguay and other Neotropical basins indicate that larvae may develop in the main channel and backwaters (Reynalte-Tataje *et al*., 2012b, 2017; Silva *et al*., 2012; Mounic-Silva *et al*., 2019), although in more limited conditions if compared to wetlands and floodplains (Agostinho, Zalewski, 1996; Suzuki *et al*., 2009). Thus, although some larvae develop in the river channel, most probably drift towards lowland areas located 130 km downstream from the confluence with the Piratinim River, where they find suitable conditions for development.

Although eggs and larvae were registered in the main channel and mouth of tributaries, we recorded spatial variations in the structure of assemblages. Studies have shown that ichthyoplankton diversity is related to morphometric aspects of the river, where smaller rivers seem less attractive to reproductive fish (Hermes-Silva *et al*., 2009; Reynalte-Tataje *et al*., 2013; Silva *et al*., 2015). In fact, low densities of eggs and larvae occurred in the mouth of the Comandaí River, the smallest system in the area. Otherwise, higher densities of eggs and larvae, at the early stages of development, occurred in the Piratinim River. This tributary is well preserved and composed of different microhabitats such as rapids, channels, islands, and pools, reinforcing the relevance of instream habitat diversity for fish reproduction (Oliveira, Ferreira, 2008; Reynalte-Tataje *et al*., 2013; Silva *et al*., 2015). However, the Ijuí River, the largest sub-basin in the study area, presented the lowest richness of migratory species. The presence of two large dams upstream may explain this pattern since flow regulation and limited connectivity negatively affect the reproduction of some migratory species (Agostinho *et al*., 1993; Agostinho *et al*., 2007). A similar pattern was observed in the upper São Francisco River, where migratory fish seem to avoid regulated tributaries (Lopes *et al*., 2019b).

One important aspect is the relevance of confluence areas for spawning and larvae drift. The importance of these environments for fish reproduction had also been verified in the upper Uruguay (Hermes-Silva *et al*., 2009; Reynalte-Tataje *et al*., 2012a). Some studies suggest that environmental variation in the confluence (temperature, electrical conductivity and flow) can act as triggers for fish spawning (Lucas, Baras, 2008; Rakowitz *et al*., 2008; Reynalte-Tataje *et al*., 2012c). These findings point to the importance of fluvial conditions and hydrological connectivity for reproduction dynamics, as this setting seems to create an adequate environment for spawning and egg/larvae transportation.

The larvae captured in this study, mainly in drifting stages, indicate that migratory species have different preferences for the spawning sites. *Pseudoplatystoma corruscans* seems to spawn mainly in the upper part of the middle Uruguay River, a pattern supported by other studies, such as Reynalte-Tataje *et al*. (2017) and Pachla *et al*. (2020). Differently, *Prochilodus lineatus* and *Megaleporinus obtusidens* spawn in regions further downstream and close to lowland regions. The dependence of larvae of this species on microcrustaceans (Paes *et al*., 2011) and juveniles on detritus (*P. lineatus*) and plant material (*M. obtusidens*) (Bayol, de Yuan, 1996; Reynalte-Tataje, Zaniboni-Filho, 2020) may partly explain the behavior of spawning in a section closer to lowland areas. Larvae of *Salminus brasiliensis* and *Pimelodus maculatus* were found mainly in the most preserved tributary river, which indicates that spawning occurred in this river. Greater activity of *S. brasiliensis* and *P. maculatus* spawning in tributary rivers was also observed in the upper Uruguay (Zaniboni-Filho, Schulz, 2003; Reynalte-Tataje *et al*., 2012a). The spatial segregation of migratory species shows that the middle Uruguay as a whole is important for this group of fish, not only because their home range is extensive, but also because each species demands specific spawning sites and nursery grounds. Nevertheless, more studies are needed to confirm these patterns, especially to follow multiple reproductive events.

Our results indicate that the middle Uruguay works as a critical site for fish reproduction. The river channel and tributaries seem to play a particular role, where migratory fishes spawn at multiple sites, with distinct preference among species. The greater flow in the Piratinim River, in particular, must attract reproductive fish and work as an important spawning ground. Habitat integrity in the middle Uruguay (*e.g.*, natural flow regime, tributaries, and riparian forests) is probably a key factor allowing the reproduction of migratory fishes. It is worth noting that we recorded 10 migratory species in the area, *i.e.*, out of 15 species existent in the whole basin (Reynalte-Tataje *et al*., 2012d). This scenario illustrates the importance of lotic stretches that preserve environmental heterogeneity for the maintenance of migratory fish populations (Lopes *et al*., 2019b; Massaro *et al*., 2019). Further studies are needed to confirm the patterns reported here, and to understand the dynamics of multiple reproductive cycles and the role of other river stretches. This information is very important considering that river regulation has disrupted the functioning of the Uruguay River basin in its upper reaches. Moreover, hydropower expansion is planned for the middle Uruguay River, such as the Garabí-Panambi complex, which would impact habitat integrity, flow and river connectivity. These dams would negatively affect populations of migratory fishes, as observed in other Neotropical basins (Agostinho *et al*., 2016; Pelicice *et al*., 2018), including endangered species such as *Brycon orbignyanus* (Valenciennes, 1850), *P. corruscans* and *S. brasiliensis* (Pachla *et al*., 2020). These results point to the need for conservation measures that preserve environmental conditions and ecological functions of rivers in the Uruguay basin. In particular, the maintenance of the hydrological connectivity between upper and lower sections of the middle Uruguay River is crucial to preserve migratory populations, as these fish need to migrate between river channels, different tributaries and the floodplain area located in the downstream section.

ACKNOWLEDGMENTS

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a scholarship, and Universidade Federal da Fronteira Sul (UFFS) for providing infrastructure. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided a research grant for Fernando M. Pelicice (Process number 312256/2020–5).

REFERENCES

- **Ahlstrom EH, Moser HG.** Eggs and larvae of fishes and their role in systematic investigations and in fisheries. Rev Trav Inst Peches Marit. 1976; 40(3):379–98. Available from: https://archimer.ifremer.fr/doc/00000/1996/
- **Agostinho AA, Mendes VP, Suzuki HI, Canzi C.** Avaliação da atividade reprodutiva da comunidade de peixes dos primeiros quilômetros a jusante do reservatório de Itaipu. Revista Unimar 1993; 15(supl.):175–89. Available from: http://repositorio.uem.br:8080/jspui/handle/1/5165

- **Agostinho AA, Zalewski M.** A planície alagável do alto rio Paraná: importância e preservação. Maringá: EDUEM; 1996.
- **Agostinho AA, Gomes LC, Pelicice FM.** Ecologia e manejo de recursos pesqueiros em reservatórios do Brasil. Maringá: EDUEM; 2007.
- **Agostinho AA, Gomes LC, Santos NCL, Ortega JCG, Pelicice FM.** Fish assemblages in Neotropical reservoirs: Colonization patterns, impacts and management. Fish Res. 2016; 173(1):26–36. http://doi:10.1016/j.fishres.2015.04.006
- **Albert JS, Tagliacollo VA, Dagosta F.** Diversification of Neotropical freshwater fishes. Annu Rev Ecol Evol Syst. 2020; 51(1):27–53. http://doi:10.1146/annurev-ecolsys-011620-031032
- **Anderson MJ.** PERMANOVA: a Fortran computer program for Permutational Multivariate Analysis of Variance. Department of Statistics, University of Auckland, New Zealand, 2005.
- **Araújo BC, Mello PH, Moreira RG, Hilsdorf AWS, Marques VH, Honji RM.** Spawning induction and embryonic development of *Salminus hilarii* (Characiformes: Characidae). Zygote 2020; 28(5):377–87. http://doi.org/10.1017/S0967199420000210
- **Ávila-Simas S, Reynalte-Tataje DA, Zaniboni-Filho E.** 2014. Pools and rapids as spawning and nursery for fish in a river stretch without floodplains. Neotrop Ichthyol. 2014; 12(3):611–22. http://doi.org/10.1590/1982-0224-20130116
- **Barthem RB, Goulding M.** The catfish connection: Ecology, migration, and conservation of Amazon predators. New York, Columbia University Press. 1997.
- **Barthem RB, Goulding M, Leite RG, Cañas C, Forsberg B, Venticinque E *et al*.** Goliath catfish spawning in the far western Amazon confirmed by the distribution of mature adults, drifting larvae and migrating juveniles. Sci Rep. 2017; 7(1):1–13. http://doi.org/10.1038/srep41784
- **Bayol V, Cordiviola de Yuan E.** Food assimilation of a neotropical riverine detritivorous fish, *Prochilodus lineatus*, studied by fatty acid composition (Pisces, Curimatidae). Hydrobiologia. 1996; 330(2):81–88. http://doi.org/10.1007/BF00019997
- **Cardini J, Zabalett A, Oliver N, Mársico D.** Efecto de los coeficientes de dispersión sobre la modelación bidimensional del transporte de contaminantes. Caso de estudio. Mecánica computacional; 2004. p.1201–14. Available from: https://amcaonline.org.ar/ojs/index.php/mc/article/download/334/321
- **Carolsfeld J, Harvey B, Ross C, Baer A.** Migratory fishes of South America: biology, fisheries and conservation status. World Fisheries Trust, British Columbia, Canada. 2003.
- **Dufrêne M, Legendre P.** Species assemblages and indicator species: the need for flexible asymmetrical approach. Ecol Monogr. 1997; 67(3):345–66. http://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2
- **Duponchelle F, Isaac VJ, Doria C, Van Damme PA, Herrera RGA *et al*.** Conservation of migratory fishes in the Amazon basin. Aquatic Conserv. 2021; 31(5)1087–105. http://doi.org/10.1002/aqc.3550
- **Fuentes CM, Gómez MI, Brown DR, Arcelus A, Ros AE.** Downstream passage of fish larvae at the salto grande dam on the Uruguay River. River Res Appl. 2016; 32(9):1879–89. http://doi.org/10.1002/rra.3030
- **Godoy MP.** Peixes do Brasil, subordem Characoidei: bacia do rio Mogi Guassu. Piracicaba: Editora Franciscana; 1975.
- **Hendry AP, Castric MT, Kinnison TP.** The evolution of philopatry and dispersal: Homing versus straying in salmonids. In: Hendry AP, Stearns SC, editors. Evolution Illuminated. Salmon and their relatives. Oxford University Press, Oxford. 2004; p.52–91.
- **Hermes-Silva S, Reynalte-Tataje DA, Zaniboni-Filho E.** Spatial and temporal distribution of ichthyoplankton in the Upper Uruguay River, Brazil. Braz Arch Biol Technol. 2009; 52(4):933–44. http://doi.org/10.1590/S1516-89132009000400017
- **Instituto Nacional de Pesquisas espaciais (INPE).** Centro de Previsão de Tempo e Estudos Climáticos. 2021. Available from: http://enos.cptec.inpe.br/
- **Lopes JM, Alves CBM, Peressin A, Pompeu PS.** Upstream and downstream migration speed of *Prochilodus costatus* (Characiformes:Prochilodontidae) in upper São Francisco basin , Brazil. Neotrop Ichthyol. 2019a; 17(2):e180072. http://doi.org/10.1590/1982-0224-20180072

- **Lopes JM, Pompeu PS, Alves CBM, Peressin A, Prado IG, Suzuki FM *et al*.** The critical importance of an undammed river segment to the reproductive cycle of a migratory Neotropical fish. Ecol Freshw Fish; 2019b; 28(9):302–16. http://doi.org/10.1111/eff.12454
- **Lucas MC, Baras E.** Migration of freshwater fishes. John Wiley & Sons Ltd. London, UK; 2008. http://doi.org/10.1002/9780470999653
- **Makrakis MC, Miranda LE, Makrakis S, Fontes Junior HM, Dias JHP, Garcia JO.** Diversity in migratory patterns among Neotropical fishes in a highly regulated River basin. J Fish Biol. 2012; 81(2):866–81. http://doi.org/10.1111/j.1095-8649.2012.03346.x
- **Massaro MV, Pachla LA, Bastian R, Pelicice FM, Reynalte-Tataje DA.** Seasonal and longitudinal variation in fish assemblage structure along an unregulated stretch of the Middle Uruguay River. Neotrop Ichthyol. 2019; 17(4):e190043. http://doi.org/10.1590/1982-0224-20190043
- **McCune B, Mefford MJ.** PC-ORD. Multivariate analysis of ecological data. Gleneden Beach, Oregon: MjM Software; 2011.
- **Mounic-Silva CE, Lopes CA, Porto-Ferreira LB, Nunes ME, Reynalte-Tataje DA, Zaniboni-Filho E.** Spawning and recruitment areas of migratory fish in the Uruguay River: applying for rivers connectivity conservation in South America. Bol Inst Pesca. 2019; 45(3):e510. http://doi.org/10.20950/1678-2305.2019.45.3.510
- **Mu H, Li M, Liu H, Cao W.** Analysis of fish eggs and larvae flowing into the Three Gorges Reservoir on the Yangtze River, China. Fish Sci. 2014; 80(3):505–15. https://doi.org/10.1007/s12562-014-0729-7
- **Nakatani K, Agostinho AA, Baumgartner G, Bialetzki A, Sanches PV, Makrakis MC *et al*.** Ovos e larvas de peixes de água doce: desenvolvimento e manual de identificação. Maringá: Eduem; 2001.
- **Nakatani K, Baumgartner G, Cavicchioli M.** Ecologia de ovos e larvas de peixe. In: Vazzoler AEAM, Agostinho AA, Hahn NS, editors. A planície inundação do alto rio Paraná, aspectos físicos, biológicos e socioeconômicos. Maringá: Eduem; 1997. p. 281–306.
- **Ninhaus-Silveira A, Foresti F, Azevedo A.** Structural and ultrastructural analysis of embryonic development of *Prochilodus lineatus* (Valenciennes, 1836) (Characiforme; Prochilodontidae). Zygote, 2006; 14(3):217–29. http://doi.org/10.1017/S096719940600373X
- **Oliveira EC, Ferreira EJG.** Spawning areas, dispersion and microhabitats of fish larvae in the Anavilhanas Ecological Station, rio Negro, Amazonas State, Brazil. Neotrop Ichthyol. 2008; 6(4):559–66. http://doi.org/10.1590/S1679-62252008000400003
- **Pachla L, Hartmann PB, Massaro M, Bastian R, Pelicice FM, Reynalte-Tataje DA.** First record of the mating behavior of the spotted surubim *Pseudoplatystoma corruscans* in the Uruguay River. J Fish Biol. 2020; 97(4):1233–37. http://doi.org/10.1111/jfb.14459
- **Paes TASV, Paes JMV, Rojas NET, Rocha O, Wisniewski JS.** Effects of liming and development of Curimbatá (*Prochilodus lineatus*) larvae on the abundance of zooplancton in fish ponds. Acta Limnol Bras. 2011; 23(4):386–93. http://doi.org/10.1590/S2179-975X2012005000017
- **Pelicice FM, Azevedo-Santos VM, Esguícero ALH, Agostinho AA, Arcifa MS.** Fish diversity in the cascade of reservoirs along the Paranapanema River, southeast Brazil. Neotrop Ichthyol. 2018; 16(2):e170150. https://doi.org/10.1590/1982-0224-20170150
- **R Development Core Team.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna; 2018.
- **Rakowitz G, Berger B, Kubecka J, Keckeis H.** Functional role of environmental stimuli for the spawning migration in Danube nase *Chondrostoma nasus* (L.). Ecol Freshw Fish. 2008; 17(3):502–14. http://doi.org/10.1111/j.1600-0633.2008.00302.x
- **Reynalte-Tataje DA, Agostinho AA, Bialetzki A.** Temporal and spatial distributions of the fish larval assemblages of the Ivinheima River sub-basin (Brazil). Environ Biol Fishes. 2013; 96(7):811–22. http://doi.org/10.1007/s10641-012-0073-7
- **Reynalte-Tataje DA, Agostinho AA, Bialetzki A, Hermes-Silva S, Fernandes R, Zaniboni-Filho E.** Spatial and temporal variation of the ichthyoplankton in a subtropical river in Brazil. Environ Biol Fishes. 2012c; 94(2):403–19. http://doi.org/10.1007/s10641-011-9955-3

- **Reynalte-Tataje DA, Barcellos RP, Hartmann PB, Scherer J, Martine G, Vlieger I *et al*.** O Médio rio Uruguai como importante area de reprodução do surubim-pintado *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae). Bol Soc Bras Ictiologia. 2017; 122(1):10–15.
- **Reynalte-Tataje DA, Hermes-Silva S, Silva PA, Bialetzki A, Zaniboni-Filho E.** Locais de crescimento de larvas de peixes na região do Alto Rio Uruguai (Brasil). In: Zaniboni-Filho E, Nuñer APO, (Eds). Reservatório de Itá. Estudos ambientais. Desenvolvimento de tecnologias e conservação da ictiofauna. Florianópolis: Editora UFSC; 2008a. p. 159–94.
- **Reynalte-Tataje DA, Nuñer APO, Nunes MC, Garcia V, Lopes CA, Zaniboni-Filho E.** Spawning of migratory fish species between two reservoirs of the upper Uruguay River, Brazil. Neotrop Ichthyol. 2012a; 10(4):829–35. http://doi.org/10.1590/S1679-62252012000400016
- **Reynalte-Tataje DA, Zaniboni-Filho E.** Biologia e identificação de ovos e larvas de peixes do alto rio Uruguai, Brasil. In: Zaniboni-Filho E, Nuñer APO, editors. Reservatório de Itá. Estudos Ambientais. Desenvolvimento de Tecnologias e Conservação da ictiofauna. Florianópolis: Editora da UFSC; 2008. p.139–54.
- **Reynalte-Tataje DA, Zaniboni-Filho E.** Biologia e cultivo de piapara, piauçu, piava e piau - espécies dos gêneros *Leporinus* e *Megaleporinus*. In: Baldisserotto B. Espécies nativas para piscicultura no Brasil. Santa Maria,RS. UFSM; 2020.
- **Reynalte-tataje DA, Zaniboni-Filho E, Bialetzki A, Agostinho AA.** Temporal variability of fish larvae assemblages: influence of natural and anthropogenic disturbances. Neotrop Ichthyol. 2012b; 10(4):837–46. http://doi.org/10.1590/S1679-62252012000400017
- **Reynalte-Tataje DA, Zaniboni-Filho E, Hermes Silva S, Machado C, Guereschi R, Nuñer APO.** Assembleia de peixes. In: Nuñer APO, Zaniboni-Filho E, editors. Reservatório de Machadinho. Peixes, pesca e tecnologias de criação. Florianópolis: Editora da UFSC; 2012d. p.11–44.
- **Reynalte-Tataje D, Zaniboni-Filho E, Muelbert B.** Stages of the embryonic development of the piavuçu *Leporinus macrocephalus* (Garavello & Britski, 1988). Act Sci . 2008b; 23(4):823–27. http://doi.org/10.4025/actascianimsci.v23i0.2614
- **Sanches PV, Nakatani K, Bialetzki A, Baumgartner G, Gomes LC, Luis EA.** Flow regulations by dams affecting ichthyoplankton: The case of Porto Primavera Dam, Paraná River - Brazil. Rivers Res Appl. 2006; 22:555–65. http://doi.org/10.1002/rra.922
- **Serra S, Loureiro M, Clavijo C, Alonso F, Scarabino F, Ríos N.** Peces del bajo Río Uruguay: Espécies destacadas. Paysandu: CARU; 2019.
- **Silva DA, Pessoa EKR, Costa SAGL, Chellappa NT, Chellappa S.** Ecologia alimentar de *Astyanax lacustris* (Osteichthyes: Characidae) na Lagoa do Piató, Assu, Rio Grande do Norte, Brasil. Biota Amazonia. 2012; 2(1):74–82. http://dx.doi.org/10.18561/2179-5746/biotaamazonia.v2n1p74-82
- **Silva PS, Makrakis MC, Miranda LE, Makrakis S, Assumpção L, Paula S *et al*.** Importance of reservoir tributaries to spawning of migratory fish in the upper paraná River. River Res Appl. 2015; 31(3):313–22. https://doi.org/10.1002/rra.2755
- **Suzuki HI, Vazzoler AEAM, Marques EE, Lizama MAP, Inada P.** Reproductive ecology os the fish assemblages. In: The Upper Paraná River and its Floodplain: physical aspects, ecology and conservation. Leiden: Backhuys Publishers, 2004. p.271–91.
- **Suzuki HI, Agostinho AA, Bailly N, Gimenes MF, Júlio-Junior HF, Gomes LC.** Inter-annual variations in the abundance of young-of-the-year of migratory fishes in the Upper Paraná River floodplain: relations with hydrographic attributes. Brazilian J Biol. 2009; 69(2):649–60. http://doi.org/10.1590/S1519-69842009000300019
- **Skov C, Brodersen J, Nilsson PA, Hansson L-A, Brõnmark C.** Inter- and size-specific patterns of fish seasonal migration between a shallow lake and its streams. Ecol Freshw Fish. 2008; 17(3):406–15. http://doi.org/10.1111/j.1600-0633.2008.00291.x
- **Tanaka S.** Stock assessment by means of ichthyoplankton surveys. FAO Fish Tech Pap. 1973; 122(1):33–51.
- **Vazzoler AEAM, Menezes NA.** Síntese de conhecimentos sobre o comportamento reprodutivo dos Characiformes da América do Sul (Teleostei, Ostariophysi). Rev Bras Biol. 1992; 52(4):627–40.

- **Winemiller KO.** Life history strategies of fishes. In: Ray GC, Ray-McCormick. Coastal Realm Conservation: Sciende and Policy. Blackwell Scientific, Oxford; 2003. p.106–07.
- **Zaniboni-Filho E, Schulz U.** Migratory fishes of the Uruguay River. In: Carosfeld J, Harvey B, Ross C, Baer A, editors. Migratory fishes of the South America: biology, social importance and conservation status. Victoria: World Fisheries Trust; 2003. p.157–94.
- **Ziober SR, Reynalte-Tataje DA, Zaniboni-Filho E.** The importance of a conservation unit in a subtropical basin for fish spawning and growth. Environ Biol Fish. 2015; 98(2):725–37. http://doi.org/10.1007/s10641-014-0307-y

AUTHORS' CONTRIBUTION

Marlon da Luz Soares: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft.

Marthoni Vinicius Massaro: Methodology, Writing-review and editing.

Paula Betina Hartmann: Methodology, Writing-review and editing.

Samuel Elias Siveris: Methodology, Writing-review and editing.

Fernando Mayer Pelicice: Formal analysis, Writing-review and editing.

David Augusto Reynalte-Tataje: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing-review and editing.

Neotropical Ichthyology

OPEN ACCESS

SBI Official Journal of the Sociedade Brasileira de Ictiologia

ETHICAL STATEMENT

CEUA – UFFS: certification number 23205.004977/2015–90.

COMPETING INTERESTS

The authors declare no competing interests.

HOW TO CITE THIS ARTICLE

- **Soares ML, Massaro MV, Hartmann PB, Siveris SE, Pelicice FM, Reynalte-Tataje DA.** The main channel and river confluences as spawning sites for migratory fishes in the middle Uruguay River. Neotrop Ichthyol. 2022; 20(3):e210094. https://doi.org/10.1590/1982-0224-2021-0094