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SPAWNING AND RECRUITMENT AREAS OF MIGRATORY FISH IN THE URUGUAY RIVER: APPLYING FOR RIVERS CONNECTIVITY CONSERVATION IN SOUTH AMERICA

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ABSTRACT

Identifying spawning and recruitment areas of Neotropical migratory fish is an important conservation issue, because it allows to define connectivity between habitats used during the initial life cycle of these species. In this sense, the objective of this study was to delimit spawning and recruitment areas of three migratory fish (*Megaleporinus obtusidens, Salminus brasiliensis* and *Prochilodus lineatus*) along of an altitude gradient of the Uruguay River. Samplings of ichthyoplankton and juveniles were applied with five different fishing devices in four river stretches, one in reservoir and three in a free section, for two consecutive years (2015-2016 and 2016-2017), during the reproductive period of the species. Larvae and juveniles were not collected in the reservoir and, in the free section, larvae in the early stages of development were found only at an intermediate stretch, and the most larvae at more advanced stages (~80%) and juveniles (~95%) were recorded in the lower sampling stretch. Based on the larval stage and abundance of juveniles, spawning-recruitment connectivity sections were presumed for these migratory species. After, these sections were associated to the location of future hydroelectric dams in the study area, with possible negative implications for the conservation of these populations in the Uruguay River.

Key words: downstream dispersion; juveniles; Neotropical migratory fish; nursery habitats.

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ÁREAS DE DESOVA E RECRUTAMENTO DE PEIXES MIGRADORES NO RIO URUGUAI: APLICAÇÃO PARA CONSERVAÇÃO DA CONECTIVIDADE DE RIOS NA AMÉRICA DO SUL

RESUMO

Identificar áreas de desova e recrutamento de peixes migradores neotropicais é um item importante de conservação, pois permite definir conectividade entre habitats utilizados durante o ciclo de vida inicial destas espécies. Neste sentido, o objetivo deste estudo foi delimitar áreas de desova e recrutamento de três peixes migradores (*Megaleporinus obtusidens, Salminus brasiliensis* e *Prochilodus lineatus*) ao longo de um gradiente de altitude do rio Uruguai. Amostragens de ictioplâncton e de juvenis foram aplicadas com cinco diferentes apetrechos de pesca em quatro trechos de rio, um em reservatório e três em uma seção livre, por dois anos consecutivos (2015-2016 e 2016-2017), durante o período reprodutivo das espécies. Larvas e juvenis não foram coletados no reservatório e, na seção livre, larvas em estágios iniciais de desenvolvimento foram encontradas somente em um trecho intermediário, e a maioria das larvas em estágios mais avançados (~80%) e juvenis (~95%) foram registrados no trecho de amostragem mais baixo. Baseando-se no estágio larval e abundância de juvenis, foram presumidas seções de conectividade desova-recrutamento para estas espécies migradoras. Posteriormente, estas seções foram associadas à localização de futuros barramentos hidrelétricos na área de estudo, com possíveis implicações negativas para a conservação destas populações no rio Uruguai.

Palavras-chave: dispersão rio abaixo; juvenis; peixes migradores neotropicais; habitats berçário.

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INTRODUCTION

In Neotropical rivers, the migratory fish is subject to intense environmental pressure caused by hydroelectric projects (Winemiller et al., 2016). The construction of large reservoirs has created ecological barriers with strong horizontal gradients, which has interrupted the dispersion of eggs and larvae downstream to recruitment areas such as floodplains, swamps, and marshes (Pelicice et al., 2015). The lack of effective technical solutions to this problem has led to continuous failures in population recruitment for this group of fish (Agostinho et al., 2011; Pelicice and Agostinho, 2012; Pompeu et al., 2012).

The downstream dispersion of migratory fish in rivers of South America differ to those of the Northern hemisphere, such as some salmonids. The latter disperse at more developed ontogenetic stages (smolts, smelts, and fry), when can swim independently (Lucas and Baras, 2001). Consequently, these species can successfully overcome hydropower pass systems and maintain population recruitment (McCormick et al., 1998; Accou et al., 2008, Havn et al., 2017).

In contrast, Neotropical migratory fish in South America are potamodromous (they complete their entire life cycle in rivers), have very small eggs and high fertility rates (Winemiller, 1989; Araújo-Lima, 1994). After spawning, the eggs drift, incubate, and hatch in the rivers (Carolsfeld et al., 2003), and their successful recruitment depends upon drifting in lotic waters to reach downstream floodplain habitats. In floodplain environments, fish larvae find food (plankton and insects) and refuges against predators (Leite et al., 2000; Suzuki et al., 2009). In Neotropical rivers of South America, migratory fish species require stronger conservation measures, such as the preservation of long, free stretches of river (Pelicice and Agostinho, 2008). Therefore, identifying areas used for spawning and recruitment is fundamental for the conservation of migratory fish in South America. It could help to identify the connection of habitats that are essential for the early life stages of these species, mainly when large hydroelectric projects are in the preliminary phase of construction (Agostinho et al., 2002).

In this way, this study aimed to identify areas of migratory fish spawning and recruitment along an altitude gradient in the main channel of the Uruguay River, in order to establish spawning-recruitment connectivity stretch(es) for three migratory fish: the piava, *Megaleporinus obtusidens* Valenciennes 1837; the dourado, *Salminus brasiliensis* Cuvier, 1816; and the grumatão, *Prochilodus lineatus* Valenciennes 1837.

MATERIAL AND METHODS

Study area

The Uruguay River basin occupies an area of approximately 365,000 km^2 and is the smallest basin among the three rivers (Paraná and Paraguay rivers) that forms the La Plata River Basin (LPRB), corresponding to approximately 12% of the total area of the LPRB (Zaniboni-Filho and Schulz, 2003). The total course is approximately 1,800 km long, approximately 800 km of which was sampled in this study.

The study area was delimited by four sampling stretches in two sectors of the Uruguay River: the upper (T1 and T2) and the middle (T3 and T4) (Figure 1). The upper Uruguay begins at the confluence of the Canoas and Pelotas Rivers and ends in the Yucumã Falls, which is the highest longitudinal waterfall in South America with 1,800 m long (Zaniboni-Filho and Schulz, 2003). This upper portion extends for approximately 400 km and is valley shaped, without floodplain areas, and is where most hydroelectric dams are concentrated. The middle portion extends from the Yucumã Falls to the Salto Grande Hydroelectric Dam in a stretch of approximately 800 km. This section is characterized by having a low longitudinal profile and includes areas of floodplain, marginal lagoons, wetlands, and marshes (Quirós, 2004). Table 1 presents some characteristics of each sampling stretch.

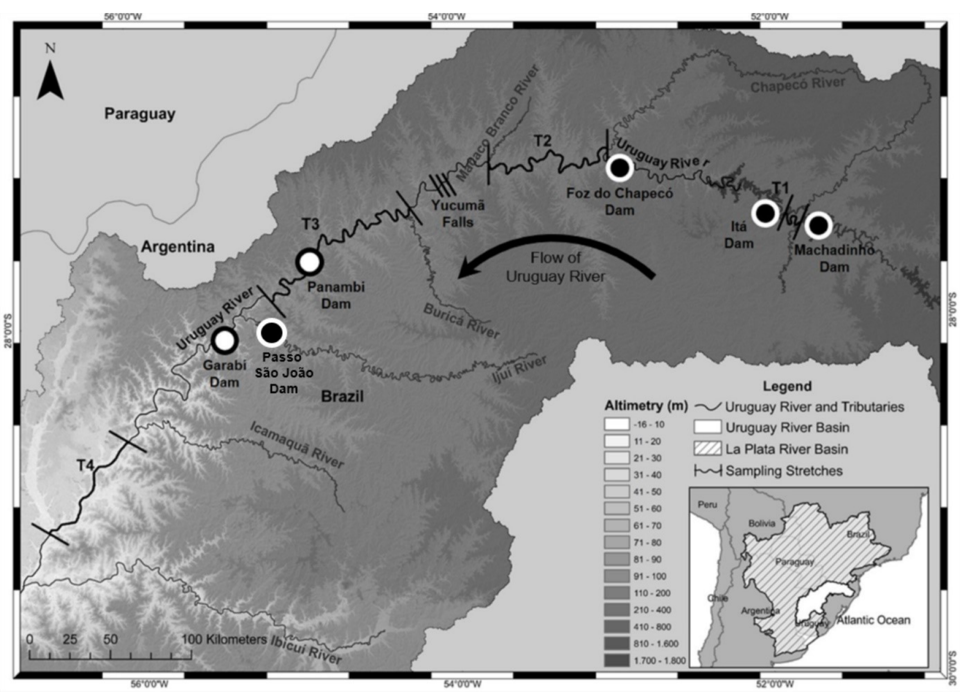

Figure 1. Altimetric map of the study area and sampling stretches (T1, T2, T3, and T4) in the Uruguay River (La Plata River Basin). Black dots with white circles indicate hydroelectric dams in operation, and white dots with black circles indicate planned hydroelectric dams. T1 comprises the semilotic, transitional area between the Itá and Machadinho Dams. The remaining stretches were downstream of hydroelectric dams that operate on the upper Uruguay River.

Table 1. Sampling stretches of the study area in the Uruguay River (upper and middle) with upper and lower limits of the sampling stretches and the general characteristics of the water flow.

Sampling Stretch	Sector of Uruguay River	Upper limit of the sampling stretch	Lower limit of the sampling stretch	Water flow of the sampling stretches
T1	Upper	Machadinho Dam	Itá Dam	Semilotic
T2	Upper	Chapecó River	Macaco Branco River	Lotic
T3	Middle	Buricá River	Ijuí River	Lotic
T4	Middle	Icamaquã River	Ibicuí River	Lotic

Sampling

Fish larvae and juveniles were sampled in four field trips conducted in November 2015 (spring), March 2016 (summer), November 2016 (spring), and March 2017 (summer), in two successive reproductive periods (2015-2016 and 2016-2017). These periods were selected based on the reproductive peak of most migratory fish species in the Uruguay River, which occurs between October and November (Hermes-Silva et al., 2012).

Each stretch on each field trip was sampled with the same fishing effort. To capture juvenile migratory fish, 10 sampling points were randomly selected in each section (40 in total) and sampled on each of the four field trips, which resulted in a total of 160 samples. The environments selected were mainly in marginal areas, where there was shrub vegetation, aquatic macrophytes, and/or other types of marginal vegetation. Sampling was conducted using four types of fishing net: gill, purse seine, hand, and casting.

The gill nets comprised two sets, one of which was placed on the bottom and the other on the surface of the water column. Each net was 60 m in length and was composed of 20-m junctions of each of three different mesh openings: 1.5, 2.0, and 2.5 mm between opposing nodes. The nets were installed at dusk for night sampling and at dawn for daytime sampling, and remained in the water for 10 h. The purse seine net was 20 m long with a 5.0-mm mesh between opposing nodes and was applied three times per sample. The hand net was composed of an iron rectangle (1.0 × 0.6 m) with a 1.0-mm mesh and was applied in vegetated areas (shrubs, aquatic macrophytes, and other types of vegetation) in the margins of the river. This net was handled simultaneously by two field technicians and applied 10 times at each sampling point. The casting net had a 4.0-m diameter and a 5.0-mm mesh between opposing nodes and was applied three times for each sample in marginal zones. The geographical coordinates of each sample were obtained using a Garmin Montana® 610 geographical positioning system.

Ichthyoplankton samples were taken at four sampling points per stretch on each of the four field trips, which totaled 64 ichthyoplankton samples. Cylindrical-conical ichthyoplankton nets were used with a 50-cm diameter of opening and a 500-µm mesh. Each sample consisted of a simultaneous trawl of two ichthyoplankton nets for 15 min with a motor boat driven at low speed (less than 1 m s^{-1}) at random points within the limits of each sampling stretch. Immediately after the trawls, the samples were packed in 500-mL plastic containers. The biological material was euthanized with a clove oil overdose (20%) and then fixed in a 4% or 10% formalin solution for eggs and larvae or juveniles, respectively. The samplings were licensed by the Ministry of the Environment (IBAMA) of the Brazilian government (Number of the licenses: 47332-1 and 47332-2 of SISBIO).

Identification and determination of migratory fish larvae and juveniles

The data were analyzed for two biological specimens groups of the three long-distance migratory fish: i) migratory fish larvae (larvae of *M. obtusidens, S. brasiliensis*, and *P. lineatus*), which considered their ontogenetic developmental stage; and ii) migratory juvenile fish, which was the total number of juveniles of the three target species.

In the laboratory, the ichthyoplankton samples were washed and screened on a Bogorov counting plate under a stereoscopic microscope Leica® - EZ4. Larvae and juveniles were identified according to the literature (Nakatani et al., 2001; Zaniboni-Filho et al., 2004), in addition to the taxonomic identification boards used by the Laboratory of Biology and Aquaculture of Freshwater Fish (LAPAD) of the Federal University of Santa Catarina (UFSC). Individuals that had completed the larval stage and were smaller than L-50 (size at which 50% of the individuals are sexually mature) were considered juveniles. The L-50 values of the target species are as follows: *M. obtusidens* = 25.0 cm (Agostinho et al., 2003); *S. brasiliensis* = 51.0 cm (Agostinho et al., 2003); *P. lineatus* = 26.0 cm (Kawakami-Resende et al., 1995). All of the juvenile migratory fish captured were deposited in the ichthyological collection of the Museum of Zoology of the State University of Londrina, Brazil.

Data analysis

The data of the migratory fish larvae and juveniles were grouped by stretch (T1, T2, T3, and T4). The ichthyoplankton was classified into four classes based on stage of ontogenetic development (Ahlstrom and Moser, 1976; Nakatani et al., 2001): larval yolk or yolk sac (LY), pre-flexion (PF), flexion (F), and post-flexion (PosF) stages.

This classification also allowed the determination of developmental stage using other morphological characteristics of the larvae, such as the presence/absence of a yolk sac and the opening of the mouth and/or anus, among others. The occurrence of migratory fish larvae in the sampling points and their developmental stage served as indicators of spawning area, by following the logic that old larvae had travelled longer distances from the spawning area than young larvae.

Based on migratory fish reproductive data under laboratory conditions (Santos and Godinho, 1996; Nakatani et al., 2001; Santos and Godinho, 2002; Borçato et al., 2004; Reynalte-Tataje and Zaniboni-Filho, 2008; Santos et al., 2016), two parameters was used to determine the spawning areas: (1) degree-hours (Santos et al., 2013) to egg hatch (average water temperature in the river x number of hours or time until the hatch), and (2) average time taken to reach each larval stage.

To presume spawning areas, a "retro-calculation" was performed using flow data (m^3 s^{-1}) and the cross-section area (m^2) of the river to obtain the river current velocity (m s^{-1}) on the sampling dates. The water temperature (degree Celsius) on the sampling dates was considered a factor in the hatching and ontogenetic developmental times, associated to degree-hours. The hydrological data (flow and cross-section) and water temperature on the sampling dates were obtained using the HIDROWEB platform, of the Water National Agency (ANA) of the Brazilian Government. The location of the spawning areas (points and stretches) from the capture points of migratory fish larvae (in km) was obtained with the following formula:

RDS = Time to hatch (h) or time to reach each larval stage (h) x 60 minutes x 60 seconds x river current velocity (m s^{-1}).

Where, RDS is the retro-calculated distance to the spawning area of migratory fish. These distances were presumed using Google Earth®. The occurrence and abundance of juvenile migratory fish in the sampling stretches (T1, T2, T3 and T4) in both reproductive years (2015-2016 and 2016-2017) was considered the indicator to presume recruitment areas.

RESULTS

Migratory fish larvae

No migratory fish larvae were caught in the Itá reservoir stretch (T1) and in the first stretch of the free section (T2). The occurrence of migratory fish larvae was restricted to T3 and T4 (Table 2), which corresponded to the middle portion of the Uruguay and the lowest altitudes in the study area. The total number of migratory fish larvae captured (*M. obtusidens, S. brasiliensis*, and *P. lineatus*) corresponded to 1.76% (39 larvae) of the total larvae caught. Three *S. brasiliensis* larvae at the LY stage were only captured in T3 in 2015-2016, and 29 *S. brasiliensis* larvae at the PF stage were captured in T4 in 2016-2017. *M. obtusidens* and *P. lineatus* larvae at the PF stage were also captured in T4 during 2016-2017 but in lower quantities, with three and four individuals, respectively. The retro-calculated spawning areas were near of falls and rapids of T3 (Table 2). Based on the sampling points of *S. brasiliensis* larvae at the LY stage, it was possible to determine the spawning sites of this species as just downstream of the Yucumã Falls and just downstream of the Chafariz Islands, a river archipelago in T3. The sampling points of *S. brasiliensis* larvae at the PF stage in T4 made it possible to determine another spawning area for this species, between T3 and T4. For *M. obtusidens* larvae at the PF stage, the beginning of the presumed spawning area was downstream of the Yucumã Falls, and for the PF larvae of *P. lineatus*, the beginning of the presumed spawning area was just downstream of the Chafariz Islands.

The main tributaries of this sector of the Uruguay River can also be considered as possible spawning areas for migratory fish, based on the calculations made. Except for the Ijuí River, in which is installed the Passo São João hydroelectric dam, about 70 km from the meeting with the Uruguay River. Due to the distance of this dam to the T4 ichthioplankton sampling points, the Ijuí River could not contain spawning stretches of *Megaleporinus obtusidens*.

Juvenile migratory fish

A total of 58 migratory fish juveniles were caught during the study: 39 *M. obtusidens*, 17 *S. brasiliensis*, and two *P. lineatus*, with the majority captured in the middle part of the river (56), in T3 and T4 (Figure 2a and 2b). A similar pattern to that of the migratory fish larvae in T1 occurred for juveniles, and no migratory fish juvenile were captured. Only two individuals were caught in the upper portion, in T2.

Of the 58 migratory juveniles, 54 were captured in T4, with *M. obtusidens* and *P. lineatus* juveniles only captured in this stretch. *S. brasiliensis* juveniles were also mainly captured in T4 but were also found in T3 and T2. This dominance of juvenile migratory fish in T4 was observed in both reproductive periods (Figure 2a and 2b). The mean lengths and weights of the captured juveniles indicated that they differed for less in size to the first gonadal maturation (Table 3).

Table 2. Data used to presume spawning areas in the Uruguay River using a retro-calculation method based on the larval development stages of three migratory fish species (*Salminus brasiliensis, Megaleporinus obtusidens*, and *Prochilodus lineatus*). WT = Water temperature on sampling date; LS = Larval stage; Sb = *S. brasiliensis*; Mo = *M. obtusidens*; Pl = *Prochilodus lineatus*; YS = Yolk sac stage; PF = Pre-flexion stage.

Stretch	Coordinates of the capture points of migratory fish larvae	Nº of larvae	Species and LS (All)	WT+ (°C)	Hours degree to hatch§	Time to hatch (h)	Time to reach PF¶ (h)	River current velocity* (m s^{-1})	Retro-calculated distance (RDS)* (Km)
T3	27°34′21.7″ S 54°42′13.6″ W	01	Sb YS	23.2	388	16.7	-----	1.16	69.7
T3	27°34′35.0″ S 54°40′53.1″ W	01	Sb YS	23.2	388	16.7	-----	1.16	69.7

T3	27°25′26.1″ S 54°20′21.8″ W	01	Sb YS	23.5	388	16.5	-----	1.21	71.9
T4	29°6′59.5″ S 56°33′48.5″ W	03	Sb PF	28.1	-----	-----	24 to 48	1.19	102.8 to 205.6
T4	29°6′59.5″ S 56°33′48.5″ W	03	Mo PF	28.1	-----	-----	96 to 120	1.19	411.3 to 514.1
T4	29°6′59.5″ S 56°33′48.5″ W	04	Pl PF	28.1	-----	-----	72 to 96	1.19	308.4 to 411.3
T4	29°6′56.3′ S 56°33′46.1″ W	26	Sb PF	28.1	-----	-----	24 to 48	1.19	102.8 to 205.6

Information source: +HIDROWEB (ANA); §Reynalte-Tataje and Zaniboni-Filho (2008); Santos and Godinho (1996), Nakatani et al. (2001), Santos and Godinho (2002), Borçato et al. (2004), Santos et al. (2016); *HIDROWEB (ANA) + ArcGIS®.

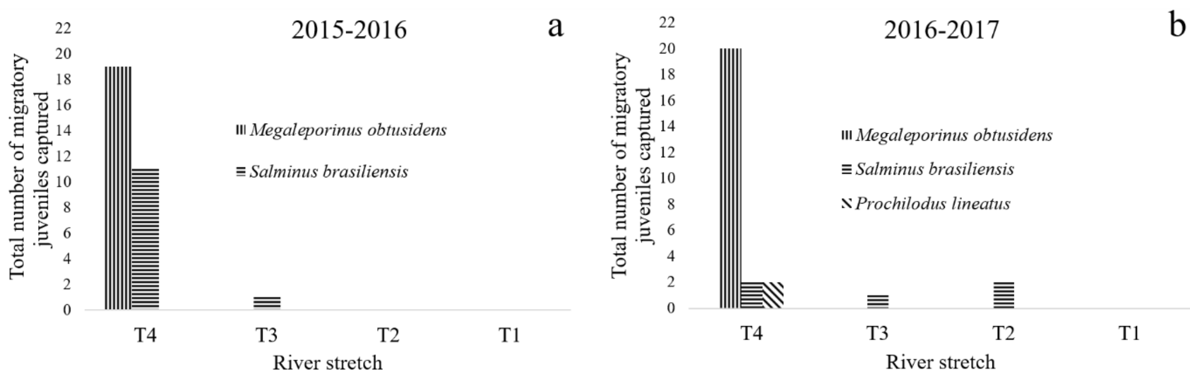

Figure 2. Total number of migratory fish juveniles (*Megaleporinus obtusidens, Salminus brasiliensis*, and *Prochilodus lineatus*) captured in four stretches of the Uruguay River in two reproductive years: a. 2015-2016 and b. 2016-2017. T1 = Itá Dam reservoir; T2 to T4 = free section, without reservoirs.

Table 3. Mean (± standard deviation) lengths and weights of the migratory fish juveniles (*Megaleporinus obtusidens, Salminus brasiliensis*, and *Prochilodus lineatus*) caught in this study.

Species	Nº of juveniles captured	Length (cm)	Weight (g)
M. obtusidens	39	16.40 (± 4.44)	113.07 (± 104.83)
S. brasiliensis	17	21.74 (± 5.20)	235.05 (± 199.51)
P. lineatus	02	20.40 (± 0.40)	252.00 (± 35.29)

A representative model is presented (Figure 3) of a 450-500-km connectivity stretches between presumed migratory fish spawning and recruitment areas, and the threats of hydroelectric dams projects for conservation of the migratory target fish, considering the main tributaries of the Brazilian side of the Uruguay River.

DISCUSSION

Due to a context with fishing pressure on migratory fish species in hydroelectric dam reservoirs of the upper Uruguay River (Schork et al., 2012), added to the presence of spawning areas in this region (Hermes-Silva et al., 2009; Reynalte-Tataje et al., 2012), the identification of recruitment areas in sectors affected by hydroelectric reservoirs is extremely necessary. Some specific rainfall conditions coupled with flooding dynamics may create unique hydrological opportunities in reservoir areas, which could facilitate sporadic recruitment processes (Zaniboni-Filho and Schulz, 2003; Reynalte-Tataje et al., 2008).

In the Itá reservoir, for example, *M. obtusidens* was among the three most abundant species in terms of well-developed larvae (in the flexion stage) in a tributary with reservoir influence, indicating that pools and rapids in tributaries would function as habitats for the recruitment of migratory fish (Ávila-Simas et al., 2014). However, a broader view that considers the ecological relationships between the upper, middle, and lower parts of the Uruguay River suggest that the upper section can be considered a spawning area for some stocks of migratory fish (Reynalte-Tataje et al., 2012; Ribolli et al., 2015). Therefore, there is no clear evidence of recruitment for migratory fish in the upper Uruguay River.

In the free section, there was an apparent effect of gradient (altitude) on the early life stages, i.e., newly hatched larvae and those at early stages of development were mainly found in the intermediate region, represented by T3, and older larvae and juveniles were mainly found in lower regions, represented by T4. The downstream dispersion of larvae between T3 and T4 seems to follow a longitudinal gradient, in accordance with the river continuum concept (Vannote et al., 1980).

The reproductive period of the dourado (*S. brasiliensis*) in the Uruguay River can extend from the end of July (mid-winter) to the beginning of May (mid-autumn), indicating that stocks of this species in this river have a longer period of reproduction than in other systems of the LPRB (Zaniboni-Filho et al., 2017). This extension of the reproductive period may be caused by a lack of marked seasonality regarding the rainy season in the region (Zaniboni-Filho and Schulz, 2003), indicating that there is a spatial effect that is as strong as the temporal effect on dourado spawning and recruitment in the Uruguay River. In this way, the presence of *S. brasiliensis* larvae at the LY stage in T3 indicates that this region may be a spawning area for migratory fish. In support of this hypothesis, piava (*M. obtusidens*) larvae was found in this region when sampling ichthyoplankton was conducted in the Turvo State Park, above T3 (Ziober et al., 2015). Moreover, the migration pattern of fish in the Paraná River after the construction of the Itaipu reservoir displays the following pattern: medium and long distances (>1,000 km) towards the headwaters of tributaries with rocky and sandy bottoms, shallower than the average depth of the main channel, and fast currents (Agostinho et al., 2003). Within the free section of the study area, T2 and T3 contained many shallow environments, rapids, and geographical anomalies, such as the Yucumã and Roncador Falls (among others), which constitute a spawning habitat for fish community (Ziober et al., 2015).

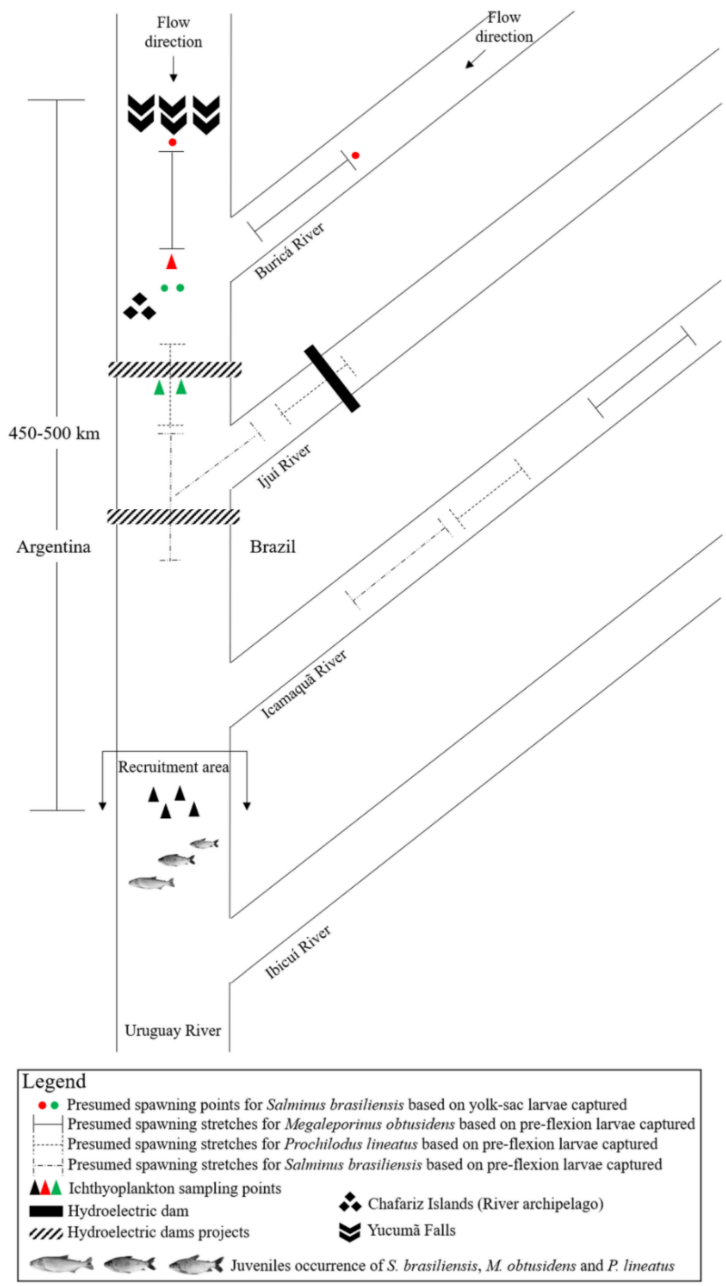

Figure 3. Representative model of connectivity stretches between spawning and recruitment areas of migratory fish, and threats of hydroelectric dams projects, considering the main tributaries of the Brazilian side of the Uruguay River.

T4 contained significantly more juvenile migratory fish and migratory fish larvae (*S. brasiliensis*, for example) than the other stretches, which indicates that T4 has strong potential as a recruitment area and confirms that T3 is a spawning area. In T4, there is a large, complex area of floodplain on the right bank of the middle Uruguay River known as Argentine Mesopotamia (Quirós and Cuch, 1989; Brea and Zucol, 2011). Neotropical floodplains contain complex aquatic habitats and are recognized as nursery areas where there is a high abundance of marginal vegetation and increased biological productivity in situ (Lowe-McConnell, 1987). These habitats provide protection from predators and a food supply for fish larvae and juveniles (Leite et al., 2000; Oliveira et al., 2015). After 25 years of LAPAD (UFSC) monitoring the ichthyofauna of the Uruguay River, it is possible, for the first time, to identify a nursery area in a certain location that contains a significative abundance of migratory fish juveniles.

Implications for conservation

These three migratory fish are the target of commercial and sport fishing in the Uruguay River (Schork et al., 2012), with conflicts, mainly in relation to dourado (*S. brasiliensis*). The dourado is the one that suffers the greatest fishing pressure in the Uruguay River basin, considered vulnerable according to the red list of endangered species of the state of Rio Grande do Sul (Marques et al., 2002). Besides, these fish species also face other environmental pressures in the Uruguay River basin, such as the destruction of habitats, the heavy pollution (Zaniboni-Filho and Schulz, 2003) and, in addition, several hydroelectric projects have been planned for this river system (Popescu et al., 2012; Palomino-Cuya et al., 2013). Some of them are between the migratory fish spawning and recruitment areas identified in this study.

Therefore, we recommend that this free section is maintained without any interruption in flow, so it will be necessary to re-locate new hydroelectric dams on the Uruguay River. This conservation procedure is necessary to maintain connectivity between current spawning and recruitment areas of the migratory fish populations of the Uruguay River, serving as an example for other Neotropical rivers of the South America.

CONCLUSION

The stretches T3 and T4, which are free of hydroelectric dams, represent an indivisible unit for the maintenance of migratory fish stocks in the region. The interconnection of these stretches allows spatial connections between spawning areas, which are characterized by the presence of rapids and falls (e.g., Yucumã Falls), and recruitment areas, which are floodplain areas in the middle Uruguay River.

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