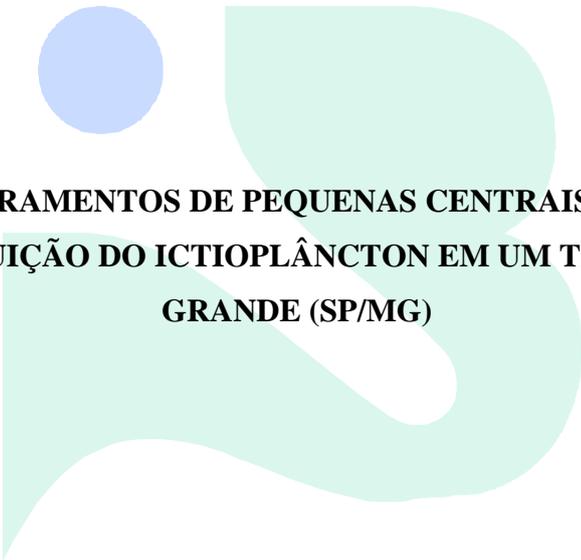


**UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”**

**CAMPUS DE BOTUCATU**

**INSTITUTO DE BIOCÊNCIAS - DEPARTAMENTO DE ZOOLOGIA**

**TESE DE DOUTORADO**



**EFEITO DOS BARRAMENTOS DE PEQUENAS CENTRAIS HIDRELÉTRICAS  
SOBRE A DISTRIBUIÇÃO DO ICTIOPLÂNCTON EM UM TRIBUTÁRIO DO RIO  
GRANDE (SP/MG)**

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HIDRELÉTRICAS SOBRE A DISTRIBUIÇÃO DO  
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(SP/MG)**

**Eduardo Meneguzzi Brambilla**

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## Resumo Geral

Uma das melhores alternativas para minimizar os impactos de barramentos na ictiofauna de grandes rios é a conservação de seus tributários, que servem como rotas alternativas para migração reprodutiva e disponibilizam novos habitats necessários para desova e crescimento. Porém, devido a alta demanda de energia elétrica no país muitos destes tributários estão sendo barrados por Pequenas Centrais Hidrelétricas (PCHs). Nesse contexto se destaca o rio Sapucaí-Mirim, um dos principais tributários do rio Grande. Uma importante ferramenta para avaliar o real impacto destes empreendimentos na reprodução e recrutamento de peixes é o estudo de ictioplâncton. Portanto, o objetivo geral deste trabalho foi avaliar os processos reprodutivos da ictiofauna, através da análise de ovos e larvas de peixe, em dois reservatórios de PCHs do rio Sapucaí-Mirim. As amostragens foram realizadas quinzenalmente, nos ciclos reprodutivos de novembro de 2015 a fevereiro de 2016 e novembro de 2016 a fevereiro de 2017 em oito pontos amostrais incluindo os trechos lótico, transição, lêntico e dentro da escada de peixes de cada um dos reservatórios. No total foram amostrados 5.004 ovos e 3.189 larvas de 22 táxons pertencentes a quatro ordens e 15 famílias. As famílias mais abundantes foram Anostomidae, Characidae, Pimelodidae e Prochilodontidae, com predominância de ovos e larvas de desenvolvimento inicial (larval vitelino e pré-flexão). A estrutura da assembléia de larvas de peixes e estágios de desenvolvimento, em ambos os ciclos reprodutivos, foram influenciados por fatores espaciais e temporais. Temporalmente, maiores densidades de ovos e larvas foram observadas em períodos com alta pluviosidade e alterações associadas nas variáveis ambientais. Espacialmente, maiores densidades de ovos foram encontradas no

reservatório mais a montante e as densidades de larvas variaram de acordo com o ciclo reprodutivo. Os trechos com maiores densidades de ovos foram lóticos em 2015-2016 e transição em 2016-2017 e para larvas foi transição em ambos os ciclos. Houve dispersão descendente de ovos e larvas ao longo do trecho estudado sendo influenciada por uma combinação de variação de chuva/vazão do rio e período do ciclo reprodutivo com pico de chuva durante o verão coincidindo com maiores abundâncias. Sob essas condições, ovos e larvas derivaram rio abaixo através dos reservatórios e atingiram as barragens. Uma vez na barragem, os indivíduos puderam passar pela escada de peixes e foram correlacionados com a densidade no reservatório imediatamente a montante, embora em densidades muito baixas em comparação com os outros locais de amostragem. Houve também uma influência de eventos climáticos extremos na reprodução dos peixes, com um período de chuvas extremas apresentando 10 vezes mais ovos e larvas e maior proporção de espécies migradoras do que um período de seca extrema. Densidades de ovos e larvas foram correlacionadas positivamente com turbidez e vazão e negativamente com temperatura, sólidos totais em suspensão, condutividade e pH. Em conclusão, este trecho do rio Sapucaí-Mirim oferece condições adequadas para desova e deriva de ovos e larvas de importantes espécies migratórias da bacia hidrográfica. Portanto, esta é uma área crítica para o recrutamento de peixes e são necessários esforços para preservá-lo.

**Palavras-chave:** biodiversidade; conservação; ictiofauna; Rio Sapucaí-Mirim; ovos e larvas de peixe

## **Apresentação**

Os barramentos de rios com fins de geração de energia hidroelétrica causam uma fragmentação abrupta dos sistemas fluviais (Nilsson et al. 2005) causando perda de habitats e consistindo em uma das maiores ameaças à biodiversidade aquática (Agostinho et al. 2016; Winemiller et al. 2016). O novo ambiente, formado após o barramento, apresenta características muito diferentes do original e as comunidades distinguem-se significativamente daquelas que ocorriam nos trechos lóticos originais ou remanescentes (Agostinho et al. 2016; Ruocco et al. 2018; Souza et al. 2019).

Os peixes estão entre os organismos aquáticos mais impactados pelos barramentos de rios, apresentando alterações na composição de espécies e estrutura das comunidades. Pode ocorrer um aumento das populações não-nativas e uma diminuição da abundância e até desaparecimento das populações naturais, principalmente de migradores de longa distância (Agostinho et al. 2016; Loures e Pompeu 2018; Pelicice et al. 2018). As barreiras criadas podem limitar ou impedir a conectividade longitudinal, diminuindo o acesso aos habitats necessários para completar o seu ciclo de vida, como locais de desova, áreas de berçário e áreas de alimentação (Pelicice e Agostinho 2008; Winemiller et al. 2016). Conseqüentemente, podendo reduzir o recrutamento populacional, resultando em declínio da população ou mesmo em extinções locais/regionais (Radinger e Wolter 2014; Agostinho et al. 2016).

Uma das alternativas mais eficientes para a mitigação dos impactos dos barramentos sobre a ictiofauna é a conservação de tributários de grandes rios barrados. Os peixes utilizam estes tributários como rotas alternativas para

migração reprodutiva e também encontram habitats essenciais para desova e crescimento de larvas e juvenis (Vianna e Nogueira 2008; Reynalte-Tataje et al. 2011; Marques et al. 2018; Lopes e Zaniboni-Filho 2019). Porém, a construção de pequenas centrais hidrelétricas (PCHs) geralmente ocorre nesses tributários, colocando em risco seu papel para a conservação da ictiofauna.

Atualmente, há uma tendência mundial de aumento na construção de PCHs (Zarfl et al. 2015), em especial no Brasil, onde existem 431 unidades (produção <30 MW e área de reservatório <13km<sup>2</sup>) em operação, gerando 5.031 MW e mais de 1.000 planejadas (ANEEL 2018). Em comparação com grandes usinas hidrelétricas, as PCHs tendem a ter menor custo de implantação sendo atraentes para uma gama maior de investidores, menor desperdício de energia na distribuição, porque abastecem centros populacionais próximos, não requerem uma grande rede de transmissão e podem ser construídos em rios menores (Sharma et al. 2013).

Independentemente do tamanho, a construção de usinas hidrelétricas tende a estar associada à degradação ecológica. Os impactos gerados por PCHs podem até mesmo serem maiores que os de grandes hidrelétricas (UHEs), especialmente quando se considera as alterações por quilowatt de energia gerada e o potencial de fragmentação causado por um grande número de PCHs dentro de uma bacia hidrográfica (Bakken et al. 2012). Considerando os impactos na fauna de peixes, as PCHs podem reduzir a abundância, o comprimento médio, o peso total e o fator de condição dos peixes; alterar a composição de espécies (Benejam et al. 2016); reduzir populações de espécies migratórias (Arcifa e Esguícero 2012; Bakken et al. 2012); interromper a livre

movimentação (Bakken et al. 2012; Kucukali 2014) e influenciar na dispersão do ictioplâncton (Suzuki et al. 2011; Pelicice et al. 2014).

Uma das alternativas para a mitigação dos impactos dos barramentos sobre a ictiofauna é a implantação dos mecanismos de transposição, como escadas, elevadores e canais seminaturais (Clay 1995; Porcher and Travade 2002; Lira et al. 2017; Baumgartner et al. 2018). Estas construções caracterizam-se como estruturas físicas que visam restabelecer as rotas migratórias de algumas espécies de peixe e o acesso a determinados habitats (Pompeu et al. 2012). Porém, sua real eficiência para grandes barramentos é controversa (Britto e Carvalho 2013; Agostinho et al. 2016; Lira et al. 2017). No caso de barramentos de pequeno porte, estudos científicos a respeito do papel destes mecanismos são escassos (Kusma e Ferreira 2010), principalmente no Brasil.

Neste cenário, destaca-se o rio Sapucaí-Mirim, tributário da margem esquerda do rio Grande (formador da bacia do Alto Paraná e que atualmente encontra-se barrado por 12 UHEs). A riqueza de espécies de peixe neste tributário é expressiva, com pelo menos 105 espécies registradas, inclusive importantes migradores como o dourado (*Salminus brasiliensis*), tabarana (*Salminus hilarii*), curimbatá (*Prochilodus lineatus*), mandi (*Pimelodus maculatus*), barbado (*Pirirampus pirinampu*) e diversas espécies de piaparas e piaus (família Anostomidae) (Oliveira et al. 2016; Brambilla et al. 2018; Diniz et al. 2019). Existem atualmente três pequenas centrais hidrelétricas dispostas em cascata em operação no canal principal do rio Sapucaí-Mirim, todas possuem escadas para peixes – PCH Palmeiras, PCH Anhanguera e PCH Retiro.

Os processos relacionados à reprodução e ao desenvolvimento das fases iniciais de vida das diferentes espécies de peixe, em especial das migradoras, bem como a eficiência dos mecanismos de transposição em PCHs, são ainda desconhecidos. Desta forma, informações sobre a ocorrência e a distribuição de ovos e larvas de peixes, período crítico do ciclo de vida destes organismos, têm importância fundamental para o entendimento da biologia das espécies e contribuem para a avaliação da integridade ecológica do ecossistema. Além disso, a importância dos estudos sobre o ictioplâncton tem aumentado significativamente nos últimos anos, principalmente pela sua eficácia na identificação das áreas e períodos de desovas, bem como de criadouros naturais (Orsi et al. 2016, Silva et al. 2017; Marques et al. 2018).

Baseando-se nas considerações anteriores, foram estabelecidos os temas de estudo dessa tese, cujos dados para o desenvolvimento da pesquisa foram obtidos na área de influência da cascata de PCHs do rio Sapucaí-Mirim (bacia do rio Grande, SP/MG).

Em termos de estrutura da tese, optou-se pela organização na forma de capítulos, os quais foram redigidos nos padrões de um de artigo científico e serão submetidos para publicação após a defesa da tese. O objetivo do primeiro capítulo foi verificar a variação espaço-temporal de ovos e larvas de peixes em um trecho do rio barrado por uma cascata de PCHs. O segundo capítulo visa avaliar a dispersão de ovos e larvas de peixes no mesmo sistema. Por fim, o terceiro capítulo busca investigar o efeito de variações climáticas extremas sobre a reprodução dos peixes na mesma área de estudo.

## Área de Estudo

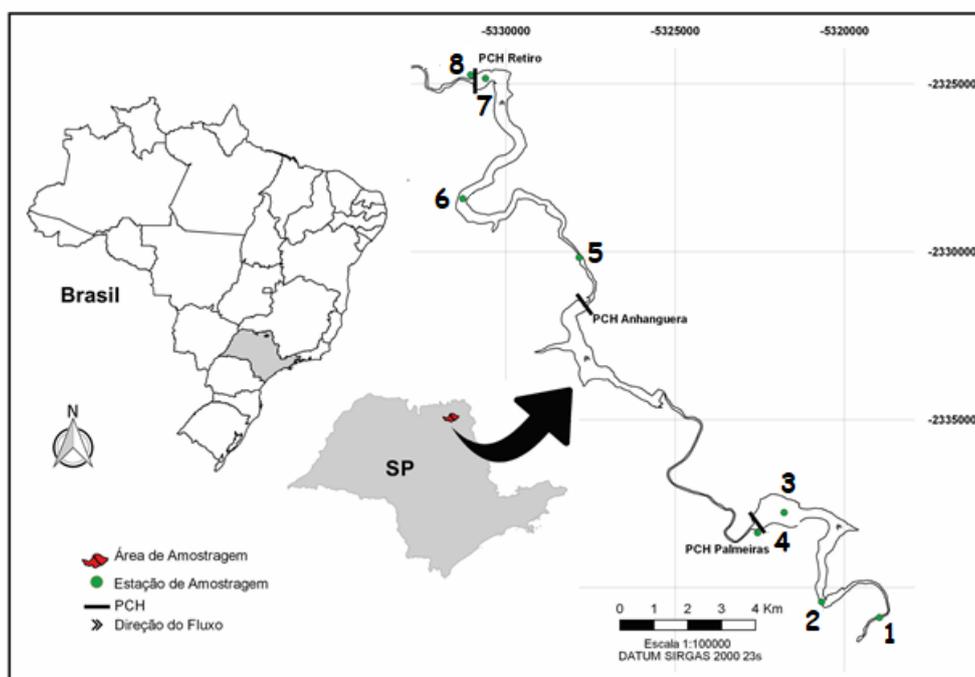
O rio Sapucaí-Mirim nasce nas encostas interiores da serra da Laje (Mantiqueira), nordeste do Estado de São Paulo, entre os municípios paulistas de Cajuru e Cássia dos Coqueiros. Possui dois tributários próximos a nascente, Esmeril e Santa Bárbara, ambos originados no Estado de Minas Gerais. Com extensão de 310 km, exclusivamente no Estado de São Paulo, trata-se de um rio de planalto, com fortes corredeiras e pequenas quedas d'água, correndo encaixado na sua porção superior e tornando-se menos veloz e turbulento na porção inferior, próximo à desembocadura na margem esquerda no reservatório da UHE Porto Colômbia, médio rio Grande (Paiva 1982).

Este rio é parte integrante da Unidade Hidrográfica de Gerenciamento de Recursos Hídricos 08 (UGRHI 08), segundo a lei paulista nº 7.663/1991, que dispõe sobre o Plano Estadual de Recursos Hídricos. A Bacia Hidrográfica do Sapucaí-Mirim tem área de drenagem de 9.125 Km<sup>2</sup>, abrangendo 23 municípios, nos quais o tipo dominante de vegetação é a Floresta Estacional Semidecidual. O rio está enquadrado na classe 2, conforme Resolução CONAMA 357, de 17 de março de 2005.

Existem atualmente uma cascata de três PCHs em operação no canal principal do rio, localizada a 168 km de distância da nascente e 118 km da foz do rio (Google Maps 2020). Entre essas, as duas localizadas na extremidade da cascata estão sob a concessão da empresa CTG Brasil – PCH Palmeiras e PCH Retiro, iniciaram a operação em 2011 e 2013, respectivamente, enquanto a outra pertencente a Central Elétrica Anhanguera S.A. – PCH-Anhanguera, iniciou em 2009. No total essas PCHs produzem 70 MW.

As PCHs Palmeiras e Retiro, por pertencerem a mesma empresa e terem programas de monitoramento similares, foram selecionadas para esse estudo. Oito pontos amostrais foram estabelecidos nas áreas de influência dessas duas PCHs: montante (região lótica) do reservatório, área de transição, reservatório (região lântica) e interior (meio) da escada para peixes (Figura 1, Tabela 1). A distância, seguindo o curso do rio, entre o ponto 1 e PCH Palmeiras é em torno de 7,5 km; entre PCH Palmeiras e PCH Anhanguera 11 km e entre PCH Anhanguera e PCH Retiro 11,5 km.

Não existem tributários ou lagoas marginais entre as PCHs. Os pequenos tributários mais próximos são Ribeirão Salgado (20 km a montante da Barragem de Palmeiras) e Ribeirão das 7 Lagoas (56 km a jusante da barragem de Retiro) (Google Maps 2020).



**Figura 1.** Distribuição dos pontos amostrais. 1 Palmeiras Montante; 2 Palmeiras Transição; 3 Palmeiras Barragem; 4 Palmeiras Escada; 5 Retiro Montante; 6 Retiro Transição; 7 Retiro Barragem e 8 Retiro Escada. Rio Sapucaí-Mirim, Sudeste, Brasil.

**Tabela 1.** Distribuição dos pontos amostrais nas PCHs Palmeiras e Retiro. Rio Sapucaí-Mirim, Sudeste, Brasil.

PCH/Trecho	Lótico	Transição	Lêntico	Escada
PCH Palmeiras	1	2	3	4
PCH Retiro	5	6	7	8

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# *Capítulo 1*

# **Spatial and temporal variation of fish eggs and larvae in a cascade of small hydropower plants in Southeast Brazil**

## **Abstract**

The construction of dams in series (cascade of dams) and seasonal variation of water flow are factors that can influence the ichthyofauna reproduction, one of the key elements in the maintenance of viable fish populations. Studies with eggs and larvae are, respectively, indicators of spawning and nursery/initial development sites in river systems, but there is a lack of knowledge regarding the impacts of small reservoirs. This study investigates the distribution of fish eggs and larvae in a river dammed by three small hydropower plants in cascade in Southeast Brazil. Six sampling sites were distributed in a stretch of the Sapucaí-Mirim River under influence of the most upstream (Palmeiras) and the most downstream (Retiro) dams, encompassing the lotic, transition and lentic sections of both reservoirs. Samples were collected fortnightly between November and February in the reproductive cycles of 2015-2016 and 2016-2017. A total of 5,004 eggs and 3,189 larvae belonging to four orders and 15 families were sampled. The most abundant families were Anostomidae, Characidae, Pimelodidae and Prochilodontidae and the most abundant early development stages were eggs and yolk-sac and preflexion larval stages. Fish larvae assemblage structure and early development stages, in both cycles, were influenced by spatial and temporal factors. Temporally, higher densities of eggs and larvae was observed in periods with high rainfall and associated changes in environmental variables. Spatially, higher densities of eggs were found in the most upstream reservoir and densities of larvae varied

according to the reproductive cycle. The sections with higher densities of eggs was lotic in 2015-2016 and transition in 2016-2017 and for larvae was transition in both cycles. This river section of Sapucaí-Mirim River can offer suitable conditions for spawning and drift of eggs and larvae of important short and long-distance migratory species of the river basin. Therefore, this is a critical area for fish recruitment and efforts to preserve it are necessary.

**Keywords:** ichthyoplankton, fish migratory species, reservoir compartmentalization, Sapucaí-Mirim River

## **Introduction**

River damming can alter the river hydrology and the physical and chemical variables of water (Winemiller et al. 2016; Nogueira and Pomari 2019). The interruption of the natural flow leads to a transformation of the longitudinal axis of the river in three distinct compartments: fluvial, transitional and lacustrine (*sensu* Thornton 1990). The fluvial zone is classified as a lotic system, with relatively high water speed, normally presenting a large quantity of suspended materials, low penetration of light and a high availability of nutrients, with a source of organic matter mainly allochthonous. The transition zone is characterized by a decrease in water flow, smaller quantities of suspended solids, and higher penetration of light, usually with increased primary productivity. Finally, the lacustrine zone is an environment typically lentic, with higher water retention time, greater depth and greater light penetration and autochthonous primary production (Nogueira et al. 1999; Jorgensen et al. 2013).

Reservoir compartmentalization affects aquatic biota distribution and composition (Jorcin and Nogueira 2008; Nogueira et al. 2008; Perbiche-Neves et

al. 2011) including ichthyofauna (Ferrareze et al. 2014; Nobile et al. 2019). Differences in fish assemblage composition and structure are found among the three different compartments (lotic, transition and lentic). There are more pronounced differences between lotic and lentic zones, with higher proportion of migratory species in lotic zone (Nobile et al. 2019). Transition zone shows the greatest species richness (Nobile et al. 2019), as expected for ecotones (Carvalho et al. 1998; Agostinho et al. 2008).

Cascade of dams can cause cumulative alterations along the river basin, varying in intensity and direction (upstream or downstream) as a function of longitudinal position of the impoundment. These alterations impact the ichthyofauna with changes in the composition and structure of species (Ferrareze et al. 2014; Loures and Pompeu 2018; Pelicice et al. 2018). A clear tendency of reduction in richness of native and migratory fish species and an increase of non-native species occurs in downstream direction (Loures and Pompeu 2018; Pelicice et al. 2018). However, migratory species richness is higher in reservoirs that presented habitats similar to lotic stretches or tributaries upstream of the impounded area (Loures and Pompeu 2018). Therefore, there is a spatial variation in species composition along the reservoir cascade systems (Pelicice et al. 2018) and fish assemblages became increasingly dissimilar as reservoirs became more distant from each other and are dominated by small and medium-sized species (Loures and Pompeu 2018).

The seasonal variation of water flow is another important factor in the structuring of fish assemblages (Agostinho et al. 2016), mainly related to reproductive events. Discharge is a hydrological variable that represents one of the main triggers for reproductive migration (Vazzoler et al. 1997). Thus, high

discharge periods stimulate reproductive activity, increase of larval density, and rate of drift, allowing larvae to rapidly reach developmental areas, thereby enhancing their survival (Lechner et al. 2016). During floods, the availability of food resources and shelter for larvae also increase, affecting positively the fish offspring (Silva et al. 2019).

One of the key elements in the maintenance of viable fish populations is the succeeded reproduction followed by effective recruitment. The presence of eggs and larvae (ichthyoplankton) are, respectively, indicators of spawning and development sites in river systems (Keckeis et al. 2018). The identification and characterization of these regions are essential for the conservation of aquatic systems, mainly in damming rivers. Thus, studies concerning fish larvae distribution constitute an important tool for the establishment of conservation actions, such as the management of fish stocks (Garcia et al. 2018; Keckeis et al. 2018), consequently supporting the protection of vulnerable and/or threatened species. Several studies evaluated the spatial and temporal variation of adult fauna in large reservoirs (dos Santos et al. 2017; Loures and Pompeu 2018; Oliveira et al. 2018; Pelicice et al. 2018), but there is a lack of knowledge regarding small reservoirs, mainly related of the initial development stages of fish (eggs and larvae).

This study was conducted to investigate the distribution of fish eggs and larvae in a river dammed by a small hydropower plant (SHP) cascade in Southeast Brazil. It was designed to answer the following questions: (i) are the variation of fish early development stages and larval assemblage structure influenced by the temporal and spatial factors? (ii) if so, which environmental variables influence the variation of eggs and larvae? The main hypothesis driven

these questions is that the period within a reproductive cycle, the position of the reservoir in the cascade and the reservoir section (longitudinal compartment) will be the main drivers that influence the variation of fish early development stages and larval assemblage structure in a river with a cascade of SHPs. Environmental variables, mainly related with rainfall, can influence this variation.

## **Material and Methods**

### ***Study area***

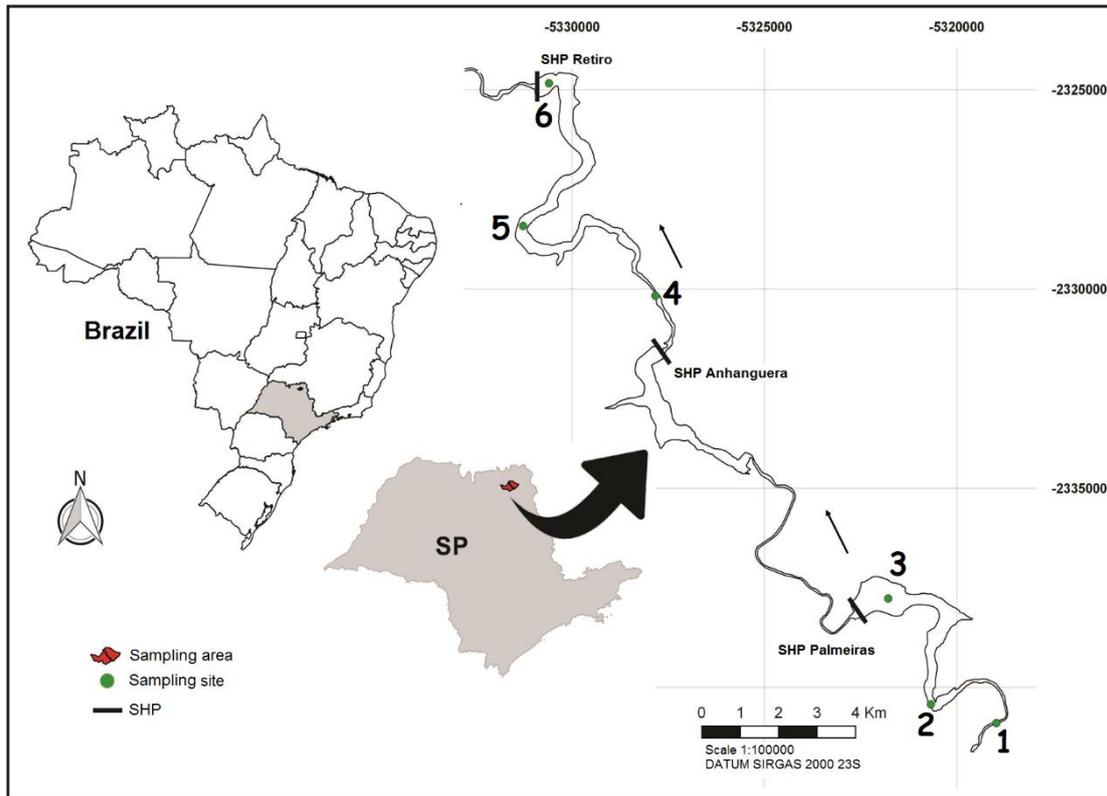
The study area is located in the Sapucaí-Mirim River (Southeast Brazil), one of the main tributaries of Grande River, upper Paraná River Basin. The river source is in the Serra da Laje (Mantiqueira) northeast of São Paulo State, runs 310 km, and its mouth is located in the left bank of Porto Colombia Reservoir, middle stretch of Grande River (Paiva 1982). There are three SHPs arranged in cascade in operation along the Sapucaí-Mirim River with a distance of 12 km between them: Palmeiras Dam (most upstream), followed by Anhanguera Dam (intermediate section) and Retiro Dam (most downstream), filled in 2011, 2009 and 2013, respectively. The SHPs are run-of-river operational systems, around three days in average of water retention time.

Palmeiras Dam is 168 km far from the source and the Retiro Dam is 118 km far from the river mouth. There are not tributaries or marginal lakes between the SHPs. The closest small tributaries are Ribeirão Salgado (20 km upstream of Palmeiras Dam) and Ribeirão das 7 Lagoas (56 km downstream of Retiro Dam) (Google Maps 2020).

### ***Samplings and processing***

Six sampling sites were distributed in the influence area of Palmeiras and Retiro Dams, encompassing the lotic zone (upstream of the reservoir, sites 1 and 4), the transition zone (in between the reservoir and the lotic area, sites 2 and 5) and the lentic zone (represented by the reservoirs, sites 3 and 6) (Figure 1).

Eggs and larvae were collected in reproductive cycles of 2015-2016 and 2016-2017, fortnightly, between November and February. The sampling period was defined because the spawning season for most of the Neotropical migratory fish commences at the end of spring and goes throughout summer (Bialetzki et al. 2005; Reynalte-Tataje et al. 2011)(Reynalte-Tataje et al. 2011; Lopes et al. 2014). Sampling was conducted at night (period of higher capture of eggs and larvae (Vianna and Nogueira 2008; Silva et al. 2017)), between 7 PM and 12 AM, and were obtained in pseudo replicates (n=3) for statistical analysis purposes, totalizing 288 samples.



**Figure 1.** Map of the study area showing the distribution of the sampling sites and arrows depicting direction of flow. Sites 1 to 3 were located upstream of Palmeiras Dam representing the lotic, transition and lentic zones, respectively. Similarly, sites 4 to 6 where located upstream of Retiro Dam.

Samples were obtained with a conical-cylindrical plankton net, with mesh size of 500  $\mu\text{m}$  and an opening area of 0.11  $\text{m}^2$ . A mechanical flowmeter (General Oceanics™) was positioned in the net opening to determine the volume of filtered water for each sample. The net was immersed in the water in the subsurface (approximately 20 cm depth) for 10 minutes. The net was trawled by a boat at low velocity in the lentic/transition sites (sites 2 and 3 and 5 and 6 in Palmeiras and Retiro Dams, respectively) and it remained stationary with the opening positioned against the direction of the flow in the lotic sites (1 and 4 at Palmeiras and Retiro Dams, respectively).

The sampled material was transferred to polyethylene bottles, the organisms collected were euthanized with an overdose (more than 283.55 mg/L) of anesthetic (eugenol) (Vidal et al. 2008) and fixed with a 4% formalin solution.

In laboratory, the samples were sorted under a stereomicroscope using a Bogorov-type plate and the fish eggs and larvae were separated from the rest of the sampled material. After separation, the larvae were identified to the lowest taxonomic level according to Nakatani et al. (2001). The taxonomic framework was based on Fricke et al. (2019).

The individuals captured were also classified in development stages, following Ahlstrom and Ball (1954) and Nakatani et al. (2001): (a) egg: from fertilization to hatch; (b) yolk-sac stage: from hatch to the eye completely or partially pigmented, the opening of the mouth and anus fully formed; (c) preflexion: from the opening of the mouth and anus to the initial flexing of the notochord, with the appearance of the first supports of the caudal fin; (d) flexion: between the initial flexing of the notochord and the appearance of the caudal fin supports to the complete flexing of the notochord and the appearance of the pelvic fin bud, and (e) postflexion: from flexing of the notochord and budding of the pelvic fin finalized to the complete formation of the rays of all the fins.

Simultaneously to the ichthyoplankton samplings were measured the following environmental variables: temperature, turbidity, dissolved oxygen (DO), electrical conductivity (K), oxide reduction potential (ORP), total dissolved solids (TDS) and pH, with a Horiba probe (U-52). Daily rainfall data was obtained from National Institute of Meteorology (INMET) using the closest station to the study area (Franca municipality, around 47 km of distance). Daily total outflow data of

each dam was obtained directly from Duke Energy Brazil, the generation company in charge of the hydroelectric plants in that occasion.

### ***Data analysis***

The density of sampled eggs and larvae was standardized as individuals per 10 m<sup>-3</sup> of filtered water, according to Tanaka (1973), modified by Nakatani et al. (2001). Larvae were grouped in taxonomical families. Data obtained in 2015-2016 and 2016-2017 cycles were analyzed separately. For each cycle we evaluated the influence of three factors on the densities of fish early development stages (eggs; yolk-sac, preflexion, flexion and postflexion larval stages) and larval assemblage structure (families): position of dam (most upstream (Palmeiras) and most downstream (Retiro)), section (lotic (sites 1 and 4); transition (sites 2 and 5) and lentic zones (sites 3 and 6)) and period (November-1; November-2; December-1; December-2; January-1; January-2; February-1 and February-2) – numbers after each month indicate whether the sample was obtained in the first -1 or second -2 fortnight.

A Permutation Multivariate Analysis of Variance (PERMANOVA) (Anderson et al. 2008) based on the Bray-Curtis similarity matrix with 0.01 dummy effect, derived from fish densities data transformed in  $\log(x+1)$ , was used to test for differences on larval assemblage structure and early development stages between the factors position of dam, section, period and the interaction among all factors.

A distance-based linear models procedure (DISTLM) (Anderson et al. 2008) was used to identify the influence of environmental variables (temperature, turbidity, DO, K, ORP, pH and TDS) on larval assemblage structure and early

development stages. For the analyses, all parameters (except pH) were log (x+1) transformed and normalized. The DISTLM analyses, using the best procedure with the Akaike Information Criterion (AIC), were performed on log (x+1) transformed biotic data, and derived from Bray-Curtis similarity matrix with 0.01 dummy effect (Anderson et al. 2008).

A Spearman correlation was used to verify correlation between total density of eggs and larvae with total outflow, rainfall in the sampling day, rainfall one day before the sampling day and accumulated rainfall three days before the sampling day.

## Results

A total (all samples from both cycles) of 5,004 eggs and 3,189 larvae of 22 taxa, belonging to four orders and 15 families were sampled (Table 1). The most abundant family, considering average density, was Anostomidae (2.45 larvae·10 m<sup>-3</sup>), followed by Characidae (0.84 larvae·10 m<sup>-3</sup>), Pimelodidae (0.60 larvae·10 m<sup>-3</sup>) and Prochilodontidae (0.42 larvae·10 m<sup>-3</sup>). The family Bryconidae was sampled only in 2015-2016 and the family Cichlidae only in 2016-2017 (Table 2).

**Table 1.** List of identified taxa sampled in reproductive cycles of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River, Southeast, Brazil.

TAXA
<b>CHARACIFORMES</b>
<b>Bryconidae</b>
<i>Salminus brasiliensis</i> (Cuvier, 1816)
<b>Anostomidae (unidentified)</b>
<i>Leporinus/Megaleporinus</i> spp.
<b>Characidae (unidentified)</b>
<i>Astyanax</i> spp.
<i>Bryconamericus</i> spp.
<i>Hyphessobrycon eques</i> (Steindachner, 1882)
<b>Curimatidae (unidentified)</b>

**Erythrinidae**

*Hoplias* spp.

**Parodontidae (unidentified)****Prochilodontidae**

*Prochilodus lineatus* (Valenciennes, 1836)

**Serrasalminidae**

*Serrasalmus maculatus* Kner, 1858

**CICHLIFORMES**

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**Cichlidae (unidentified)****GYMNOTIFORMES**

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**Gymnotidae**

*Gymnotus* spp.

**Sternopygidae**

*Eigenmannia* spp.

**SILURIFORMES**

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**Auchenipteridae**

*Tatia neivai* (Ihering, 1930)

**Callichthyidae**

*Hoplosternum littorale* (Hancock, 1828)

**Heptapteridae (unidentified)**

*Rhamdia quelen* (Quoy & Gaimard, 1824)

**Pimelodidae (unidentified)**

*Pimelodus* spp.

*Pimelodus maculatus* Lacépède, 1803

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In the reproductive cycle of 2015-2016, 4,189 eggs (12.66 ind·10 m<sup>-3</sup>) and 2,376 larvae (6.85 ind·10 m<sup>-3</sup>) were sampled, with predominance of larvae in yolk-sac (3.96 ind·10 m<sup>-3</sup>) and preflexion stages (2.70 ind·10 m<sup>-3</sup>). In 2016-2017, 815 eggs (4.26 ind·10 m<sup>-3</sup>) and 813 larvae (2.53 ind·10 m<sup>-3</sup>) were sampled, with predominance of preflexion (1.45 ind·10 m<sup>-3</sup>) and yolk-sac stages (0.91 ind·10 m<sup>-3</sup>).

Fish larvae assemblage structure and early development stages, in both cycles, were influenced by all factors and interaction among them ( $p < 0.05$ ), except for the interaction between reservoir and section for larval assemblage structure in 2016-2017 (Table 3). The variability on larval assemblage structure in 2015-2016 was better explained by pH (9.0%), K (7.7%) and ORP (6.8%). In

2016-2017 it was explained by turbidity (8.2%), K (6.5%) and TDS (5.7%). The variability of early development stages in 2015-2016 was better explained by temperature (15.3%), turbidity (14.3%) and K (10.6%). In 2016-2017 variability was explained by the same variables: temperature (15.3%), turbidity (13.2%) and K (5.7%) (Table 4).

**Table 2.** Average density (individuals·10 m<sup>-3</sup>) of larval families grouped by reservoir (Palmeiras and Retiro), section (Lentic, Transition, Lotic) and period (from first fortnight of November (Nov-1) to second fortnight of February (Feb-2) in reproductive cycles of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River, Southeast, Brazil. \* values < 0.01

Cycle	Reservoir	Characiformes						Gymnotiformes		Cichliformes		Siluriformes				
		Anostomidae	Bryconidae	Characidae	Curimatidae	Erythrinidae	Parodontidae	Prochilodontidae	Serrasalminidae	Gymnotidae	Sternopygidae	Cichlidae	Auchenipteridae	Callichthyidae	Heptapteridae	Pimelodidae
2015-2016	Palmeiras	5.76	*	1.38	*	0.08	0.06	0.87	*	0.02	-	-	*	0.03	0.09	1.30
	Retiro	2.60	0.02	0.33	0.02	0.18	0.02	0.40	0.08	*	0.02	-	0.03	-	0.03	0.20
2016-2017	Palmeiras	0.39	-	0.63	-	0.01	0.01	0.05	0.02	-	-	0.02	-	-	0.04	0.42
	Retiro	1.05	-	1.00	0.02	0.04	0.04	0.35	0.02	0.02	*	-	0.04	0.01	0.10	0.48
<b>Section</b>																
2015-2016	Lentic	2.18	0.04	0.76	0.01	0.11	0.08	0.96	0.03	0.02	-	-	-	0.04	0.07	0.82
	Transition	8.71	-	1.68	0.02	0.28	0.02	0.60	0.10	0.03	0.03	-	0.04	0.01	0.09	0.87
	Lotic	1.63	-	0.13	*	*	0.02	0.35	0.01	-	*	-	*	-	0.02	0.56
2016-2017	Lentic	0.74	-	1.03	-	0.03	0.03	0.39	-	-	-	0.02	0.02	-	0.10	0.15
	Transition	0.78	-	1.22	0.02	0.03	0.04	0.10	0.07	-	*	-	0.03	0.02	0.08	0.43
	Lotic	0.64	-	0.19	-	0.02	0.01	0.11	-	0.02	-	*	-	*	0.03	0.76
<b>Period</b>																
2015-2016	Nov-1	0.45	-	0.16	0.03	0.11	0.03	0.02	0.17	-	-	-	-	0.03	-	0.45
	Nov-2	0.71	-	0.13	-	-	0.02	0.04	-	-	-	-	0.02	0.02	-	0.26
	Dec-1	5.86	-	0.10	-	0.07	-	0.06	0.06	0.03	0.08	-	0.09	-	-	4.47
	Dec-2	2.70	0.01	0.31	-	0.18	0.05	0.98	*	0.01	-	-	-	0.04	-	0.37
	Jan-1	3.73	0.03	0.48	-	0.10	0.04	2.16	0.08	0.03	0.02	-	-	-	0.20	0.18
	Jan-2	19.74	0.07	4.98	0.08	0.02	0.19	1.83	0.02	0.04	-	-	0.02	0.04	0.29	0.11
	Feb-1	0.18	-	0.51	-	0.40	-	-	-	-	-	-	*	-	-	0.14
	Feb-2	0.04	-	0.21	-	0.18	-	-	0.02	-	-	-	-	-	-	0.01
2016-2017	Nov-1	0.20	-	0.42	-	-	-	-	-	-	-	-	-	-	-	0.06
	Nov-2	0.09	-	0.14	-	-	-	0.10	-	-	-	-	0.05	-	-	0.09
	Dec-1	0.99	-	-	-	0.05	0.03	0.04	0.09	-	-	0.02	0.03	-	-	1.38

Dec-2	0.27	-	-	-	0.09	0.03	-	0.06	0.07	-	-	0.04	-	-	0.03
Jan-1	0.26	-	0.19	-	-	-	-	0.04	-	-	-	-	-	-	0.34
Jan-2	1.91	-	5.14	0.02	-	0.09	0.79	-	-	-	0.04	0.02	0.01	0.54	0.95
Feb-1	1.64	-	0.39	0.04	0.04	0.07	0.70	-	-	-	-	-	0.04	0.02	0.62
Feb-2	0.39	-	0.22	-	0.02	-	-	-	-	0.02	-	-	-	-	0.11

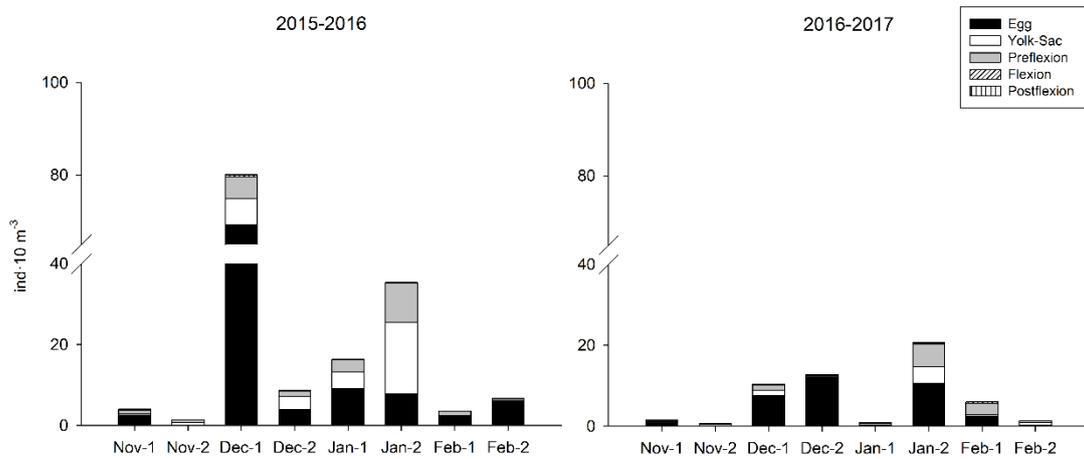
**Table 3.** Values of Pseudo-F and coefficient of significance (P(perm)) of Permutation Multivariate Analysis of Variance (PERMANOVA) performed with fish larvae assemblage structure and early development stages data, considering the factors dam, section, period and the interaction among them in reproductive cycle of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River, Southeast, Brazil.

Source	Fish larvae assemblage structure				Early development stages			
	2015-2016		2016-2017		2015-2016		2016-2017	
	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Dam	2.233	0.041	8.103	0.001	7.256	0.001	6.565	0.001
Section	3.463	0.001	4.639	0.003	23.175	0.001	14.920	0.001
Period	9.225	0.001	5.478	0.001	8.997	0.001	6.387	0.001
Dam X Section	4.490	0.001	1.837	0.072	7.927	0.001	6.005	0.001
Dam X Period	2.808	0.001	3.718	0.001	3.875	0.001	5.963	0.001
Period X Section	2.140	0.001	2.154	0.001	3.292	0.001	2.697	0.001
Dam X Section X Period	1.438	0.016	1.937	0.001	2.192	0.001	2.579	0.001

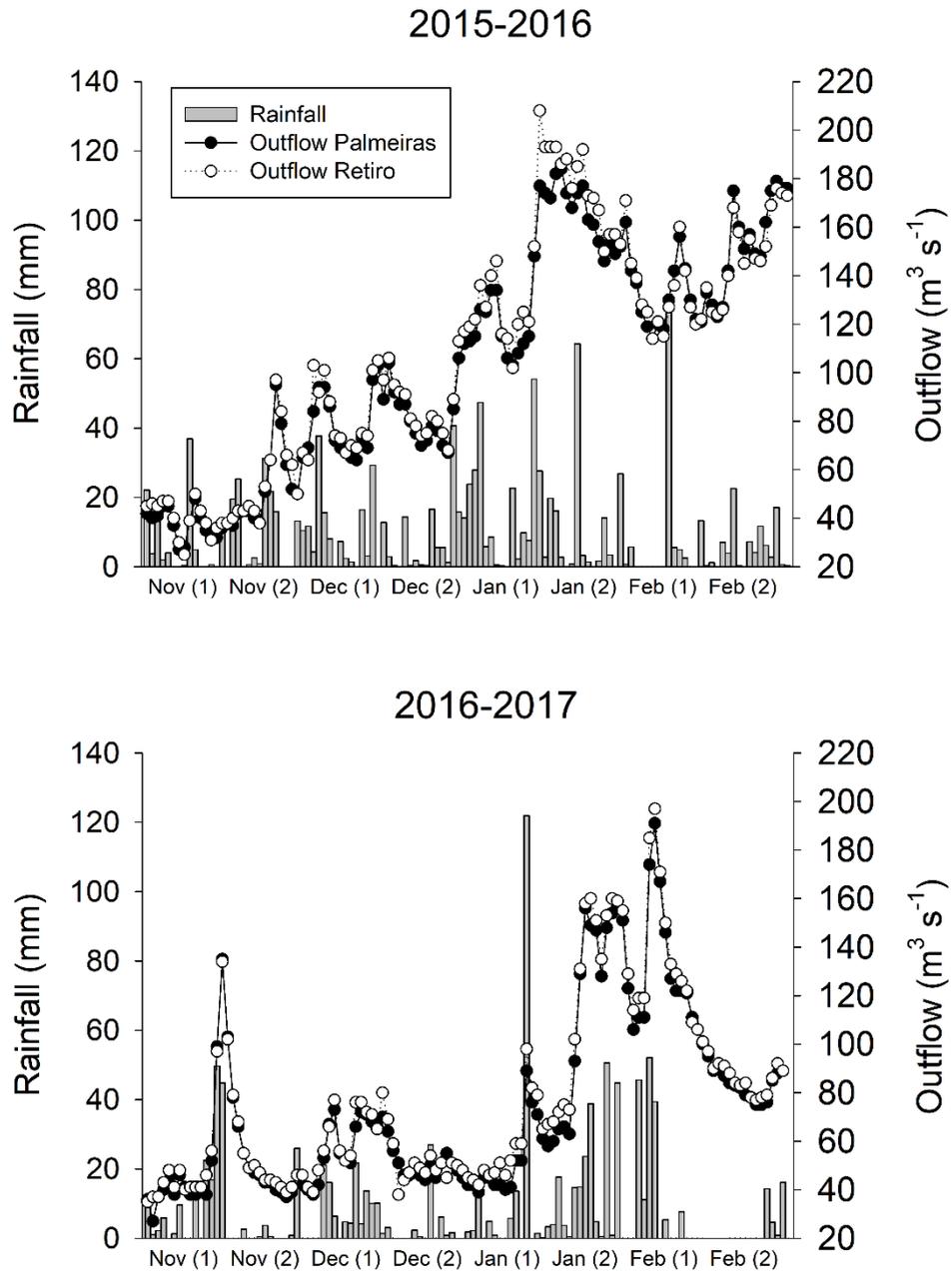
**Table 4.** Values of Pseudo-F, coefficient of significance (P) and proportion of explanation (Prop.%) of marginal test presented by DISTLM test performed between environmental variables and fish larvae assemblage structure and early development stages data in the reproductive cycles of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River, Southeast, Brazil.

Variable	Fish larvae assemblage structure						Early development stages					
	2015-2016			2016-2017			2015-2016			2016-2017		
	Pseudo-F	P	Prop.%	Pseudo-F	P	Prop.%	Pseudo-F	P	Prop.%	Pseudo-F	P	Prop.%
Temperature	2.834	0.009	5.8	1.383	0.202	2.9	8.335	0.001	15.3	8.334	0.001	15.3
pH	4.541	0.002	9.0	1.084	0.336	2.3	5.013	0.001	9.8	2.113	0.067	4.4
ORP	3.371	0.002	6.8	0.627	0.737	1.3	4.130	0.004	8.2	1.535	0.189	3.2
Conductivity (K)	3.849	0.001	7.7	3.182	0.003	6.5	5.464	0.002	10.6	2.760	0.030	5.7
Turbidity	2.572	0.013	5.3	4.127	0.001	8.2	7.679	0.001	14.3	6.989	0.001	13.2
Dissolved Oxygen	2.163	0.029	4.5	1.550	0.128	3.3	1.633	0.157	3.4	0.959	0.411	2.0
TDS	3.296	0.004	6.7	2.781	0.009	5.7	5.241	0.002	10.2	2.566	0.029	5.3

Higher densities of eggs and larvae were observed, for both cycles, in December and January (Figure 2). In 2015-2016 they were positively correlated with total outflow ( $p < 0.05$ ,  $S = 0.517$ ) and accumulated rainfall three days before sampling day ( $p < 0.05$ ,  $S = 0.509$ ) and only with total outflow ( $p < 0.05$ ;  $S = 0.653$ ) in 2016-2017. Figure 3 shows daily rainfall and outflow.



**Figure 2.** Average density (individuals·10 m<sup>-3</sup>) of fish early development stages in each period of reproductive cycles of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River, Southeast, Brazil.

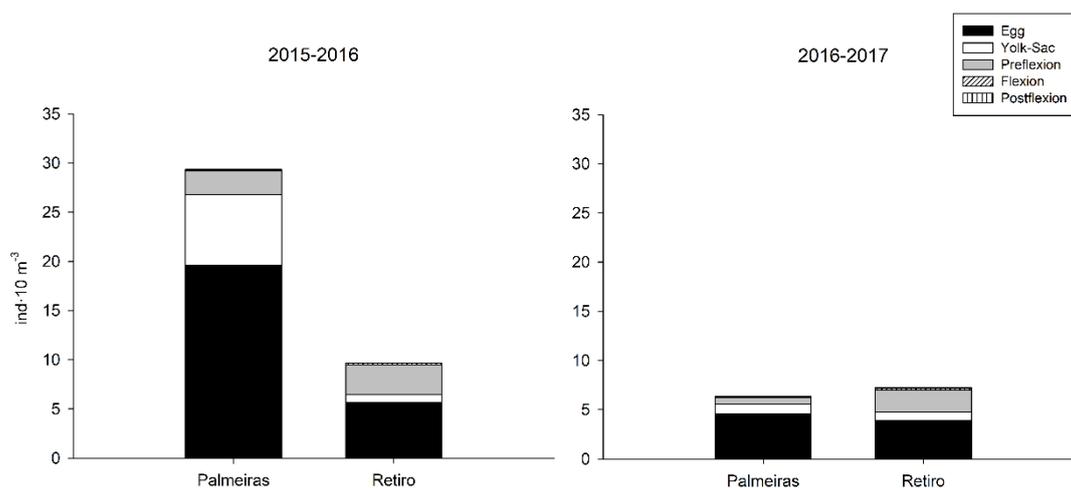


**Figure 3.** Daily values of rainfall (nearest meteorological station, Franca municipality - 47 km) and outflow of Retiro and Palmeiras dams in the reproductive cycles of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River, Southeast, Brazil.

In 2015-2016 Anostomidae and Prochilodontidae presented higher densities in December and January, Characidae in January-2 and Pimelodidae in December-1. In 2016-2017 Anostomidae and Prochilodontidae presented

higher densities in January-2 and February-1, Characidae in January-2 and Pimelodidae in December-1 (Table 2).

Eggs were the most abundant development stage in both dams and both reproductive cycles. Palmeiras, in both cycles, presented higher number of eggs than Retiro, around four times more in 2015-2016 and slightly higher in 2016-2017. For larvae, Palmeiras presented more than twice times than Retiro in 2015-2016 and in 2016-2017 Retiro presented around twice times than Palmeiras. For both cycles there was a predominance of yolk-sac larvae in Palmeiras and preflexion larvae in Retiro (Figure 4).

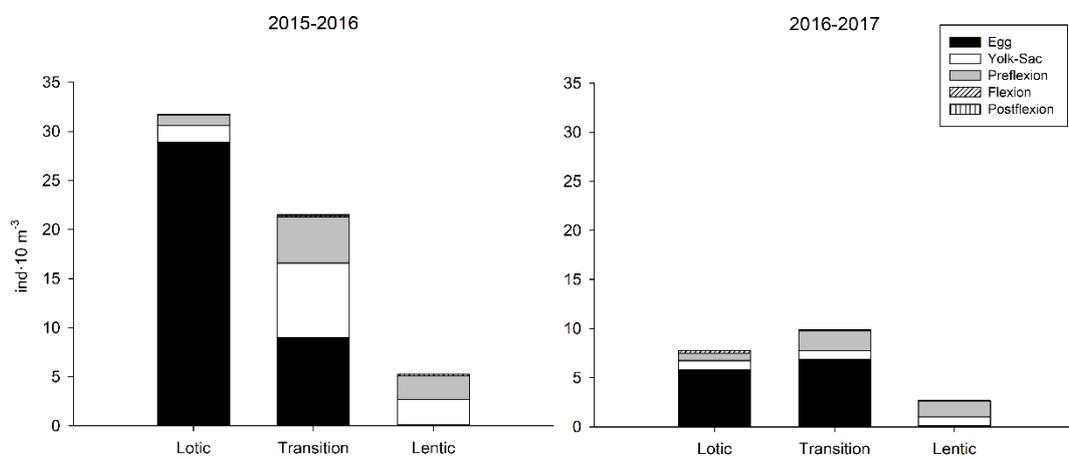


**Figure 4.** Average density (individuals · 10 m<sup>-3</sup>) of fish early development stages in each studied reservoir of the Sapucaí-Mirim River, Southeast, Brazil during the reproductive cycles of 2015-2016 and 2016-2107.

The most abundant families in both dams and cycles were Anostomidae, Characidae, Pimelodidae and Prochilodontidae. In 2015-2016 Palmeiras presented around six times more Pimelodidae, four times more Characidae and twice more Anostomidae and Prochilodontidae than Retiro. Callichthyidae was the only family exclusive from Palmeiras and Sternopygidae from Retiro. In 2016-

2017 Retiro presented around seven times more Prochilodontidae, twice times more Anostomidae and higher density of Characidae and Pimelodidae than Palmeiras. In this cycle Retiro presented five exclusive families and Palmeiras only one (Table 2).

Considering reservoir sections for both reservoirs there was a decreasing amount of eggs in 2015-2016, with higher number in lotic section, intermediate values in transition section and less eggs in lentic section. In 2016-2017, transition section presented the highest density of eggs, followed by lotic and then lentic section. Density of larvae was lower in lotic zone and higher in transition in both cycles. In both cycles there was the predominance of initial development larvae (yolk-sac and preflexion stages) in all sections (Figure 5)



**Figure 5.** Average density (individuals · 10 m<sup>-3</sup>) of fish early development stages per section of both studied reservoirs in the Sapucaí-Mirim River, Southeast, Brazil, during the reproductive cycles of 2015-2016 and 2016-2017.

For all sections and both cycles the most abundant families were also Anostomidae, Characidae, Pimelodidae and Prochilodontidae. In both cycles Anostomidae and Characidae were more abundant in transition section,

Prochilodontidae in lentic section and Pimelodidae in transition section in 2015-2016 and lotic section in 2016-2017. In 2015-2016 the family Bryconidae was sampled only in transition section. In 2016-2017 Gymnotidae was sampled only in lotic section and Curimatidae, Serrasalminidae and Sternopygidae only in transition section (Table 2).

## **Discussion**

In the cascade of SHPs of Sapucaí-Mirim River the fish early development stages and larval assemblage structure were influenced by temporal (periods of a reproductive cycle), spatial factors (position of dam and section) and the interaction among them in both analyzed reproductive cycles. The peak of spawning was observed in periods with higher rainfall and associated changes in the river environmental variables. The position of dams in the cascade and the reservoirs compartments determined differential reproductive conditions for distinct families and respective early development stages.

Comparing with other fish larval studies in Neotropical region, Sapucaí-Mirim River presented similar number of families found in undammed river stretches of central and southern Brazilian: Ivinhema River (15 families) (Reynalte-Tataje et al. 2011), Anhambá River (10), Ivaí River (15), Paraná River (15) (Baumgartner et al. 2008) and Chapecó River (14) (Corrêa et al. 2011). The four most abundant families (Anostomidae, Characidae, Pimelodidae and Prochilodontidae) are represented by some species with external fertilization, short or long reproductive migratory behavior and without parental care (Agostinho et al. 2003; Suzuki et al. 2005; Langeani and Rêgo 2014). Long distance migrators migrate upstream searching for adequate spawning habitats,

usually in the period that coincide with the beginning of the rainy season when river flows increased (Agostinho et al. 2016; Makrakis et al. 2019).

The fish early development stages predominant in the studied system are eggs and early larval stages (yolk-sac and preflexion), meaning that this stretch of river is an area of fish spawn and intensive drifting. The cascade of SHPs seems to be not an obstacle for the drift of larvae, because they are small reservoirs (extension between 5 to 10 km) with short retention time (around three days in average). Downstream passage of larvae was also reported in small reservoirs with low retention time (Pompeu et al. 2011; Suzuki et al. 2011; Fuentes et al. 2016).

Temporal variation have been reported as one of the most influent factor on the assemblage structure and/or density of fish eggs and larvae (Baumgartner et al. 2018; Silva et al. 2019), and was clearly observed in our study. During periods of intensive rainfall, and consequently floods, the river environmental conditions are modified, some abiotic factors change and the availability of food resources and shelters increases (Sanches et al. 2006; Gogola et al. 2010; Suzuki and Pompeu 2016) . This condition acts as a trigger to spawning of some species, because increases offspring survival (Vazzoler et al. 1997), resulting in high densities of eggs and larvae.

The environmental variables with higher influence on the early development stages and larval assemblage structure variation in the Sapucaí-Mirim River, were temperature and turbidity, considered the main abiotic factors responsible for temporal variation on the densities of eggs and larvae in unregulated river stretches (Suzuki and Pompeu 2016). An increase in

temperature along the year are related with gonadal development (Nascimento and Nakatani 2006). Higher water temperatures increase the fish metabolism and accelerate the development of early stages (Baumgartner et al. 2008; Gogola et al. 2010). Early life stages of fish are fragile and short time in these stages means higher survival. Spawning in periods of high turbidity/low transparency has been identified as a behavior adaptation to avoid visual predators, thus increasing the survival rate of offspring (Agostinho et al. 2016).

Other environmental variables, such as electrical conductivity, pH, TDS and ORP also explained development stage and larval variation in the Sapucaí-Mirim River. It seems these variables are more related with reproduction, representing an indirect effect in the occurrence of eggs and larvae. Electrical conductivity appears to influence spawning, when they have high values (Agostinho et al. 2007) and acts as a synchronizing factor for final maturation, spawning and fertilization (Vazzoler 1996). In the floodplain of the upper Paraná River, electrical conductivity was found to be positively correlated with ichthyoplankton density (Rosa et al. 2018). Electrical conductivity and pH have been mentioned as the main factors influencing rheophilic fish spawning (Reynalte-Tataje et al. 2012). Although it is not clear how pH and electrical conductivity affect the reproductive process, in some manner they induce spawning for some species (Baumgartner et al. 2008). Higher values of TDS may increase spawning too (Agostinho et al. 2007; Rosa et al. 2018). For ORP, our study may be the first to find some relationship between this variable and larval assemblage structure variation.

In the Sapucaí-Mirim cascade of SHPs were found a mix of what is observed for longitudinal variation of undammed rivers and compartmentalized

reservoirs. In many rivers and tributaries there is a longitudinal spatial pattern of eggs and larvae distribution, with a reducing number of eggs and increasing number of larvae when going from upstream to downstream (Vianna and Nogueira 2008; Gogola et al. 2010). In the compartments of a reservoir higher amount of eggs occurs in lotic environments, while larvae are more common in transitional and lentic environments (Lopes and Zaniboni-Filho 2019).

Lotic environments may be suitable for spawning because the low water transparency protects against visual predators and thus increases offspring survival rate (Pompeu et al. 2012). Also, the water currents maintain pelagic and semidense eggs in suspension, transporting them downstream the river to reach nursery and growth areas, avoiding conditions that would block metabolic changes, such as decantation, substrate abrasion and inorganic-matter accumulation (Worthington et al. 2014).

In general, transitional between lotic and lentic environments have biological, physical and chemical conditions that are very suitable for the growth of larvae. Characteristics as food availability, low water velocity, higher temperatures, shallow environments, low water transparency, microhabitat heterogeneity and shelter presence (Silva et al. 2012; Price et al. 2013). Most larvae found in lentic and transition sections of the Sapucaí-Mirim River reservoirs were in early development stages (yolk-sac and preflexion stages), probably drifting and not using these sections to growth. However, these compartments can also serve as a nursery area for some species, since their larvae were found in advanced developed stages (flexion and postflexion).

The reproductive cycles of 2015-2016 and 2016-2017 in the Sapucaí-Mirim River exhibited more similarities than differences. Differences between reproductive cycles are, generally, related to the amount and distribution of rainfall and environmental variables influenced by it. Fluctuations in reproductive output can also occur due to a large number of spawners in a given reproductive season, or even a small number finding ideal conditions for spawning, offspring development, or both (Silva et al. 2019).

In summary, particular time/condition within the reproductive cycle, position of dam and the different sections of the reservoirs are the main drivers that influence the distribution of the early development stages and the larval assemblage structure in an atypical system with a cascade of three SHPs. This river section of Sapucaí-Mirim River can offer suitable conditions for spawning and drift of eggs and larvae of important short and long-distance migratory species of the river basin. Therefore, this is a critical area for fish recruitment and efforts to preserve it are necessary.

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## *Capítulo 2*

# **Dispersal of fish eggs and larvae in a cascade of small hydropower plants with fish ladders**

## **Abstract**

It is essential that eggs and larvae of migratory Neotropical fish disperse downstream to reach critical areas for their development and later recruitment into juvenile and adult stages. Some large reservoirs can act as barriers for downstream dispersal of eggs and larvae, but in some small reservoirs, downstream dispersal can effectively occur. This study was conducted to investigate eggs and larvae dispersal in a river stretch influenced by SHPs in Southeast Brazil. Eggs and larvae were collected fortnightly, between November 2016 and February 2017, from locations upstream of two the dams, including lotic, transition and lentic zones. Additional samples were collected inside the fish ladders at each dam. Downstream dispersal of eggs and larvae was influenced by a combination of rainfall/flow variation and stage of the cycle, with peak of rainfall during the summer coinciding with higher abundances. Under these conditions, eggs and larvae drifted downstream through the reservoirs and reached the dams. Once in the dam, they can pass through the fish ladder. This passage was correlated with larval density in the reservoir immediately upstream, although in very low densities compared with the other sampling locations.

**Keywords:** fish passage, ichthyoplankton, river conservation, Sapucaí-Mirim River

## **Introduction**

There is a global boom in the construction or renovation of small hydropower plants (SHP). However, there is not a universal definition of SHP, especially in terms of the upper production range (up to 100 MW for USA) (Premalatha et al. 2014; Spänhoff 2014; Kelly-Richards et al. 2017). In Brazil there are 431 SHPs (production < 30 MW and reservoir area < 13km<sup>2</sup>) in operation, generating 5,031 MW, and about 1,000 new more plants have been already planned (ANEEL 2018). It was argued that, compared to large hydropower plants, SHPs would have lower construction cost, being attractive to a wider range of investors; have a more efficient distribution system, because they can supply nearby population centers do not requiring a large distribution grid, and can be built in smaller rivers (Sharma et al. 2013). Nevertheless, irrespective of size, hydropower plants are often associated with ecological degradation (Zarfl et al. 2015) and have been a long-lasting threat to the aquatic biota globally (Dudgeon et al. 2006).

The extent of the environmental impacts caused by SHPs can be even slight higher than those of large hydropower plants, especially when considering alterations per kilowatt of power generated and the potential fragmentation caused by large numbers of SHPs within a catchment (Bakken et al. 2012). Considering the impacts on fish fauna, SHP construction has been associated with reduction in abundance, average length, total weight and fish condition; species composition changes (Benejam et al. 2016); decrease in populations of migratory species (Arcifa and Esguícero 2012; Bakken et al. 2012) and interruption of fish movements (Bakken et al. 2012; Kucukali 2014). These

impacts are largely documented for adults but relatively little information exists on eggs and larval stages.

Upstream and downstream movements are essential parts of the life cycle of several Neotropical potamodromous migratory fish species, with different life stages dispersing between critical habitats throughout a river basin (Godinho and Kynard 2009). It is crucial that eggs and larvae disperse downstream to reach important areas for their development and later recruitment into juvenile and adult stages (Agostinho et al. 2016), but the absence of flow in large reservoirs can prevent downstream dispersal of eggs and larvae that normally would be passively transported. Eggs and larvae sink in lentic waters of the reservoir and cannot finish their development or are exposed to higher predation pressure due to high water transparency (Agostinho et al. 2007; Suzuki et al. 2011; Pelicice et al. 2015). However, in some small reservoirs, downstream dispersal of fish eggs and larvae might be able to effectively occur, but will be reliant on the water retention time and reservoir area (Pelicice et al. 2015). The minimum reservoir dimensions in which downstream passage of eggs and larvae have been recorded in Neotropical systems was 7.5 km<sup>2</sup> and two days of retention time (Pompeu et al. 2011; Pelicice et al. 2015). Therefore, given the characteristics of SHPs (small reservoir area and short water retention time), downstream passage of eggs and larvae may be possible, but information is scant, particularly for the Neotropical region.

In light of such scenario, studies focused on understanding the dynamics of eggs and larvae in SHP reservoirs becomes paramount for monitoring and development of management plans focusing on fish conservation. At river systems where barriers impede connectivity of fish with essential habitats, it is

critical to determine whether spawning and dispersal of early life stages are occurring along the affected area. For instance, fish passages have been installed at dams as a management tool to reestablish connectivity for migratory fish populations (Clay 1995; Porcher and Travade 2002; Silva et al. 2012) and are expected to provide access to critical habitats (Pompeu et al. 2012; Makrakis et al. 2019), especially to the ones upstream located. This management technology would be particularly beneficial in situations where spawning sites are located upstream and nursery sites downstream of a barrier (Pompeu et al. 2012). Nevertheless, its success would depend on having eggs and larvae dispersing downstream, reaching the dam and safely passing through it. The lack of downstream passage may compromise the use of fishways as a management tool (Pelicice and Agostinho 2008; Pelicice et al. 2015), even at SHPs.

If eggs and larvae, particularly in small reservoirs, could drift downstream and reach the dam, their ability to survive downstream passage will depend on the route of passage (e.g. through the turbines, spillways, fishways, bypasses), which can be critical to guarantee further recruitment success (Pompeu et al. 2011; Suzuki et al. 2011). High mortality rates can occur if eggs and larvae move downstream through turbines and spillways due to adverse hydraulic conditions (Brown et al. 2013; Boys et al. 2016) and passage through fishways is unlikely to occur (Agostinho et al. 2011). The loss of a great numbers of eggs and larvae during downstream passage can severely compromise migratory fish populations, even if appropriate management tools are in place to provide for reproduction. Nevertheless, very little is known in regards to the downstream dispersal of eggs and larval through reservoirs in tropical systems, particularly in SHPs, and only passage through turbines or spillways have been recorded on

either small or large hydropower (Pompeu et al. 2011; Suzuki et al. 2011; Fuentes et al. 2016). However, considering small dams, there is a knowledge gap whether eggs and larvae can potentially move downstream through a fish passage, and this aspect is yet to be quantified in tropical systems.

This study was conducted to investigate eggs and larvae dispersal in a river stretch influenced by SHPs in Southeast Brazil. It was designed to answer the following questions: (i) can free flowing eggs and larvae disperse downstream, through the reservoir, and reach the dams? (ii) if so, what are the main factors influencing downstream dispersal? (iii) would eggs and larvae pass through the fish ladders? The main hypothesis driving these questions is that the downstream dispersal of free flowing eggs and larvae is likely to occur given the small size of the studied reservoirs and passage through the dam may occur, with the fish ladder contributing to it.

## **Material and Methods**

### ***Study area***

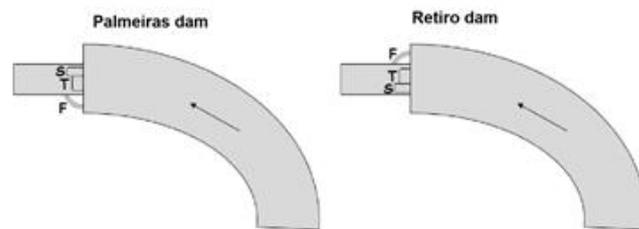
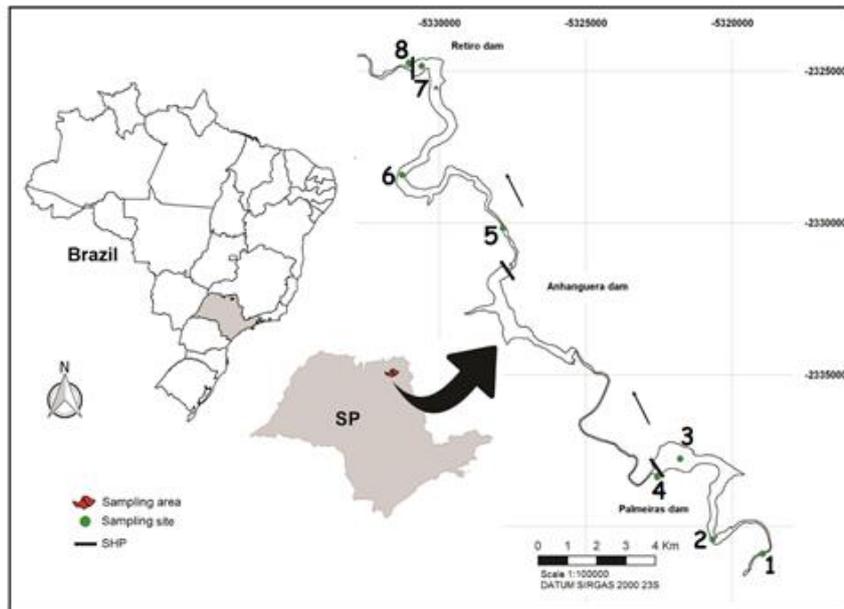
The study was conducted in the Sapucaí-Mirim River (Southeast Brazil), one of the main tributaries of the Grande River, Upper Paraná River basin (Figure 1). The river total length is 310 km, entirely in the State of São Paulo, and its main tributaries are Esmeril (43 km of the source) and Santa Bárbara (74 km of the source) (Google Maps 2020).

Between 168 km from this source and 118 km from this mouth there is a cascade of three SHPs currently in operation in the main channel of the Sapucaí-Mirim River (Google Maps 2020). Palmeiras Dam is the most upstream in the

cascade (reservoir filled in 2011), and Retiro Dam (2013) the most downstream, with Anhanguera Dam (2009) in between them. All dams have a weir-and-orifice type fish ladder installed with the purpose of maintaining connectivity along the river system.

Palmeiras and Retiro Dams belong to the same stakeholder (CTG Brazil) and have similar monitoring requirements set by the Environmental Company of São Paulo State (CETESB). Therefore, these two reservoirs and respective fish ladders were selected for this work. Both Palmeiras and Retiro are run-of-river dams, their reservoirs presenting similar area (less than 3km<sup>2</sup>) and with short retention time (around three days in average). Also, the fish ladders at both dams have similar length, number of pools and flow characteristics (Table 1).

There are not tributaries or marginal lakes between the SHPs. The closest small tributaries are Ribeirão Salgado (20 km upstream of Palmeiras Dam) and Ribeirão das 7 Lagoas (56 km downstream of Retiro Dam) (Google Maps 2020).



**Figure 1.** Map of the study area showing the distribution of the sampling sites and arrows indicate direction of flow. Sites 1 to 3 were located upstream of Palmeiras dam representing the lotic, transition and lentic zones, respectively, whilst site 4 was located within the fish ladder. Similarly, sites 5 to 7 were located upstream of Retiro dam, whilst site 8 was within the fish ladder. Schematic of location and satellite image (Google Earth™, scale 1:116 m) of fish ladder (F), turbine (T) and spillway (S) at Palmeiras Dam (left) and Retiro Dam (right).

**Table 1.** Reservoir area and volume, water retention time in days (average and range), turbine type, operation and type of flow and date of filling of Palmeiras and Retiro Small Hydropower Plants (SHPs). Characteristics of the fish ladder for both dams are also presented.

<b>DAM FEATURES</b>	<b>SHP</b>	
	<b>Palmeiras</b>	<b>Retiro</b>
RESERVOIR AREA (km <sup>2</sup> )	2.5	2.8
RESERVOIR VOLUME (m <sup>3</sup> . 10 <sup>6</sup> )	17.71	17.87
RETENTION TIME (DAYS)*	3.4 (1.1-7.6)	3.3 (1.0-5.9)
TURBINE TYPE	Kaplan (PIT)	Kaplan (PIT)
OPERATION AND TYPE OF FLOW	run-of-river	run-of-river
DATE OF FILLING	2011	2013

<b>FISH LADDER FEATURES</b>		
TYPE	Weir and Orifice	Weir and Orifice
LENGTH (m)	253	250
SLOPE (m)	2% initial and 8 % final	2% initial and 8 % final
FLOW (m <sup>3</sup> /s)	1.0 - 2.0	1.2 - 2.5
Nº of POOLS	45	46
WEIR DIMENSION (M)	3.7(L)x3(W)x1.3(H)	3.7(L)x3(W)x1.3(H)
ORIFICE DIMENSION (M)	0.5(W)x0.4(H)	0.5(W)x0.4(H)
LOCATION OF THE ENTRANCE (downstream)	Tailrace, just downstream of the turbines	Tailrace, just downstream of the turbines
LOCATION OF THE EXIT (upstream)	Forebay, near the turbine intake in the left bank	Forebay, near the turbine intake in the right bank

\* Between 01/11/2016 and 28/02/2017

### ***Sampling design and processing***

Eight sampling sites were distributed upstream and also within the fish ladder of both Palmeiras and Retiro Dams (Figure 1). The distance between site 1 and 7 is 30 km and between each dam is 12 km (Google Maps 2020). The sites were distributed from upstream to downstream in order to evaluate dispersal, encompassing the lotic (upstream of the reservoir, sites 1 and 5), the transition

(between the reservoir and the lotic area, sites 2 and 6) and the lentic (represented by the reservoirs, sites 3 and 7) zones. Sampling was also conducted in one location (mid-section) inside the fish ladder of each dam (sites 4 and 8) (Figure 1).

To evaluate the dispersal of eggs and larvae, the sites were sampled, sequentially, from upstream to downstream (e.g. site 1, 2 and 3 at Palmeiras Dam) and fortnightly between November 2016 and February 2017, totaling eight samples for each site. The sampling period was defined based on the spawning season for most of the Neotropical migratory fish, commencing at the end of spring (October) until the end of summer (March) (Reynalte-Tataje et al. 2011; Lopes et al. 2014). Sampling was conducted at night, between 7 PM and 12 AM, at those sites located upstream of the dams. At the fish ladders, sampling occurred early in the morning because of work safety requirements. Three pseudo replicates were obtained for each sampling site, except for the fish ladders where a single sample was obtained due to operational constraints, totalizing 160 samples.

All samples were obtained with a conical-cylindrical plankton net, with mesh size of 500  $\mu\text{m}$  and an opening area of 0.11  $\text{m}^2$ . A mechanical flowmeter (General Oceanics™) was positioned in the opening of the net to determine the volume of filtered water for each sample. The net was soaked in the water in the subsurface (approximately 20 cm below the surface) for 10 minutes. In the lentic/transition sites (sites 2 and 3 and 6 and 7 at Palmeiras and Retiro Dams, respectively) the net was trawled by a boat at low velocity whereas for the lotic sites (1 and 5 at Palmeiras and Retiro Dams, respectively) it remained stationary with the opening positioned against the flow direction.

The sampled material was transferred to polyethylene bottles and fixed with a 4% formalin solution. An overdose (over 283.55 mg/L) of anesthetic (eugenol) was added to euthanize the organisms collected before fixing with formalin (Vidal et al. 2008).

### ***Abiotic and operational data***

Measurements of temperature, turbidity, dissolved oxygen (DO), electrical conductivity (K), oxide reduction potential (ORP), total dissolved solids (TDS) and pH were taken immediately before retrieving the nets, using a Horiba probe (U-52). Daily rainfall data were obtained from the National Institute of Meteorology (INMET) <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>, accessed 29 November 2018) using the closest station to the study area (Franca municipality, around 47 km of distance). Duke Energy Brazil (the generation company in charge of the hydroelectric plants in that occasion) provided the operational data for each dam, including daily mean turbine and spillway flows, and total inflow and outflow. Retention time (days) was defined as the ratio of reservoir volume and the outflow calculated using the formula:  $TRT = V / (Q \times 86,400)$ , where  $V$  = reservoir volume ( $m^3$ );  $Q$  = mean flow ( $m^3.s^{-1}$ ); and 86,400 = number of seconds contained in a day (Nogueira and Pomari 2019).

In the laboratory, all samples were sorted under a stereomicroscope using a Bogorov-type plate, and the eggs and larvae were separated from the rest of the sampled material. After separation, the larvae were identified to the lowest taxonomic level according to Nakatani et al. (2001). Identified taxonomic groups and species names were confirmed and updated based on Fricke et al. (2019).

The larvae captured were also classified in development stages, following Ahlstrom and Ball (1954) and Nakatani et al. (2001).

### ***Data analysis***

To evaluate potential dispersal through the study area, only larvae of species with external fertilization, absence of parental care and short or long reproductive migration (Agostinho et al. 2003; Suzuki et al. 2005; Langeani and Rêgo 2014) were considered for further analyses in this study. Species presenting such traits have free flowing eggs and larvae and, therefore, would depend on downstream dispersal to complete their life cycles (Nakatani et al. 2001). A list of species (Appendix I) occurring in the Sapucaí-Mirim River was compiled from previous studies (Freitas-Souza 2014; Oliveira et al. 2016; Brambilla et al. 2018) to assure that eggs and larvae not identified at the species level still pertained to species with the aforementioned traits, so they could be reliably treated as having free flowing embryos in the analyses.

The density of eggs and larvae was calculated and standardized as individuals per 10 m<sup>-3</sup> of filtered water, according to Tanaka (1973), modified by Nakatani et al. (2001) and larvae were pooled together at the family level. Data obtained at Palmeiras and Retiro Dams were analyzed separately, given that they are separated in the system by Anhanguera Dam and could be considered independent. For each dam were evaluated the influence of two factors in the density of eggs and larvae: section [lotic (sites 1 and 5); transition (sites 2 and 6) and lentic zones (sites 3 and 7)] and period (November-1; November-2; December-1; December-2; January-1; January-2; February-1 and February-2 –

numbers after each month indicate whether the sample was obtained in the first -1 or second -2 fortnight).

To identify spatial-temporal trends in spawning activity a factorial analysis of variance (two-way ANOVA) was used to test differences in the density of eggs and larvae between sections, periods and the interaction of both factors. A post-hoc Holm-Sidak test was used for pairwise comparisons of density of eggs and larvae between factors.

A distance-based linear models procedure (DISTLM) (Anderson et al. 2008) was used to identify the influence of abiotic attributes, both physical (temperature, turbidity, TDS, rainfall in the sampling day, rainfall one day before sampling day and accumulated rainfall five days before sampling day) and chemical (K, DO, ORP and pH), on the density of eggs and larvae. For this analysis, all parameters (except pH) were  $\log(x+1)$  transformed and normalized. Draftsman plots were then examined to determine autocorrelation between abiotic attributes and those with high correlation ( $r = |0.8|$ ) were removed from the analysis (only K were removed). The DISTLM was performed on  $\log(x+1)$  transformed biotic data, and derived from Euclidean similarity matrices with 0.1 dummy effect (Anderson et al. 2008), using the best procedure with the Akaike Information Criterion (AIC).

Multiple linear regressions were used to verify whether operational parameters and the density of eggs and larvae in the lentic zone (i.e. closest site to the dam) influenced the density of those life stages within the fish ladders. For this procedure, turbine outflow, spillway outflow and density in the lentic zone

were used as independent variables and the density within the fish ladders were treated as the dependent variable.

Finally, to verify the contribution of the fish ladders to the downstream dispersal of larvae, a passage proportion was calculated by dividing the density of larvae within the fish ladder of both Palmeiras and Retiro Dams by the density of larvae in the reservoir immediately upstream. A percentage value was obtained and the contribution of the fish ladder for the downstream dispersal of larvae was defined as low (< 33%), intermediate (33% < x < 66%) and high (> 67%). The significance level adopted for all statistical analyses was  $p < 0.05$ .

## Results

A total of 815 eggs and 658 larvae of five families (Anostomidae, Characidae, Heptapteridae, Pimelodidae, Prochilodontidae) were sampled in the study area. For larvae, the most abundant families, considering average density were Characidae (0.73 ind. $\cdot$ 10 m<sup>-3</sup>), Anostomidae (0.66 ind. $\cdot$ 10 m<sup>-3</sup>) and Pimelodidae (0.42 ind. $\cdot$ 10 m<sup>-3</sup>) (Table 2). Considering the Anostomidae family, only 2.3% was identified at the genus level as *Megaleporinus* spp. and *Leporinus* spp. Within the Characidae family, identified taxa included *Bryconamericus* spp. (4.5%); *Astyanax* spp. (3.6%); and *Hyphessobrycon eques* (0.5%). Larger proportion of identified taxa included *Pimelodus* spp. (10.6%) within the Pimelodidae family, *Rhamdia quelen* (26.3%) for Heptapteridae, and *Prochilodus lineatus* (100%) as representative of the Prochilodontidae family.

The density of eggs in Palmeiras Dam was influenced by all tested factors, section ( $F = 15.187$ ;  $p < 0.001$ ), period ( $F = 7.219$ ;  $p < 0.001$ ) and the interaction

between both ( $F = 5.073$ ;  $p < 0.001$ ). Higher density of eggs was observed in the transition zone in December-1 and January-2 (Figure 2;  $p < 0.001$ ). Similar trend was observed for the density of eggs in Retiro Dam with also section ( $F = 17.095$ ;  $p < 0.001$ ), period ( $F = 14.881$ ;  $p < 0.001$ ) and the interaction between both factors ( $F = 9.726$ ;  $p < 0.001$ ) influencing abundance. Nevertheless, for Retiro Dam, the lotic zone showed higher density of eggs in December-2 (Figure 3;  $p < 0.001$ ). Eggs were not collected from the fish ladder at Palmeiras Dam, whilst they sparsely occurred in the Retiro Dam fish ladder. For the latter, eggs were collected in the fish ladder during three sampling events: November-1 ( $0.97 \text{ ind.} \cdot 10 \text{ m}^{-3}$ ), December-1 ( $9.73 \text{ ind.} \cdot 10 \text{ m}^{-3}$ ) and February-1 ( $0.82 \text{ ind.} \cdot 10 \text{ m}^{-3}$ ).

**Table 2.** Average density of eggs and larvae (individuals·10 m<sup>-3</sup>) of family groups with species showing reproductive migratory behavior (long or short) and external fertilization in each section of small hydropower plants (SHPs) Palmeiras and Retiro in the Sapucaí-Mirim River, Southeast, Brazil.

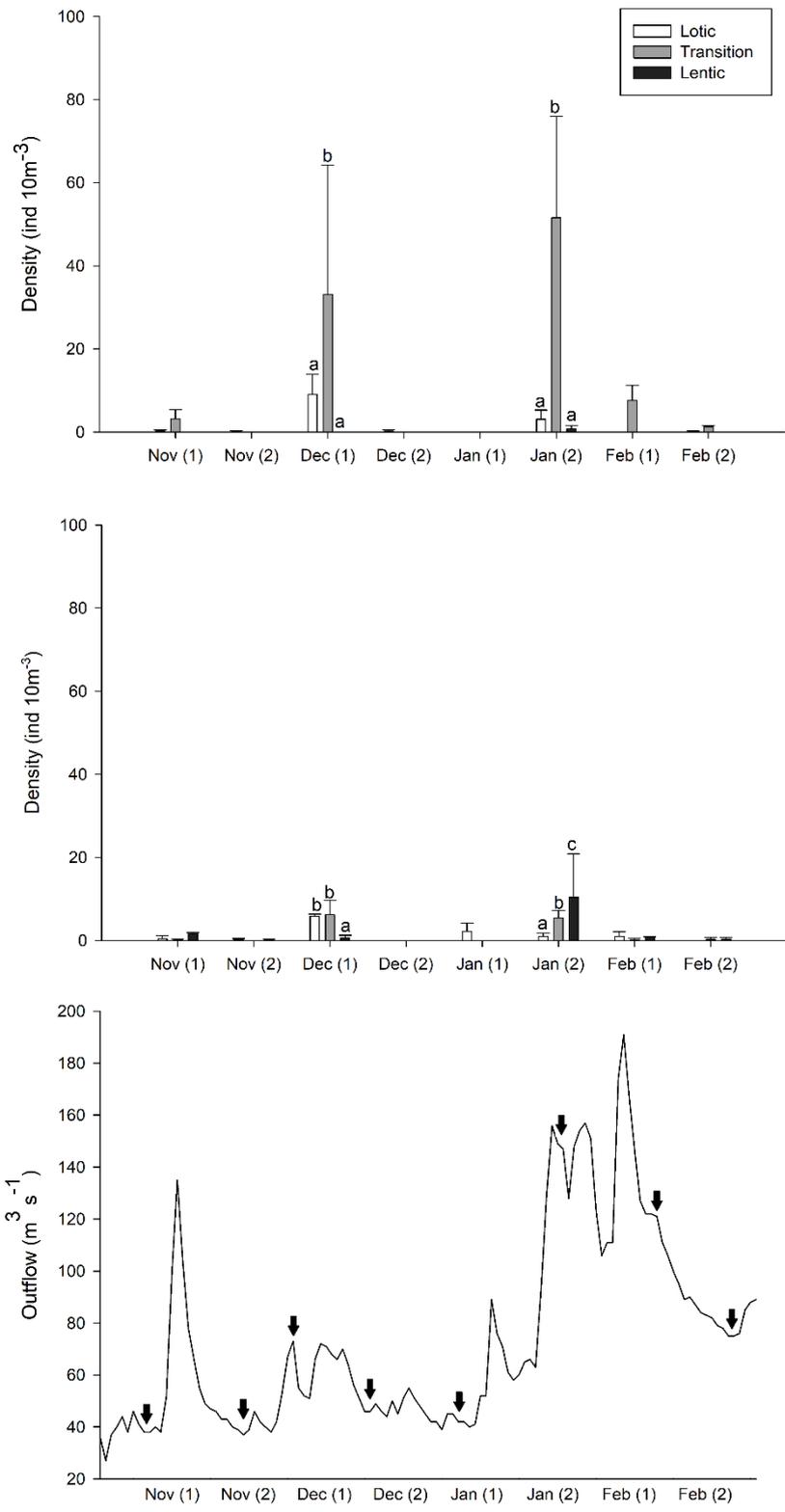
SHP	Section	Eggs	Larvae				
			Anostomidae	Characidae	Heptapteridae	Pimelodidae	Prochilodontidae
Palmeiras	Lotic	1.59	0.65	0.09	0.00	0.56	0.02
	Transition	12.07	0.36	0.41	0.00	0.62	0.14
	Lentic	0.10	0.15	1.39	0.13	0.08	-
	Ladder	-	-	-	-	0.19	-
Retiro	Lotic	10.06	0.63	0.29	0.06	0.96	0.20
	Transition	1.66	1.19	2.02	0.16	0.25	0.07
	Lentic	0.07	1.33	0.68	0.07	0.23	0.79
	Ladder	0.48	0.28	-	-	0.03	-

For density of larvae, the factors period ( $F = 7.302$ ;  $p < 0.001$ ) and the interaction between period and section ( $F = 2.632$ ;  $p = 0.006$ ) were significant in Palmeiras Dam. The lentic zone in Palmeiras showed the lowest density of larvae in December-1 and the highest in January-2, the latter corresponding to the same period when the lowest density was registered in the lotic zone (Figure 2;  $p <$

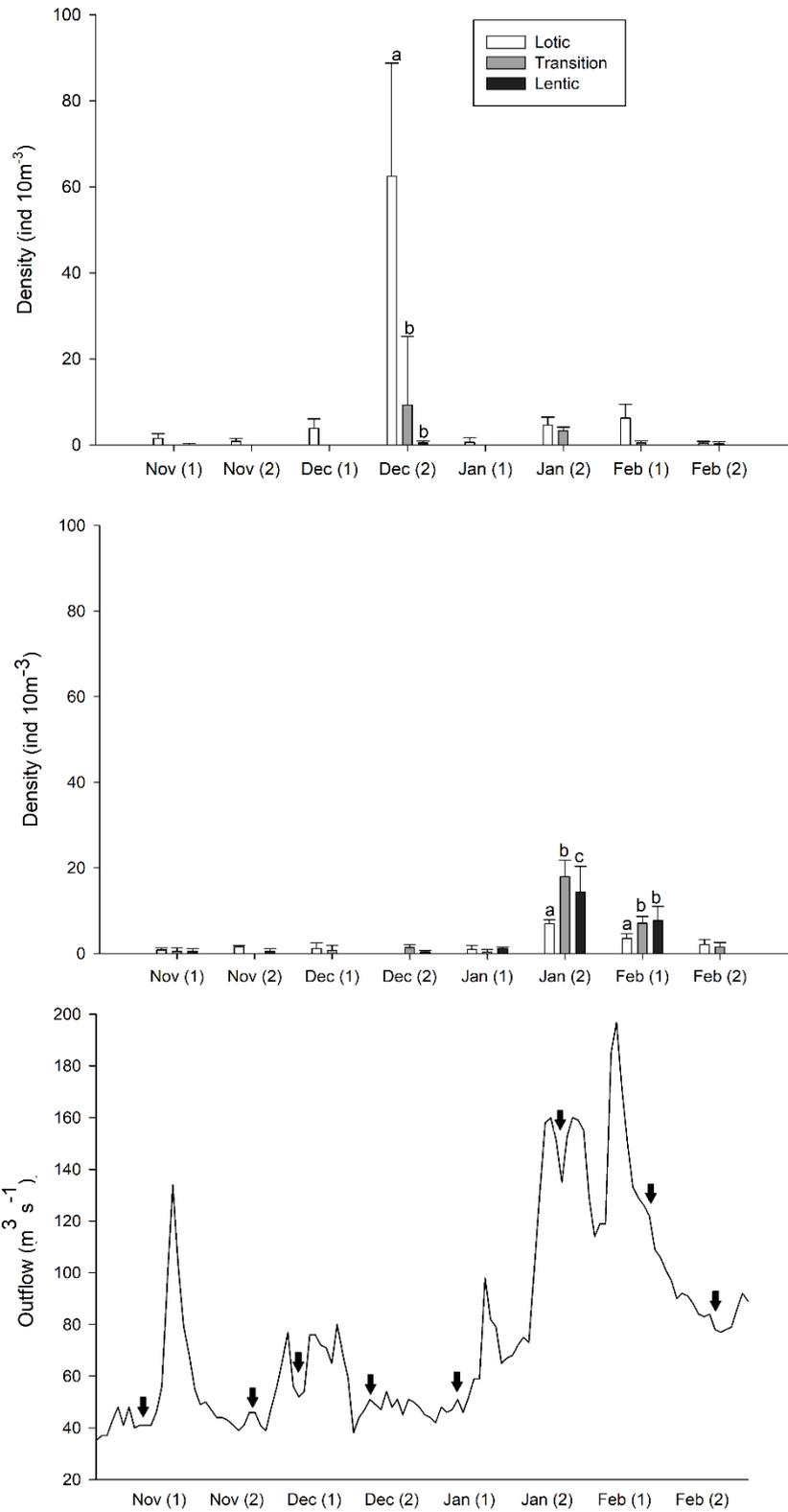
0.05). In Retiro Dam, the factors influencing density of larvae were section ( $F = 4.700$ ;  $p = 0.014$ ), period ( $F = 57.855$ ;  $p < 0.001$ ) and also the interaction of both factors ( $F = 4.700$ ;  $p < 0.001$ ). In January-2, the transition zone for Retiro Dam showed the highest density of larvae whilst the lowest was recorded in the lotic zone. The latter zone also showed the lowest densities in February-1 (Figure 2;  $p < 0.05$ ).

Rainfall peaks resulted in higher densities of larvae (Figure 2 and 3). In Palmeiras Dam, larvae were sampled in the fish ladder only in January-2 ( $1.52 \text{ ind.} \cdot 10 \text{ m}^{-3}$ ) at preflexion stage. In Retiro Dam, larvae were captured in December-2 ( $0.26 \text{ ind.} \cdot 10 \text{ m}^{-3}$ ) at flexion stage and in January-2 ( $6.82 \text{ ind.} \cdot 10 \text{ m}^{-3}$ ) at preflexion stage. For both dams the presence of larvae in the fish ladder in January-2 coincided with high density in the reservoir immediately upstream and, also, peak of rainfall (Figure 3).

Turbidity (41%), rainfall in the sampling day (29%) and rainfall one day before the sampling day (26%) were the main factors explaining the variability of density of eggs in Palmeiras Dam as well as larvae (45%, 35%, 40%, respectively). For Retiro Dam, density of eggs was influenced by temperature (27%), ORP (23%) and turbidity (22%). On the other hand, TDS (51%), turbidity (46%) and accumulated rainfall 5 days before sampling day (25%) were the main factors influencing density of larvae (Table 3).



**Figure 2.** Average and standard error (bars and whiskers) of density of eggs (upper graph) and larvae (middle graph) and daily outflow (solid line) (lower graph) in Palmeiras Dam located in the Sapucaí-Mirim River, Southeast, Brazil. Different letters show significant differences ( $p < 0.05$ ) between sections within each sampling period (months) according to post-hoc Holm-Sidak test and arrows represent the sampling day ( $p < 0.05$ ).



**Figure 3.** Average and standard error (bars and whiskers) of density of eggs (upper graph) and larvae (middle graph) and daily outflow (solid line) (lower graph) in Retiro Dam located in the Sapucaí-Mirim River, Southeast, Brazil. Different letters show significant differences ( $p < 0.05$ ) between sections within each sampling period (months) according to post-hoc Holm-Sidak test and arrows represent the sampling day ( $p < 0.05$ ).

**Table 3.** Values of Pseudo-F, coefficient of significance (p) and proportion of explanation presented by DISTLM test performed between abiotic attributes and density of eggs and larvae in Palmeiras and Retiro Small Hydropower Plants (SHPs) in the Sapucaí-Mirim River, Southeast, Brazil. In bold the three highest proportions of explanation for eggs and larvae in each dam.

Abiotic attributes	PALMEIRAS DAM						RETIRO DAM					
	EGGS			LARVAE			EGGS			LARVAE		
	Pseudo-F	p	Prop.	Pseudo-F	p	Prop.	Pseudo-F	p	Prop.	Pseudo-F	p	Prop.
Temperature	20.2	0.001	0.22	17.4	0.001	0.20	25.9	0.001	<b>0.27</b>	5.0	0.027	0.07
pH	4.9	0.040	0.07	4.3	0.047	0.06	1.1	0.250	0.02	9.5	0.004	0.12
ORP	0.1	0.739	0.00	0.4	0.539	0.01	21.0	0.001	<b>0.23</b>	1.4	0.265	0.02
Turbidity	50.0	0.001	<b>0.42</b>	58.4	0.001	<b>0.45</b>	20.5	0.001	<b>0.23</b>	61.6	0.001	<b>0.47</b>
Dissolved Oxygen	2.7	0.097	0.04	1.0	0.321	0.01	0.4	0.551	0.01	20.2	0.001	0.22
TDS	8.6	0.005	0.11	5.9	0.011	0.08	1.8	0.176	0.03	74.2	0.001	<b>0.51</b>
Rainfall in the day	29.3	0.001	<b>0.30</b>	37.7	0.001	<b>0.35</b>	0.9	0.338	0.01	9.3	0.007	0.12
Rainfall one day before sample	25.0	0.001	<b>0.26</b>	47.1	0.001	<b>0.40</b>	0.4	0.543	0.01	23.0	0.001	<b>0.25</b>
Accumulated rainfall five days before sample	12.6	0.002	0.15	21.2	0.001	0.23	0.4	0.533	0.01	23.2	0.001	<b>0.25</b>

The presence of larvae in Palmeiras Dam fish ladder was highly correlated ( $R^2 = 0.978$ ,  $p < 0.001$ ) with turbine outflow, spillway outflow and density of larvae in the lentic zone (Table 4). The model showed that presence of larvae in the fish ladder can be predicted by the density of larvae in the reservoir ( $p = 0.002$ ). Nevertheless, the proportion of larvae observed in the fish ladder of Palmeiras Dam compared to the density registered in the lentic zone was considered low (14.6%) for the passage event occurred in January-2. For Retiro Dam, the model also presented high correlation ( $R^2 = 0.859$ ,  $p = 0.035$ ) with turbine outflow, spillway outflow and density of larvae in the lentic zone. Also, the density of larvae in the fish ladder of Retiro Dam can be predicted by the density of larvae in the reservoir ( $p = 0.026$ ). An intermediate (65%) proportion of larvae was registered in the fish ladder in December-2 and a low proportion (15.8%) in January-2 in relation to the densities observed in the lentic zone. For eggs, no relationship was noted ( $R^2 = 0.174$ ).

**Table 4.** Values of  $\beta$ ,  $\beta$  standard error (SE), coefficient of determination ( $R^2$ ) and coefficient of significance ( $p$ ) presented by multiple linear regression performed between operational parameters and the density of eggs and larvae within the fish ladders in Palmeiras and Retiro Small Hydropower Plants (SHPs) in the Sapucaí-Mirim River, Southeast, Brazil.

Parameters	$\beta$	$\beta$ SE	$R^2$	$p$
<b>PALMEIRAS DAM</b>				
Density of larvae within the fish ladder			0.978	<0.001
	-			
Turbine outflow	0.000635	0.00248		0.810
Spillway outflow	0.000143	0.00146		0.926
Density of larvae in the lentic zone	0.146	0.0198		0.002
<b>RETIRO DAM</b>				
Density of larvae within the fish ladder			0.859	0.035
Turbine outflow	-0.0161	0.00989		0.180
Spillway outflow	0.0147	0.0273		0.619
Density of larvae in the lentic zone	0.247	0.0716		0.026

## Discussion

The conceptual model presented in Figure 4 summarizes the dynamic of eggs and larvae in the study stretch of the Sapucaí-Mirim River which is fragmented by three SHPs. Basically, spawning activity of migratory fish occurs throughout the area currently influenced by the dams. Spawning sites (locations with high densities of eggs) are likely to be located in the upstream reaches within the lotic or transition zones between the dams and may be triggered by rainfall peaks in the most upstream reach (upstream of the reservoir at Palmeiras Dam). Once spawning occurs, the eggs would drift towards the lentic zone and hatch along the way. Hatching would occur mainly in the transition zone, with some in the lotic zone and the abundance of eggs markedly reduce in the lentic zone towards the dams. Drift of eggs and larvae is apparently affected by a combination of rainfall and period of the reproductive cycle, with peak of rainfall during the summer coinciding with higher abundances of eggs and larvae in the system. Under these conditions, hatched larvae would maintain drifting downstream through the reservoir, reaching the dams. Larvae may get trapped upstream in the reservoir or can, potentially, move downstream through the dam if a passage route is found (turbines, spillway or fish ladders). Larvae, in very low densities, can disperse downstream through the fish ladders and this event is likely to occur when density in the reservoir immediately upstream increases. The higher the density of larvae in the lentic zone the higher is the likelihood of downstream dispersal through the fish ladder, which can be the safest route through the dam. Nevertheless, this passage event occurs randomly and is likely higher in Retiro Dam because of the fish ladder location in the right bank, where the highest flows occur due to the river channel morphology (see Figure 1).



presenting low retention time (around three days in average). As shorter is the retention time the similarity with a river is higher. This kind of dam does not store water, consequently has a fixed volume and level and an intensive flow (Nogueira et al. 2012). Consequently, in this kind of system eggs and larvae of fish species could potentially drift downstream.

Spawning activity was detected throughout the study area and four main taxonomic groups were identified (Anostomidae, Characidae, Pimelodidae and Prochilodontidae). Among these groups, at least one long distance migratory species, *P. lineatus* (Prochilodontidae), was identified to species level. Potentially, spawning of another three migratory species can also be occurring in the system since a proportion of the sampled eggs and larvae were identified as *Pimelodus* spp. and *Megaleporinus* spp. and species from these genera have been identified as migratory in the Upper Paraná Basin (Vono et al. 2002). Previous studies in the same area which sampled adult fish (Freitas-Souza 2014; Oliveira et al. 2016; Brambilla et al. 2018) have registered the occurrence of *Pimelodus maculatus* (Pimelodidae), *Megaleporinus obtusidens* and *Megaleporinus piavussu* (Anostomidae) (see Appendix 1) which, therefore, strongly indicates that the eggs and larvae identified in this study can belong to these species.

Studies in the Paraná River indicate that *P. lineatus* migrates, in general, 450 to 500 km upstream to spawn, after which free embryos are expected to drift downstream and develop (Agostinho et al. 2003). Downstream drifting is expected to occur at the early life stages (eggs and larvae in yolk-sac and preflexion stages, see Nakatani et al. (2001) for ontogeny, until morphological development provide for structures that favor swimming and obtainment of

exogenous food (flexion stage of larvae). Therefore, given the expected drifting stage of this species and the identification of embryos sampled in the study area, it was retained as a model to discuss downstream drift in the Sapucaí-Mirim River. For instance, ontogeny of *P. lineatus* at water temperature of 27°C is expected to promote hatching at 16h after fecundation, yolk-sac development at 22h, and preflexion larvae fully formed at 76h post fecundation (personal observation). Considering that the distance between sites 1 (lotic area upstream of Palmeiras Dam) and 7 (reservoir of Retiro Dam) is 30 km and that *P. lineatus* larvae in initial developing stages is present throughout the study area, it is reasonable to assume that adults can spawn upstream of Palmeiras and Retiro Dams and eggs and larvae would drift downstream, passing through dams. Other evidence that *P. lineatus* are migrating upstream of Palmeiras Dam to spawn is the presence of adults of this species in the fish ladders of all dams of the cascade (personal observation).

The density of eggs and larvae in the study area was influenced by spatial (sections) and temporal (periods) factors and the interaction between them, except for Palmeiras Dam where section did not show influence in density of larvae. Influence of sampling period in the abundance of eggs and larvae is more likely a response to other abiotic attributes, mainly rainfall and turbidity, since sampling was only undertaken during the spawning season of Neotropical migratory fish. The fortnightly frequency certainly provided for sampling at different environmental conditions. Indeed, spatial differences were only observed for the sampling periods when rainfall peak have occurred. Increases in rainfall may have triggered adult fish to spawn, consequently increasing the abundance of eggs and larvae in the system, as observed in other studies in the

Neotropical region (Reynalte-Tataje et al. 2011; Suzuki and Pompeu 2016). Rainfall generally provide a set of environmental modifications that can maximize larval survival. Alterations caused by rainfall include: (a) increase in river discharge favoring larval displacement through the system as a consequence of higher water velocities (Cheshire et al. 2016); (b) increase water level, therefore promoting connection with littoral habitats, which may increase the availability of food and refuge (Gogola et al. 2010); and (c) increase turbidity, which can reduce predation (Sanchez et al. 2006; Suzuki and Pompeu 2016). Indeed, the dynamic of eggs and larvae in the Sapucaí-Mirim system was influenced by rainfall and other abiotic attributes directly related to it, such as turbidity and TDS.

The distribution of eggs and larvae observed in this study is similar to the expected trend for undammed rivers where the abundance of eggs may decrease from the upper to the lower sites whereas larvae follow an inverse pattern with higher abundances in the lower stretches (Vianna and Nogueira 2008; Gogola et al. 2010). This similar trend may have been observed because of the low retention time of the water in the reservoirs of the studied SHPs. Because of the small area of the reservoirs and the short retention time, it is possible to hypothesize that in certain conditions (e.g. high rainfall and increased water discharge) a homogenization of the system may occur reducing compartmentalization effects (lotic, transition and lentic) and contributing to the dispersal of eggs and larvae. This has been particularly noticed for Palmeiras Dam where the spatial scale (section factor) did not influence larvae abundance. On the other hand, a different trend is expected for large reservoirs where the abundance of eggs and larvae are expected to decline severely from the upper to the lower stretches, which suggests that the downstream drift of eggs and

larvae is imperiled by the hydraulic conditions of the reservoir (Pelicice and Agostinho 2008). According to Pelicice et al. (2015), the absence or reduced flow depending on the size of reservoirs can prevent the downstream drift of eggs and larvae that normally would be passively transported to suitable habitats for development. In this scenario, non-buoyant eggs and larvae may sink in lentic waters of the reservoir and are either deposited in harsh environments that are inappropriate for development or exposed to higher predation pressure due to high water transparency (Suzuki et al. 2011; Marques et al. 2018).

Apparently, the small reservoirs of Palmeiras and Retiro Dams do not impede the drift of larvae because of the high abundances observed near the dams, particularly at Palmeiras Dam. Possibly, the potential homogenization of the system at certain conditions (e.g. high rainfall and increased flows) because of the low retention time (around three days in average) and small size of the reservoirs (less than 3 km<sup>2</sup> in area) would permit the continuation of the larvae drift. Similarly, successful drift and downstream passage of eggs and larvae are reported at other hydropower plants with comparable characteristics (Suzuki et al. 2011; Fuentes et al. 2016). A fundamental condition to maintain this ecological process is high water discharge. On the other hand, the passage of eggs and larvae through small reservoirs can be unlikely in low flow conditions (Rosa et al. 2018). Indeed, this has also been noticed for the study area where, at certain sampling periods coinciding with lower flows, the lotic zones of both dams were the sections with lowest eggs and larvae densities. During these periods of low flow there is a reduction of the useful volume of the reservoir, so there is an increase in the retention time of water, consequently the favorable conditions to larval and egg survival decrease (Rosa et al. 2018).

Furthermore, the abiotic attributes investigated in this study influenced the density of eggs and larvae differently between the two dams. This may be a consequence of the connectivity disruption caused by the dams, since only turbidity was a common factor influencing density in both Palmeiras and Retiro. Since Palmeiras Dam is located in the upper section of the Sapucaí-Mirim River, the influence of factors that are related to the increase in water level and flow rates (rainfall in the sampling day and one day before) triggering spawning and drift was more pronounced upstream of Palmeiras Dam. Once flow is disrupted by the dams, the density of eggs and larvae in the most downstream dam in the study area (Retiro Dam) becomes influenced by other factors, such as temperature and ORP. Temperature and ORP may be influenced by the operation of the dams upstream.

Based on the dynamic of eggs and larvae observed in this study and the capture of these early stages at the fish ladders of both Palmeiras and Retiro Dams, it is possible to infer the role of these fishways installed at both SHPs of the Sapucaí-Mirim River. The conceptual model suggests that adult fish is possibly migrating and spawning upstream in the system and eggs and larvae are drifting downstream and passing through the reservoirs and respective dams. This assumption suggests that spawning sites are located upstream and nursery areas may be downstream of the dams. Therefore, adult fish are likely to be moving upstream through the fish ladders and eggs and larvae are drifting downstream reaching the dams. For the Neotropical region fish ladders construction would mostly benefit fish in such scenario (Pompeu et al. 2012). However, the early life stages would need to survive passage through the dam and reach the nursery habitats downstream. The likelihood of survival will,

therefore, be highly dependent on the route of passage (e.g. turbines, spillways or fishways, if present), with high survival rates expected for eggs and larvae moving through a fishway.

Nevertheless, for the studied SHPs, once larvae disperse downstream through the reservoir and reach the dams, passage randomly occurs through the fish ladders. Larvae were detected in the fish ladders only when there was high abundance in the reservoir immediately upstream. If larvae are truly passive drifters and follow flow accordingly it would be expected that a small proportion may move through the fish ladder with higher passage occurring through the turbines and spillways. This is to be expected because the flow within the fish ladder is significantly lower than the spillways or turbines. Moreover, as fish ladders are not designed to allow downstream passage of fish (Pelicice et al. 2015; Celestino et al. 2019), the passage of larvae through the fish ladders at Retiro and Palmeiras Dams may indeed represent only a small proportion of passage for the entire community drifting downstream. However, since the fish ladder is likely to have more favorable hydraulic conditions for passage when compared to turbines or spillways, larvae moving through that route have a higher chance of survival. Indeed, Brown et al. (2014) have indicated the need to better understand the effects of hydraulic conditions on the survival of eggs and larvae passing through dams. In a recent study, Boys et al. (2016) showed the influence of pressure changes, likely to occur during passage through turbines and spillways, on the survival of eggs and larvae of three Australian species, indicating no influence on eggs and high survival rates of larvae exposed to pressure changes 40% or more of the pressure to which they will be acclimated prior to passage.

Considering that fish ladders are likely to result in higher downstream survival, but passively moving fish will follow flow, there needs to be an engineering solution to divert fish from the spillway/turbine to the fish ladder. Many fish protection and safe downstream passage techniques have been developed, such as screens or diversion pipes (Geiger et al. 2018). These technologies can be used either alone or in combination to provide safe egress downstream (Amaral et al. 2018). However, before suggesting such technologies for Retiro and Palmeiras Dams, there is a need to understand the actual proportion and the fate of eggs and larvae moving through each one of the available routes during downstream drift. This is an information of paramount importance for the management of fish populations in the studied area. Also, it is important to determine the likelihood of passage through each route given the location of each structure in relation to the flow approaching the dam.

For instance, the difference in the proportion of larval passage through the fish ladder of Palmeiras and Retiro Dams can, potentially, be explained by the location of the fish ladder (Figure 1) in the dam and in relation to the flow vectors upstream. Areas with higher current speeds may concentrate more eggs and larvae, mainly if they are considered as passive drifters (Pavlov 1994; Pavlov et al. 2008; Lechner et al. 2016). In Palmeiras Dam, where less passage events and lower percentage of passage through the fish ladder were recorded, the position of the fish ladder is in the left bank where low flow occurs, given the river bend immediately upstream of the dam. Consequently, the majority of the larvae are directed to the turbine or spillway. On the other hand, in Retiro Dam, the location of the fish ladder is in the right river bank with likely high flows because it is on

the same side of the river bend upstream of the dam. Therefore, the likelihood of having more larvae being directed to the fish ladder is higher than in Palmeiras.

In summary, reproductive activity of migratory species do occurs in the Sapucaí-Mirim River stretch directly influenced by the SHPs cascade. The early life stages can successful drift downstream the river and reach the dams. Once near the dam, a small proportion of the larvae can pass through the fish ladder, but only when there is a high abundance of larvae and, therefore, passage is likely to be occur randomly. Consequently, it is hypothesized that most larvae approaching the vicinity of the dam may be primarily passing via turbines and spillways, routes where high mortality rates are expected. Although the migratory species are spawning upstream, recruitment may be imperiled because eggs and larvae need to reach suitable habitats downstream of the dams but are moving primarily through the turbines and spillways. In this case, conservation of migratory species in the area may still be compromised, despite the potential of the fish ladders to move fish upstream to spawning sites. The results of this study also indicate, clearly, that fishway investigation needs a holistic approach to understand the contribution of fish passages to the conservation of migratory fish populations, going beyond the establishment of passage rates and focus on the fishway structure itself (Wilkes et al. 2019). It also shows that the decision-making process to determine the need of a fishway for tropical systems has to consider upstream and downstream movements of all life stages of fish, which may lead to the need to design and implement passage technologies that would allow movements through the dam in both directions.

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**Appendix I.** Occurrence of fish species of Anostomidae, Characidae, Prochilodontidae, Heptapteridae and Pimelodidae families in the Sapucaí-Mirim River, based on Freitas-Souza 2014; Oliveira et al. 2016; Brambilla et al. 2018 and information regarding migration and fecundation based on Agostinho et al. 2003; Suzuki et al. 2005; Langeani and Rêgo 2014.

Order	Family	Species	Migration	Fecundation
Characiformes	Anostomidae	<i>Leporinus amblyrhynchus</i> Garavello & Britski, 1987	Short distance	External
Characiformes	Anostomidae	<i>Leporinus friderici</i> (Bloch, 1794)	Short distance	External
Characiformes	Anostomidae	<i>Leporinus lacustris</i> Amaral Campos, 1945	Short distance	External
Characiformes	Anostomidae	<i>Leporinus octofasciatus</i> Steindachner, 1915	Short distance	External
Characiformes	Anostomidae	<i>Leporinus striatus</i> Kner, 1858	Short distance	External
Characiformes	Anostomidae	<i>Megaleporinus obtusidens</i> (Valenciennes, 1837)	Long distance	External
Characiformes	Anostomidae	<i>Megaleporinus piavussu</i> (Britski, Birindelli & Garavello, 2012)	Long distance	External
Characiformes	Anostomidae	<i>Schizodon nasutus</i> Kner, 1858	Short distance	External
Characiformes	Characidae	<i>Astyanax bockmanni</i> Vari & Castro, 2007	Short distance	External
Characiformes	Characidae	<i>Astyanax fasciatus</i> (Cuvier, 1819)	Short distance	External
Characiformes	Characidae	<i>Astyanax lacustris</i> (Lütken, 1875)	Short distance	External
Characiformes	Characidae	<i>Astyanax schubarti</i> Britski, 1964	Short distance	External
Characiformes	Characidae	<i>Bryconamericus iheringii</i> (Boulenger, 1887)	Short distance	External
		<i>Bryconamericus turiuba</i> Langeani, Lucena, Pedrini & Tarelho-Pereira, 2005	Short distance	External
Characiformes	Characidae	<i>Galeocharax gulo</i> (Cope, 1870)	Short distance	External
Characiformes	Characidae	<i>Hyphessobrycon eques</i> (Steindachner, 1882)	Short distance	External
Characiformes	Characidae	<i>Knodus moenkhausii</i> (Eigenmann & Kennedy, 1903)	Short distance	External
Characiformes	Characidae	<i>Oligosarcus pintoii</i> Amaral Campos, 1945	Short distance	External
Characiformes	Characidae	<i>Piabarchus stramineus</i> (Eigenmann, 1908)	Short distance	External
Characiformes	Characidae	<i>Piabina argentea</i> Reinhardt, 1867	Short distance	External
Characiformes	Characidae	<i>Planaltina britskii</i> Menezes, Weitzman & Burns, 2003	No information	
Characiformes	Prochilodontidae	<i>Prochilodus lineatus</i> (Valenciennes, 1836)	Long distance	External
Siluriformes	Heptapteridae	<i>Imparfinis mirini</i> Haseman, 1911	No information	

Siluriformes	Heptapteridae	<i>Imparfinis schubarti</i> (Gomes, 1956)	No information	
Siluriformes	Heptapteridae	<i>Pimelodella avanhandavae</i> Eigenmann, 1917	Short distance	External
Siluriformes	Heptapteridae	<i>Pimelodella gracilis</i> (Valenciennes, 1835)	Short distance	External
Siluriformes	Heptapteridae	<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	Short distance	External
Siluriformes	Pimelodidae	<i>Iheringichthys labrosus</i> (Lutken, 1874)	Short distance	External
Siluriformes	Pimelodidae	<i>Pimelodus maculatus</i> Lacépède, 1803	Long distance	External
Siluriformes	Pimelodidae	<i>Pimelodus microstoma</i> Steindachner, 1877	Short distance	External

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## *Capítulo 3*

# **Extreme climatic events affecting freshwater fish reproduction in the Neotropics**

## **Abstract**

Climate change, which result in more often extreme climatic events (unusual droughts and floods), is a growing threat to freshwater ecosystems and their species. Such events affect the river hydrologic conditions, disturbing the ichthyofauna structure and reproductive processes. A possibility to evaluate the impact of extreme climatic events on ichthyofauna is the assessment of reproductive events succeeded through the study of eggs and larvae distribution and abundance. This kind of evaluation contributes significantly to understand the ecology of fish populations, indicating partially spawning-stock biomass and recruitment in adult fish stocks. The aim of this work was to evaluate the effect of extreme climatic events on fish reproduction by comparison of the density of eggs and larvae in two consecutives, but contrasting, fish reproductive cycles, one atypically dry and other with intensive rains caused by an strong El Niño event. Samples of eggs, larvae and environmental variables were collected fortnightly between December and February of 2014-2015 (drought cycle) and 2015-2016 (wet cycle) in six sampling sites along the Sapucaí-Mirim River. The wet cycle presented higher density of eggs and larvae (around 10 times more) than the drought cycle ( $p < 0.05$ ) with a higher proportion of families with migratory species. Principal Component Analysis explained 74.2% of environmental data variability and all the environmental variables showed statistical differences between the two reproductive cycles, except for pH and oxide reduction potential. Extreme climatic events clearly affect the reproduction of freshwater fish, with a negative

effect during drought periods and the opposite for wet periods. A drought period can be a lost reproductive cycle, mainly to species that are more dependent of flooding. Nevertheless, this reproductive failure can be compensated by a prolonged period of intensive rain and flooding.

**Keywords:** drought, El Niño, flood, ichthyoplankton, Sapucaí-Mirim River

## **Introduction**

Climate change, which result in more often extreme climatic events (unusual droughts and floods), is a growing threat to natural systems and their species (Pecl et al. 2017; Ruaro et al. 2019). In particular, freshwater environments are drastically affected because changes in rainfall regimes alter flooding dynamics and hydrologic characteristics of freshwater habitats (van Vliet et al. 2011; Bertoncin et al. 2019). Hydrological cycle fluctuations have important effects on the ecological functioning of a river ecosystem as flow is a key factor which integrates complex environmental conditions directly related to ecological patterns and processes, including alterations in the physical habitat and trophic resource availability (Ríos-Pulgarín et al. 2016). In this way, extreme hydrological conditions are considered to be disturbances to the structure of aquatic communities (Campos et al. 2019), including ichthyofauna (Oberdorff et al. 2001).

The rainfall and the river hydrology influence fish reproduction (Gogola et al. 2010). The increase in fluviometric level is a trigger for spawning, signaling favorable conditions that maximize offspring survival. When river level rises, food availability and refuges increase and some limnological alterations occur (e.g. increase in turbidity) and consequently higher survival is expected for fish eggs and larvae. If there is no flood, some species do not spawn or, if spawn, there is

a low survival of eggs and larvae mainly because they are more susceptible to predation by fish and other aquatic organisms (Sanches et al. 2006).

The southeast region of Brazil, specially São Paulo State, suffered significant rainfall deficits during the summers of 2013-2014 and 2014-2015, resulting in drought conditions and a water crisis impacting several sectors of society (Coelho et al. 2016; Buckeridge and Ribeiro 2018). Summer of 2013-2014 was classified as exceptionally dry with overly early termination of the rainy season and was considered the driest summers since 1961-1962. In the summer of 2014-2015 similar deficits occurred (Coelho et al. 2016). In contrast, Brazilian summer of 2015-2016 was characterized by one of the strongest El Niño events of history, with high values of rainfall in São Paulo State (Pereira et al. 2017).

An alternative to evaluate the impact of extreme climatic events on ichthyofauna is the study of eggs and larvae distribution and abundance. This kind of study contributes significantly to understand the ecology of fish populations, indicating partially spawning-stock biomass and recruitment in adult fish stocks (Nakatani et al. 2001). Initial development stages of fish are vulnerable and are influenced by physical and biological processes such as hydrological conditions, transport processes, seasonal variability, spawning patterns of adults, food availability and specially climate effects (Primo et al. 2011).

The aim of this study was to evaluate the effect of extreme climatic events on fish reproduction by the comparison of eggs and larvae densities in two consecutive and contrasting reproductive cycles, an atypical drought and a wet cycle caused by an El Niño event. This study was designed to answer the following questions: (i) environmental variables are different between the two

contrasting reproductive cycles? (ii) are there differences in the density of eggs and larvae between the two reproductive cycles? (iii) if so, which environmental variables are related with these differences? The main hypothesis driving these questions is that there are higher densities of fish eggs and larvae in wet cycles than in drought cycles and these differences are related with environmental variables associated with rainfall.

## **Material and Methods**

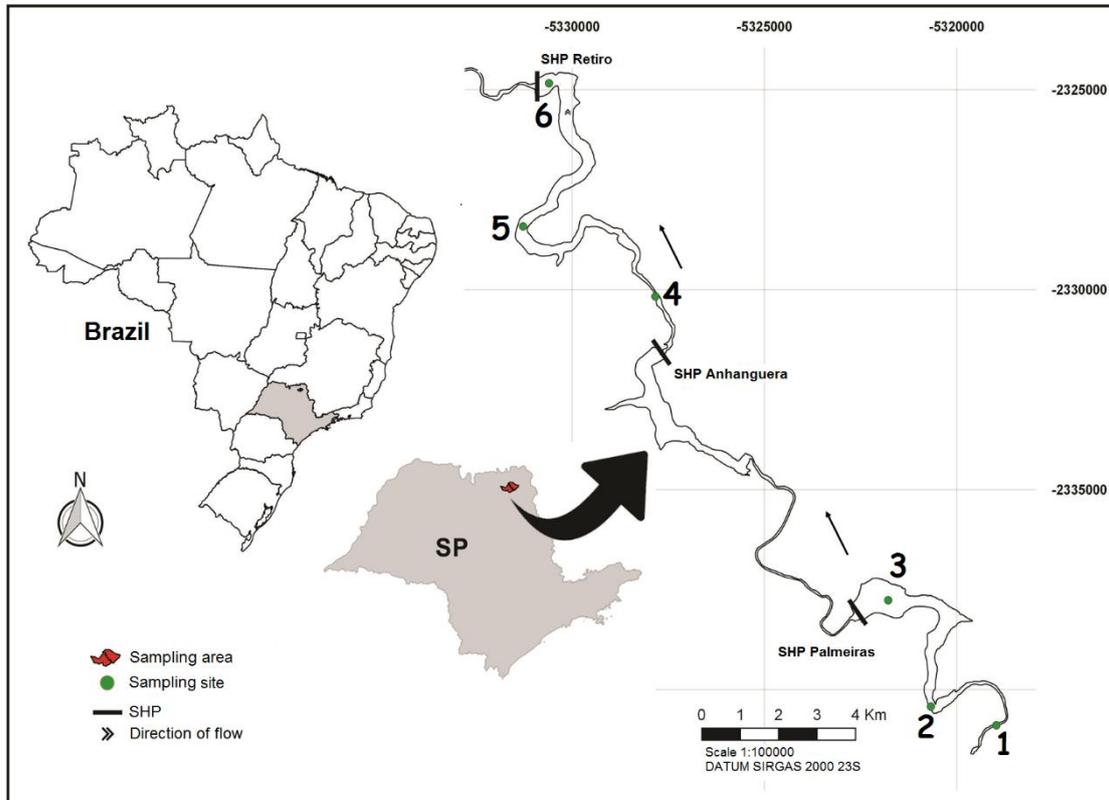
### ***Study area***

The study area is located in the Sapucaí-Mirim River (Southeast Brazil), one of the main tributaries of Grande River, Upper Paraná River Basin. The river source is in the Serra da Laje (Mantiqueira), runs 310 km entirely in the State of São Paulo, and its mouth is located in the left bank of Porto Colombia Reservoir, middle stretch of Grande River (Paiva 1982). There are three small hydropower plants (SHPs) in operation arranged in a cascade along the Sapucaí-Mirim River with run-of-river operational systems and about three days in average of reservoir water retention time. This cascade of dams does not affect the hydrological dynamics of this river.

### ***Sampling and processing***

Six sampling sites were distributed in the influence area of most upstream and most downstream dams, encompassing the lotic zone (upstream of the reservoir, sites 1 and 4), the transition zone (in between the reservoir and the lotic

area, sites 2 and 5) and the lentic zone (represented by the reservoirs, sites 3 and 6) (Figure 1).



**Figure 1.** Map of the study area showing the distribution of the sampling sites and arrows indicating direction of flow. Sites 1 and 4 representing the lotic zone; 2 and 5 transition zone; 3 and 6 lentic zone of the Sapucaí-Mirim River, Southeast, Brazil.

Eggs and larvae were collected in two consecutive and contrasting fish reproductive cycles: (1) a drought cycle (December 2014 to February 2015), period after two successive years of high deficits in spring/summer rainfalls (October to March of 2013-2014 and 2014-2015) and (2) a wet cycle (December 2015 to February 2016) period of rainfall higher than the historical average (1961-2010) (Coelho et al. 2016).

A total of 72 samples were collected, six for each site per cycle. The sampling period was defined because the spawning season for most Neotropical migratory fish commences in the end of spring and goes throughout summer (Vazzoler 1996; Reynalte-Tataje et al. 2011). Sampling was conducted at night (period of day with higher capture of eggs and larvae (Vianna and Nogueira 2008; Silva et al. 2017)), between 7 PM and 12 AM.

All samples were obtained with a conical-cylindrical plankton net, with mesh size of 500  $\mu\text{m}$  and an opening area of 0.11  $\text{m}^2$ . A mechanical flowmeter (General Oceanics™) was installed in the net opening to determine the volume of filtered water for each sample. The net was positioned in the water subsurface (approximately 20 cm depth) for 10 minutes. In the lentic/transition sites (sites 2, 3, 5 and 6) the net was dragged by a boat at low velocity whereas for the lotic sites (1 and 4) it remained stationary with the opening positioned against the direction of the flow.

The sampled material was transferred to polyethylene bottles and fixed with a 4% formalin solution. An overdose (more than 283.55 mg/L) of anesthetic (eugenol) (Vidal et al. 2008) was added to euthanize the organisms collected before fixing with formalin.

The samples were sorted, in the laboratory, under a stereomicroscope using a Bogorov-type plate and the eggs and larvae separated from the rest of the sampled material. After separation, the larvae were identified to the lowest taxonomic level according to Nakatani et al. (2001). The taxonomic framework was based on Fricke et al. (2019).

Environmental variables temperature, turbidity, dissolved oxygen (DO), electrical conductivity (K), oxide reduction potential (ORP), total dissolved solids (TDS) and pH were also sampled immediately before retrieving the nets. Samples were performed with a Horiba probe (U-52). Data of accumulated rainfall per month was obtained from National Institute of Meteorology (INMET) using the closest station to the study area (Franca municipality about 47 km). Daily total outflow data of each dam was obtained directly from Duke Energy Brazil, the generation company in charge of the hydroelectric plants in that occasion.

### ***Data analysis***

For environmental variables data, an ordination and identification of variability between the wet and drought reproductive cycles were performed using a Principal Components Analysis (PCA) with data transformed in  $\log x + 1$  (except pH) and normalized (Anderson et al. 2008).

The samples of each environmental variable were categorized in two groups: wet and drought reproductive cycles and data were transformed in  $\log x + 1$  (except pH) prior analyses. The data normality and data equal variance were checked using Kolmogorov-Smirnov test and Levene median test, respectively. To evaluate differences of each environmental variable between the two groups a t-test or a Mann-Whitney rank sum test was performed depending on the results of previously normality and equal variance tests.

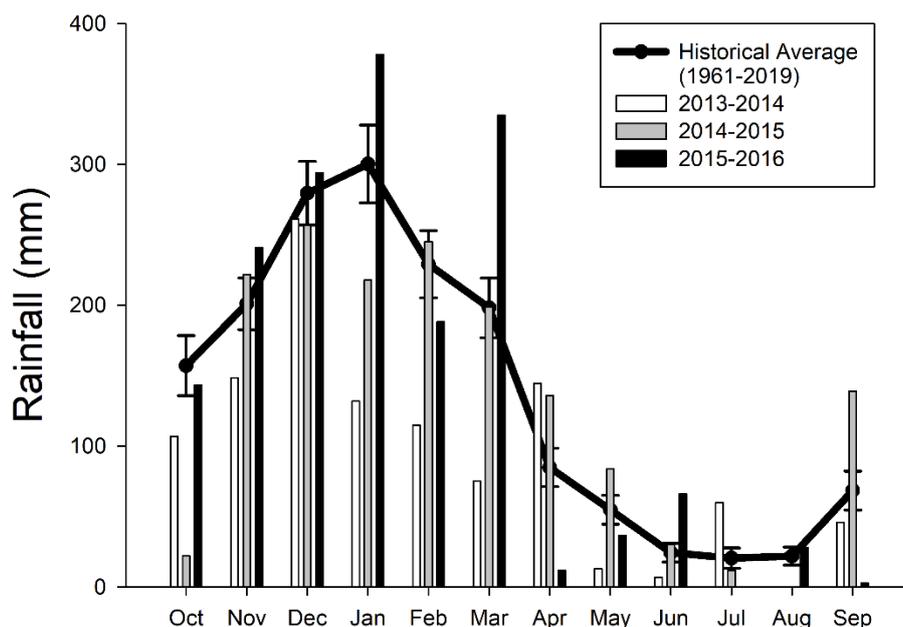
The abundance of sampled eggs and larvae was standardized as individuals per  $10 \text{ m}^{-3}$ , according to Tanaka (1973), modified by Nakatani et al. (2001). To evaluate differences of density of eggs and larvae between the two reproductive cycles a Mann-Whitney rank sum test was performed. Previously

Kolmogorov-Smirnov test and Levene median test were used to check for data normality and equal variance, respectively. The significance level considered in all analysis was 0.05.

A Spearman correlation was used to verify correlation between total density of eggs and larvae with environmental variables.

## **Results**

The rainfall values of late spring and summer (October, November, December, January, February and March) comparing with historical average (1961-2019) indicated for 2013-2014 a total deficit of 526.7 mm and for 2014-2015 a deficit of 202.8. On the other hand, for 2015-2016 there was a surplus of 214.3 (Figure 2). Considering the period of late spring and summer from 1961-1962 to 2018-2019 (58 periods), 2013-2014 was the driest and 2014-2015 the seventh driest periods. Deficits higher than 200 mm only occur seven times. The period of 2015-2016 was the eighth rainiest period. Surplus higher than 200 mm only occur 10 times (Table 1).



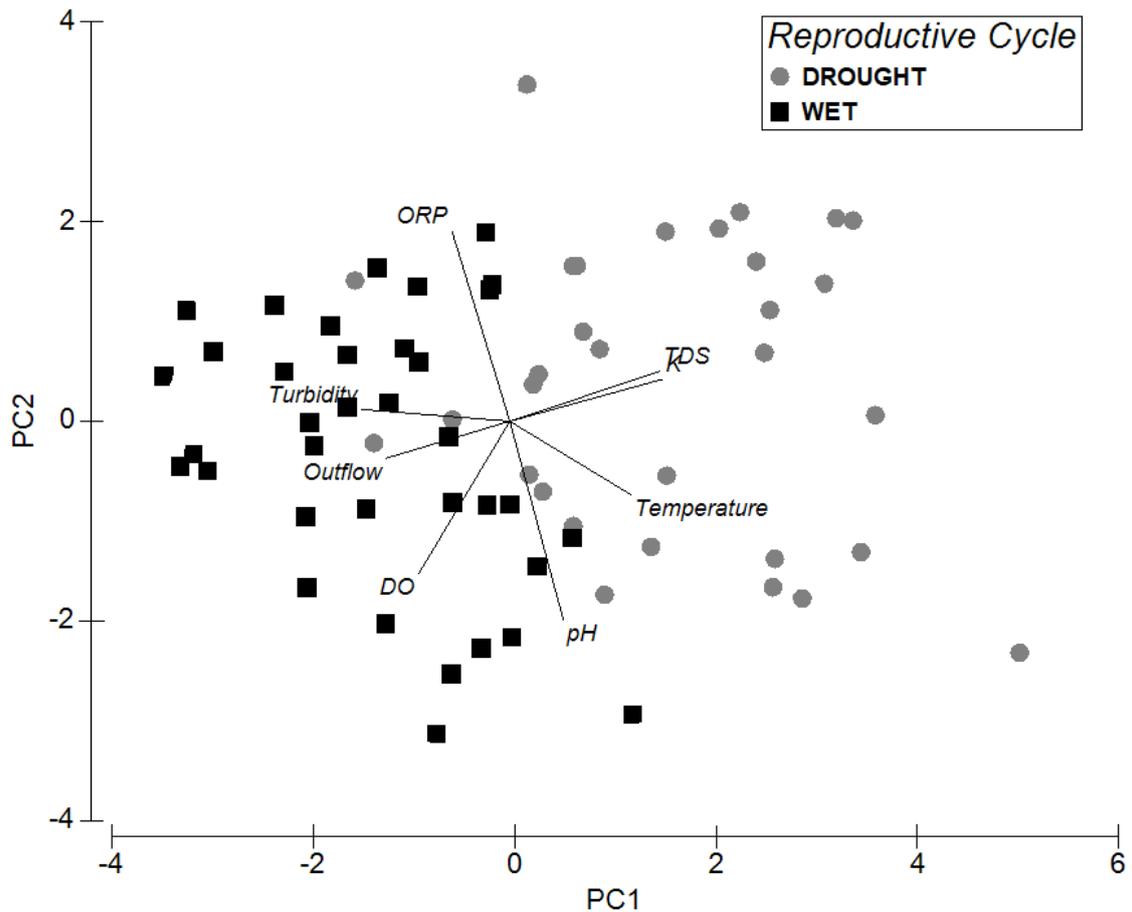
**Figure 2.** Accumulate rainfall values per month between October 2013 and September 2016 (bars) and historical average (1961-2019) (line) with 5% confidence interval. Data obtained from National Institute of Meteorology (INMET) using the closest station to the study area (Franca municipality about 47 km).

**Table 1.** Rainfall deficits and surplus higher than 200 mm compared with historical average of 1365,3 mm (1961-2019) considering late spring and summer (October, November, December, January, February and March) from 1961-1962 to 2018-2019. Data obtained from National Institute of Meteorology (INMET) using the closest station to the study area (Franca municipality about 47 km). Studied periods in bold.

PERIOD	RAINFALL (mm)	DEFICIT	SURPLUS	CLASSIFICATION
<b>2012-2013</b>	<b>838,6</b>	<b>-526,7</b>		<b>1°</b>
1970-1971	852,2	-513,1		2°
1963-1964	905,5	-459,8		3°
1965-1966	1016,8	-348,5		4°
1999-2000	1030,1	-335,2		5°
1985-1986	1137,5	-227,8		6°
<b>2013-2014</b>	<b>1162,5</b>	<b>-202,8</b>		<b>7°</b>
1980-1981	1975,6		610,3	1°
2005-2006	1955,7		590,4	2°
1981-1982	1885,9		520,6	3°
1966-1967	1845,5		480,2	4°
1964-1965	1700,1		334,8	5°
2008-2009	1675,2		309,9	6°

1983-1984	1586,8	221,5	7°
<b>2014-2015</b>	<b>1579,6</b>	<b>214,3</b>	<b>8°</b>
1998-1999	1571,8	206,5	9°
2004-2005	1570,1	204,8	10°

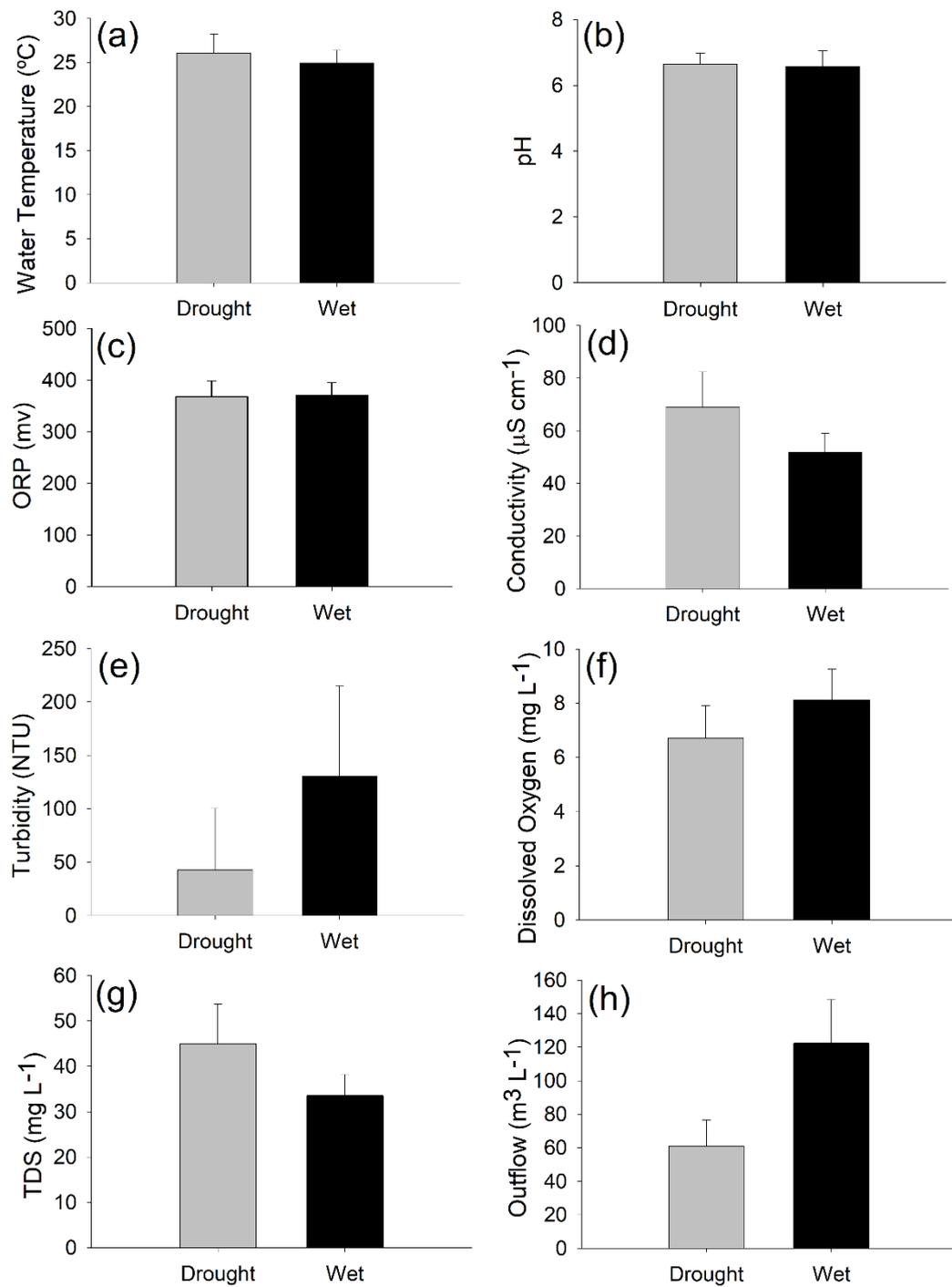
The PCA explained 74.2% of environmental data variability, 48.7% in the PC1 and 25.5% in the PC2. A clear separation between reproductive cycles were observed in the first axis. The drought cycle was located in the positive axis, more related with high values of K, TDS and temperature. On the negative axis it was located the wet cycle, more related with high values of turbidity and outflow (Figure 3; Table 2). All the environmental variables presented statistical differences between the two reproductive cycles ( $p < 0.05$ ), except for pH and ORP. Temperature, K, TDS were higher in the drought and turbidity, DO and outflow in the flood cycle (Figure 4).



**Figure 3.** Principal components analysis (PCA) showing the distribution of the sampling sites of the Sapucaí-Mirim River, Southeast, Brazil, for the reproductive cycles of 2014-2015 (drought) and 2015-2016 (flood), according to the environmental variables.

**Table 2.** Eigenvectors and eigenvalues of the first two axis for each environmental variable of Principal Component Analysis performed for the reproductive cycles of 2014-2015 (drought) and 2015-2016 (wet) in the Sapucaí-Mirim River, Southeast, Brazil.

Variable	PC1	PC2
Temperature	-0.363	-0.222
pH	-0.16	-0.599
ORP	0.173	0.574
Conductivity	-0.456	0.127
Turbidity	0.442	0.035
Dissolved Oxygen	0.272	-0.459
TDS	-0.447	0.15
Outflow	0.369	-0.112
Eigenvalues	3.90	2.04



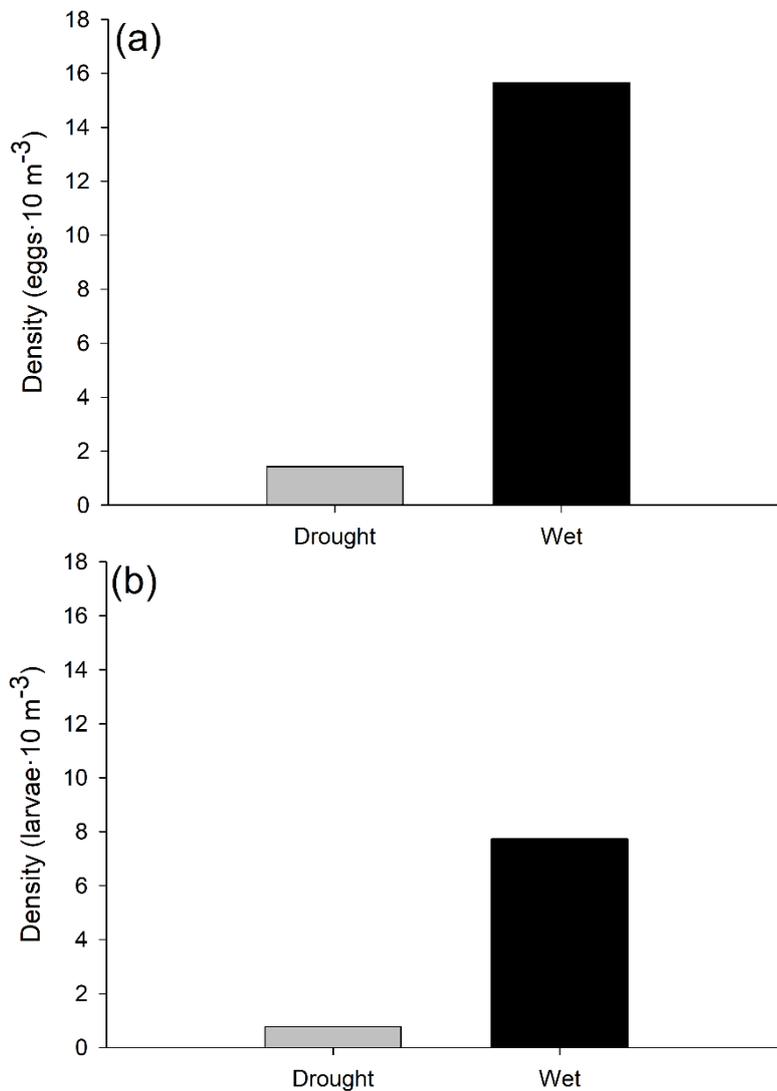
**Figure 4.** Mean and standard deviation for (a) water temperature, (b) pH, (c) ORP, (d) electrical conductivity, (e) turbidity, (f) dissolved oxygen, (g) TDS and (h) outflow for the reproductive cycles of 2014-2015 (drought) and 2015-2016 (wet) in the Sapucaí-Mirim River, Southeast, Brazil.

In the drought cycle it was sampled 277 eggs (1.43 individuals·10 m<sup>-3</sup> in average) and 78 larvae (0.78 individuals·10 m<sup>-3</sup> in average) of 10 taxa, belonging to two orders and nine families. In the wet cycle were sampled 1,250 eggs (15.65 individuals·10 m<sup>-3</sup> in average) and 523 larvae (7.72 individuals·10 m<sup>-3</sup> in average) of 14 taxa, belonging to three orders and 12 families (Table 3). The wet cycle presented higher density of eggs and larvae (around 10 times more) than the drought cycle (p<0.05) (Figure 5). The most abundant families of the drought cycle were Anostomidae (41.39%), followed by Pimelodidae (16.72%), Characidae (14.5%) and Parodontidae (12.64%). In the wet cycle the most abundant families were Anostomidae (58.69%), followed by Pimelodidae (20.71%), Characidae (8.88%) and Prochilodontidae (8.13%). The families Gymnotidae, Sternopygidae, Curimatidae and Prochilodontidae were sampled only in the flood cycle (Table 4).

**Table 3.** List of identified taxa sampled in reproductive cycles of 2014-2015 (drought) and 2015-2016 (wet) in the Sapucaí-Mirim River, Southeast, Brazil.

TAXA	CYCLE	
	DROUGHT	WET
<b>CHARACIFORMES</b>		
<b>Anostomidae (unidentified)</b>	X	X
<b>Characidae (unidentified)</b>	X	X
<i>Bryconamericus</i> spp.	X	X
<b>Curimatidae (unidentified)</b>		X
<b>Erythrinidae</b>		
<i>Hoplias</i> spp.	X	X
<b>Parodontidae (unidentified)</b>		X
<i>Apareiodon</i> spp.	X	
<b>Prochilodontidae</b>		
<i>Prochilodus lineatus</i> (Valenciennes, 1836)		X
<b>Serrasalminidae</b>		
<i>Serrasalmus maculatus</i>	X	X
<b>GYMNOTIFORMES</b>		

<b>Gymnotidae</b>		
<i>Gymnotus</i> spp.		X
<b>Sternopygidae</b>		
<i>Eigenmannia</i> spp.		X
<b>SILURIFORMES</b>		
<b>Auchenipteridae</b>		
<i>Tatia neivai</i> (Ihering, 1930)	X	
<b>Heptapteridae (unidentified)</b>	X	
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)		X
<b>Loricariidae (unidentified)</b>	X	X
<b>Pimelodidae (unidentified)</b>	X	X
<i>Pimelodus</i> spp.		X



**Figure 5.** Mean densities of (a) eggs and (b) larvae for the reproductive cycles of 2014-2015 (drought) and 2015-2016 (wet), in the Sapucaí-Mirim River, Southeast, Brazil.

**Table 4.** Average density (individuals·10 m<sup>-3</sup>) and % (per reproductive cycle) of larval families grouped by reproductive cycles, 2014-2015 (drought) and 2015-2016 (flood) in the Sapucaí-Mirim River, Southeast, Brazil. \* values < 0.01.

Order	Family	DROUGHT		WET	
		Density	%	Density	%
Characiformes	Anostomidae	0.27	41.39	4.26	58.69
	Characidae	0.09	14.50	0.65	8.88
	Curimatidae	-	0.00	*	0.05
	Erythrinidae	0.01	1.10	0.09	1.22
	Parodontidae	0.08	12.64	0.02	0.31
	Prochilodontidae	-	0.00	0.59	8.13
	Serrasalminidae	0.02	2.89	*	0.03
Gymnotiformes	Gymnotidae	-	0.00	0.03	0.39
	Sternopygidae	-	0.00	0.05	0.68
	Auchenipteridae	0.03	4.83	0.04	0.52
Siluriformes	Heptapteridae	0.01	2.03	0.02	0.32
	Loricariidae	0.03	3.89	*	0.08
	Pimelodidae	0.11	16.72	1.50	20.71

Density of eggs were positively correlated with turbidity, followed by outflow and negatively correlated with temperature, followed by TDS, K and pH. Density of larvae were positively correlated with turbidity, followed by outflow and ORP and negatively correlated with TDS, followed by K, pH and temperature (Table 5).

**Table 5.** Spearman correlations among densities of eggs and larvae and environmental variables for the reproductive cycles of 2014-2015 (drought) and 2015-2016 (wet), in the Sapucaí-Mirim River, Southeast, Brazil. In bold the correlations that are statistically significant (p<0.05).

	Eggs	Larvae
Temperature	<b>-0.414</b>	<b>-0.321</b>
pH	<b>-0.252</b>	<b>-0.351</b>
ORP	0.212	<b>0.360</b>
Conductivity	<b>-0.280</b>	<b>-0.437</b>
Turbidity	<b>0.493</b>	<b>0.484</b>
Dissolved Oxygen	0.183	0.086
TDS	<b>-0.285</b>	<b>-0.443</b>
Outflow	<b>0.412</b>	<b>0.449</b>

## **Discussion**

Environmental variables were different between the drought and the wet reproductive cycles. Also, the density of eggs and larvae were different between the two contrasting cycles with higher values in the wet cycle, which are related with some environmental variables that are influenced by rainfall, such as water temperature, K, TDS, turbidity, DO and outflow. Thus, extreme climatic events influence fish reproduction.

The clear difference in the environmental variables between the two reproductive cycles can be explained by the amount of rainfall. The drought cycle was affected by two successive years of a significant deficit of rainfall in spring/summer occasioning in a severe drought condition in the southeast region of Brazil. During these periods of low rainfall the water volume of river is lower and the dilution effect is decreased resulting in higher values of some variables like TDS and K (Brambilla et al. 2018). The water temperature is also higher in these periods because the inputs from rain are usually colder than river water temperature. On the other hand, the wet cycle was influenced by an El Niño event, markedly by intense rainfall in southeast Brazil. In periods of rain considerable exchanges of water, sediment and nutrients occur because the high loads of external material from the drainage basin, especially in small and medium size rivers, consequently the river water turbidity increases. More water into the system increases the river level and the total outflow (Junk et al. 1989).

Freshwater ecosystems, mainly rivers, are highly affected by flooding (Agostinho et al. 2016). Some environmental variables, such as turbidity, outflow

and temperature influence fish reproduction (Sanches et al. 2006; Gogola et al. 2010; Suzuki and Pompeu 2016). The beginning of rains is a trigger to migration and spawning and the water level changes signaling for favorable conditions to offspring survival and it is a signal for spawning (Bailly et al. 2008; Ruaro et al. 2019). The modifications caused by floods in rivers increase the survival of eggs and larvae because more refuge and food area are available and the predation pressure decrease, consequently higher offspring survival is expected (Röpke et al. 2017). In this study is clear how these changes affect the density of eggs and larvae, with around 10 times more eggs and larvae sampled in the wet cycle than in the drought cycle.

Droughts have negative effects on fish recruitment (Bailly et al. 2008; Oliveira et al. 2014). Bailly et al. (2008) reported an important role of floods in the recruitment of species in Cuiabá River. They observed a correlation between the abundance of young-of-the-year of species of all reproductive strategies, except the small migrators, with the attributes of floods (duration, time of year, delay and intensity of floods). Oliveira et al. (2014) studying the Paraná River and two tributaries of the right bank (Baía and Ivinhema Rivers) observed that several migratory species (*Brycon orbignyanus*, *Megaleporinus piavusu*, *Megaleporinus macrocephalus*, *Megaleporinus obtusidens* and *Prochilodus lineatus*) exhibited an exponential increase in young-of-the-year abundance associated with flood duration. In the study the hydrologic cycle of the 2009–2010 coincided with a strong El Niño phenomenon, representing a year of exceptional flooding in the Paraná River. This resulted in high recruitment of all the studied species; however, some species demonstrated a greater requirement for exceptional flooding events in order to significantly increase their recruitments. Conversely, it

would be expected that, when flooding is absent, short or delayed, spawning does not occur and the maturing gametes are reabsorbed (Bailly et al. 2008; Oliveira et al. 2014).

Some species that migrates long distances and have total spawn, like *P. lineatus*, only reproduce when the river conditions are adequate to a higher survival of offspring (Carolsfeld et al. 2003; Suzuki et al. 2005; Lopes et al. 2019). When the conditions are not adequate, they do not reproduce (Agostinho et al. 2016). Frequently cycles of low rainfall can promote a sharp decline of these populations, mainly in impacted ecosystems, for example dammed rivers. In this study *P. lineatus* was not sampled in the drought cycle, but it was in high density in the posterior year characterized by intensive rains. In addition, families with some long distance migratory species like Anostomidae and Pimelodidae were sampled in higher density and proportions in the cycle of floods compared to the drought cycle.

Extreme climatic events clearly affect the reproduction of freshwater fish, with a negative effect during drought periods and a contrasting positive effect on periods of floods. An intense drought period can result in a lost reproductive cycle for fish recruitment, mainly for species that are more dependent of rainfall. However, a prolonged period of intensive floods can compensate this lost cycle.

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## Considerações finais

Na região do rio Sapucaí-Mirim com a presença de uma cascata de três pequenas centrais hidrelétricas (PCHs) há a atividade reprodutiva de diferentes famílias de peixes, incluindo migradores reprodutivos de curta e longa distância. Através do estudo do ictioplâncton ao longo desse sistema, observamos áreas de desova, deriva de ovos e estágios iniciais de larva e condições para o desenvolvimento de estágios mais desenvolvidos.

Diversos fatores atuam na distribuição da assembleia de larvas e estágios de desenvolvimento iniciais, entre eles fatores temporais, incluindo variação entre períodos e ciclos reprodutivos e fatores espaciais, como a posição da barragem na cascata e os diferentes trechos de cada barragem. Algumas variáveis ambientais, principalmente relacionadas com a chuva, ajudam a explicar essas variações.

As PCHs e seus pequenos reservatórios não atuam como barreira para dispersão de ovos e larvas de peixes. Inclusive, as escadas para peixes atuam como possíveis caminhos para a passagem descendente de ovos e larvas. Essa condição só é possível devido ao pequeno tamanho do reservatório e baixo tempo de retenção. Em períodos de alta vazão do rio essa dispersão se torna mais eficiente.

Eventos climáticos extremos afetam a reprodução dos peixes nesse sistema. Alterações em variáveis ambientais que influenciam a sobrevivência de ovos e larvas dos peixes foram claramente identificadas. Conseqüentemente, isso se reflete na abundância de ovos e larvas, com valores mais elevados em

períodos de maior pluviosidade. Vale ressaltar que algumas espécies migradoras de longa distância só se reproduzem em períodos onde há condições ideais de sobrevivência, deixando de se reproduzir quando as chuvas são escassas.

Há um efeito do barramento de PCHs sobre a distribuição de ovos e larvas de peixes no rio Sapucaí-Mirim. Indicando atividade reprodutiva de diversas espécies, com áreas de desova, deriva e berçário. Estudos complementares, incluindo comparações com a fauna adulta e as condições de passagem de ovos e larvas através de turbinas e vertedouros, são necessários para avaliar se as populações de peixes, principalmente de espécies migradoras, conseguem se manter viáveis nesse sistema.