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Genetic evidence supports polygamous mating system in a wild population of *Prochilodus lineatus* (Characiformes: Prochilodontidae), a Neotropical shoal spawner fish

Correspondence:
Josiane Ribolli
josianeribolli@gmail.com

Josiane Ribolli[1], Carolina Isabel Miño[2], Bianca Maria Soares Scaranto[1], David Augusto Reynalte-Tataje[3] and Evoy Zaniboni Filho[1]

Behavioral observations made on fish have revealed remarkably diverse reproductive strategies, including polygamy by both sexes. Still, to date, most Neotropical species remain unstudied as to whether the observed reproductive behavior in natural populations correlates with their genetic mating systems. Here, we investigated the genetic mating system of a wild population of *Prochilodus lineatus* settled in the Middle Uruguay River basin. By using sibship reconstruction and parental inference methods based on microsatellites' genotypes, we inferred 45 females and 47 males as potential parents of the 87 larvae analyzed. We found evidence supporting polygamous mating in both sexes: while a high percentage of males (44.7%) fertilized the eggs of one female, 55.3% of the inferred males fertilized eggs of up to four females. Likewise, while 44.5% of the inferred females had their eggs fertilized by one only male, 55.5% of females were fertilized by multiple males. The estimated *proxy* of the effective population size (*Nb*) was 126, exhibiting moderate to high levels of genetic diversity. The genetic evidence contributed in this study complements earlier behavioral observations of formation of spawning *nuclei* of aggregating breeders, which may be promoting a polygamous mating strategy in this long-distance migratory fish.

Keywords: Genetic mating system, Microsatellites, Migratory fish, Parentage, Relatedness.

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1 Laboratório de Biologia e Cultivo de Peixes de Água Doce, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rodovia SC 406-Km3, 3532, Lagoa do Peri, 88066-260 Florianópolis, SC, Brazil. (JR) josianeribolli@gmail.com (corresponding author); (BMS) biascaranto@gmail.com; (EZF) evoy@lapad.ufsc.br.

2 Instituto de Biología Subtropical, IBS - Universidad Nacional de Misiones/CONICET, Félix de Azara 1552, N3300LQH Posadas, Misiones, Argentina. (CIM) carolinaianido@yahoo.com.ar.

3 Universidade Federal da Fronteira Sul, Av. Jacob Reinaldo Haupenthal, 1580 - Bairro São Pedro, 97900-000 Cerro Largo, RS, Brazil. (DART) david.tataje@uffs.edu.br.

Observações do comportamento de peixes neotropicais têm revelado estratégias reprodutivas marcadamente variáveis, incluindo poligamia nos dois sexos. Ainda assim, até então, a correlação entre comportamento reprodutivo observado em populações naturais e sistemas de acasalamento genético permanece pouco explorada para maioria de espécies Neotropicais. Neste estudo investigamos o sistema genético de acasalamento de *Prochilodus lineatus* em uma população natural estabelecida no Médio rio Uruguai. Utilizando métodos de reconstrução de grupos familiares e inferências parentais baseados em genótipos de microssatélites, inferimos 45 fêmeas e 47 machos como os possíveis parentais das 87 larvas amostradas. Encontramos evidência que permite apoiar a ocorrência de acasalamento poligâmico em ambos os sexos: enquanto uma percentagem alta de machos (44,7%) fertilizou somente uma fêmea, 55,3% dos machos inferidos fertilizaram mais de uma fêmea (até quatro por macho). Da mesma forma, enquanto que 44,5% das fêmeas inferidas tiveram seus ovos fertilizados por apenas um único macho, 55,5% das fêmeas tiveram ovos fertilizados por múltiplos machos. A estimativa do tamanho populacional efetivo (*Nb*) foi 126, exibindo níveis entre moderados e altos de diversidade genética. A evidência genética que apresentamos nesse estudo complementa observações iniciais da formação de núcleos de desova que podem promover estratégias de acasalamento poligâmico nessa espécie migratória de longa distância.

Palavras-Chave: Microssatélites, Parentesco, Paternidade, Peixes migradores, Sistema genético de acasalamento.

INTRODUCTION

The mating system of a given species (also known as breeding system; Reynolds, 1996) can be defined as a behavioral strategy for obtaining mates, and is influenced by many factors, such as life-history traits, ecology and behavior (Klug, 2011). This central biological trait of organisms can influence a number of other which, in turn, determine the effective population size, *Ne* (Nunney, 1993; Karageorge, Wilson, 2017), and, thus, shape evolutionary processes. From a conservation standpoint, the mating system can accurately predict the extinction probability (Legendre *et al*., 1999). Mating systems can govern the population growth rate during and after periods of intense exploitation, and could be used as surrogates to assess the extinction risks and to settle conservation strategies, particularly in species exploited as economic resources (Rowe, Hutchings, 2003).

Breeding systems of teleostean fishes vary widely and are extraordinarily diverse, from self-fertilization and social monogamy to promiscuous behavior and group spawning (DeWoody, Avise, 2001; Haddeland *et al*., 2015; Ribolli *et al*., 2016; Smith, Wootton, 2016). All possible social mating systems can be found among these fishes: monogamy by both sexes, polygyny (male's polygamy), polyandry (females' polygamy), polygynandry (polygamy of both sexes) and promiscuity (both sexes mate with multiple partners) (DeWoody, Avise, 2001; Avise *et al*., 2002; Haddeland *et al*., 2015; Smith, Wootton, 2016). Furthermore, studies have demonstrated that pheromones may regulate various aspects of the reproductive behavior of teleostean fishes, such as stimulation of mating ceremonies (Liley, Stacey, 1983), synchronization of reproduction (Liley, Stacey, 1983; Kobayashi *et al*., 2002), spawning and increase of shoals (Chung-Davidson *et al*., 2010). However, in fishes, as it occurs in other vertebrates, such as birds (Griffith *et al*., 2002), the observed reproductive behavior may not correspond to the genetic mating system: for example, both males and females may take part in extra-pair spawnings (Wootton, Smith, 2014).

The Curimbatá *Prochilodus lineatus* (Valenciennes, 1837) (=*Prochilodus scrofa* Steindachner, 1881) is commercially exploited, and represents one of the most important freshwater resources in South America (Espinach Ros, Fuentes, 2000; Avigliano *et al*., 2016). Distributed in the Plata basin (Paraná, Paraguay, Uruguay, De La Plata River, among others), it is exploited by fisheries in Argentina, Brazil, Bolivia, Paraguay and Uruguay. Species of the genus *Prochilodus* Agassiz, 1829 are potamodromous, exhibit high fertility and lack of parental care (Vazzoler, 1996; Winemiller, 2005). *Prochilodus lineatus* displays total spawning (Lowe-McConnell, 1999; Sato, Godinho, 1999): females spawn over a short time-frame and no further eggs are shed in that breeding season (Smith, Wootton, 2016). The Curimbatá also has external fertilization which takes place under continuous water flow (Lowe-McConnell, 1999; Sato, Godinho, 1999). During the reproductive period, *P. lineatus* forms large fish aggregations, which are usually found downstream of waterfalls or dams (Godoy, 1975). As other Neotropical migratory species, the Curimbatá performs upward reproductive migrations towards adequate spawning grounds; they release their eggs into running water, and eggs drift freely with the currents (Harvey, Carolsfeld, 2003). Initial reports of observations of numerous *P. lineatus* breeding events described large fish shoals, composed of hundreds of individuals, resembling a "mating dance", in which fish jump out of the water in an event that can last several hours (Godoy, 1975). Decades later, Godinho *et al.* (2017) described that males of a congeneric species *Prochilodus argenteus* Spix, Agassiz, 1829 perform characteristic spawning sounds, suggesting the occurrence of "lek choruses" (it is a call behavior performed by male, in chorus). However, despite the existence of some visual reports made on wild populations of this migratory fish species, details of its spawning and breeding system remain unclear. Therefore, investigating the genetic mating system of *P. lineatus* can provide useful information to support conservation and management of this species.

Describing the reproductive strategies of fishes in nature is often precluded by the complex biology of these organisms, which prevents direct behavioral observations of the individuals involved in reproductive encounters. In the wild, *P. lineatus* inhabits large and deep rivers, with high turbidity, and this precludes accurate observations of the spawning events and reproductive behavior of individuals (Godinho *et al*., 2017). In such situation, the mating system can be verified or inferred using DNA-based methods, mostly aimed at reconstructing kinship amongst individuals in an array, based on their *multilocus* genotypes (Jones, Ardren, 2003). When eggs are laid in masses so that the progeny may occur in family groups – as in some fishes – additional analytical power is gained by the kinship reconstruction analyses (Flanagan, Jones, 2019). This approach is therefore a keystone of molecular ecology, providing important insights into behavior, ecology and evolution (Flanagan, Jones, 2019), as well as surprising revelations about the behavior of species in their natural environments (Griffith *et al*., 2002), providing cues to broaden the knowledge on evolutionary aspects of such a fundamental biological trait. Indeed, during the last two decades, molecular markers have contributed to clarify the reproductive modes and unraveled the genetic mating systems of several fish species, such as, for example, some salmonids (MacCrimmon, Gots, 1979; Martinez *et al*., 2000; Serbezov *et al*., 2010; Weir *et al*., 2010; de Mestral *et al*., 2012), trout (Fraser *et al*., 2005; Serbezov *et al*., 2010; Kanno *et al*., 2011), and sturgeon (Duong *et al*., 2013; Jay *et al*., 2014). To the extent of our knowledge, the molecular-parentage approach has only been applied to investigate the mating strategies of *Salminus brasiliensis* (Cuvier, 1816) (Ribolli *et al*., 2016). Thus, in contrast to the many investigations focusing on reproductive strategies and mating behavior of such commercially important organisms, the knowledge on Neotropical migratory fishes is still incipient.

The genetic mating system of fishes showing external fertilization in the freshwater courses, as the Curimbatá, can be approached by sibship reconstruction based on genotypic information. Here, we assessed aspects of the breeding system of wild *P. lineatus* by means of molecular markers and kinship reconstruction of larvae sampled at the Uruguay River, South Brazil. Our aims were to: i) estimate the number of breeding males and females in the population; ii) assess the most prevalent genetic mating system in both sexes; and iii) estimate the reproductive contribution of males or females during spawning.

MATERIAL AND METHODS

Field sampling and laboratory procedures. We sampled *P. lineatus* eggs at a site located in the Middle Uruguay River basin (27°15'50.16"S; 54°03'16.97" W), located between Turvo State Park (Brazil) and Yabotí Biosphere Reserve (Argentina), which are both National protected areas. This area represents the largest dam-free stretch in the Middle Uruguay River (*ca*. over 800 km), and is of paramount importance for the reproduction of long-distance migratory fishes (Reynalte-Tataje *et al*., 2012; Ziober *et al*., 2015; Reynalte-Tataje *et al*., 2017; Ribolli *et al*., 2018). Sampling was conducted in March 2014 (summer season in the southern hemisphere), at night time (around 9:00 PM), using conical-cylindrical plankton nets (0.5 mm mesh and mouth area of 0.11 m^2) following methods described in Hermes-Silva *et al.* (2009). All eggs were sampled simultaneously and using the same plankton net, and were immediately incubated in plastic bags supplied with water flow and oxygen input. *Prochilodus lineatus* eggs were captured at the initial segmentation stage (about 8–10 hours after fertilization), thus confirming that all eggs derived from the same spawning event, and the larvae were cultivated in the laboratory until juvenile stage (3-month-old) according to the *live ichthyoplankton* methodology (Reynalte-Tataje, Zaniboni-Filho, 2008). A fin fragment of each 87 juveniles were cut using sterilized surgical scissors and preserved in ethanol 96% at -20 °C until processed. Voucher of *P. lineatus* from Uruguay River is deposited in the Museum of the State University of Londrina (MZUEL11729).

DNA extraction and marker amplification. Genomic DNA was isolated from fin samples following an universal salt extraction protocol (Aljanabi, Martinez, 1997).

Samples were amplified at 11 microsatellite *loci*, using fluorescently-labeled primers (see details in Additional File S1). Polymerase Chain Reactions were carried out in 10 µL mixes containing: 50 ng of template DNA, 15 mM of $MgCl_2$, 1U *Taq* DNA polymerase (Uniscience, Brasil), 1X PCR buffer, 2 mM of each *forward* and *reverse* primer, and 1.1 mM of dNTPs (Invitrogen, Brasil). Standard cycling parameters were: initial denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 30 sec, annealing temperature of each primer (File S1) for 45 s, extension at 72 °C for 30 sec, followed by a final elongation step at 72 °C for 20 min. Allele sizing was carried out by capillary electrophoresis using 1 µL of diluted PCR product in ultrapure water (2:15 µL), added to 0.25 µL of GS600 LIZ® and 8.75 µL of formamide HIDI™ and run in an ABI 3500XL Sequencer (Applied Biosystems). Genotyping of each sample was carried out using automatic bins in Gene Mapper v.3.2 software (Applied Biosystems) and eye-checked.

Statistical analyses. The genotypic dataset was analyzed for the presence of null alleles, allelic dropout and stutter peaks using MICRO-CHECKER v2.2.3 (van Oosterhout *et al*., 2004). Departures from Linkage Equilibrium or Hardy-Weinberg Equilibrium (*HWE*) were investigated using GENEPOP 1.2 (Raymond, Rousset, 1995) and GenAlEx v6.4 (Peakall, Smouse, 2006), respectively. Mean number of alleles per locus, and mean observed (*Ho*) and expected (*He*) heterozygosity values were calculated using GenAlEx. Allelic richness (Ar; Leberg, 2002), inbreeding coefficients (F_{IS}; Weir, Cockerham, 1984) and tests for heterozygotes' excess (*pL*) or deficit (*pS*) were calculated using FSTAT 2.9.3.2 (Goudet, 2001).

Given that our aim was to maximize our ability to correctly reconstruct sibship arrays, in order to accurately infer the genetic mating system of *P. lineatus*, we first conducted an analysis in KinInfor v.1 (Wang, 2006), to determine the effect of the number of loci used on the power of relationship analysis (*PWR*). For this, we simulated 1,000,000 genotypes of full-siblings as primary hypothesis and unrelated individuals as null hypothesis, used empirical allele frequencies, a genotypic error of 0.01 for each marker, and 0.05 alpha level.

We then used COLONY v2 (Jones, Wang, 2010) to infer potential parentage and full- and half-sibship arrays based on larvae genotypes, by running a full-likelihood method and assuming, separately: i) a polygamous mating system without inbreeding for both sexes, ii) a polygamous mating system for males and monogamy for females, iii) a polygamy for females and monogamy for males and iv) monogamy for both sexes. COLONY v2 allows for deviations from Hardy-Weinberg equilibrium. We run five replicate runs for each of the different mating system assumptions and only recorded the resulting full- and half-sibling assignments with a probability >0.80 which were consistently recovered in all runs (*i.e*., the five replicates of each of the four types of mating system). Finally, we used the Sibship Assignment method implemented in COLONY to estimate the effective number of breeders, *Nb* (and its corresponding 95% Confidence Interval) as *proxy* of the population effective size (*Ne*), assuming an unknown number of parents, very high likelihood precision algorithm and polygamy in both-sexes.

RESULTS

Population genetic parameters, larvae sibship arrays and inference on parentage patterns. The 11 microsatellites were polymorphic (total of 178 alleles, 4–33 allele/*locus*) (Tab. 1). Loci Pli34 and Plin139 deviated significantly from Linkage Equilibrium (*P* = 0.00), therefore, locus Pli34 was excluded from sibship reconstruction analyses and estimates of *Nb*, which were then performed with a set of 10 unlinked microsatellites. Six *loci* deviated significantly from *HWE* (Tab. 1). The mean *He* ranged from 0.13 to 0.95 and *Ho* ranged from 0.13 to 0.93 (Tab. 1). The *multilocus PWR* to discriminate between full-siblings and unrelated individuals was 0.99. COLONY analyses recovered the number of sires producing each full-sib array with a high probability (minimum = 0.95, the value obtained for the two broods sired by four males, see below). Likewise, the number of mothers producing each progeny array was recovered with a high probability (minimum = 0.95).

Our analyses inferred 47 males as sires of the larvae, 21 of which (44.7%) sired one larva, while 14 (29.8%) sired two larvae, 10 males (21.3%) sired three and two of males (4.2%) sired four larvae (Fig. 1A). Forty-five females were inferred as dams, 19 of which (42.2%) mothered only one larva, while 14 (31.1%) produced two, nine (20.0%) sired three larvae, two females (4.4%) produced four larvae, and one (2.3%) mothered five larvae (Fig. 1A). While 21 males fertilized the eggs from only one female, 16 males (34.0%) fertilized eggs of two females each, nine males (19.2%) fertilized eggs of three females each, and a single male (2.1%) fertilized eggs from up to four females (Fig. 1B). Likewise, while females (44.5% of the inferred females) had their eggs fertilized by a single male, 13 females (28.8%) had their eggs fertilized by two males, 10 females (22.2%) by three, and two (4.5%) by four males (Fig. 1B). From these figures, the number of males siring a brood varied from one to four and the population frequency of multiple paternity was 55.5% (sum of the sibship arrays sired by two or more males).

TABLE 1 | Summary population genetic diversity for eleven microsatellite loci genotyped in *Prochilodus lineatus* larvae collected in the Middle Uruguay River basin, Southern Brazil. Number of individuals genotyped (*N*), Number of alleles (*A*), observed heterozygosity (*Ho*), expected (*He*) heterozygosity, inbreeding coefficient (F_{IS}) and probabilities of tests for deviation from *Hardy-Weinberg* equilibrium (*PHWE*) are shown (the asterisks denote significant values, $P < 0.05$).

Primer	*N*	*A*	*Ho*	*He*	F_{IS}	*PHWE*
Plin14	79	16	0.76	0.81	0.16*	0.00*
Plin25	85	8	0.93	0.58	0.03	0.03
Pli34	71	12	0.78	0.80	0.02	0.25
Plin64	84	19	0.88	0.91	0.02	0.55
Plin119	87	33	0.67	0.95	0.34*	0.00*
Plin139	87	28	0.62	0.94	0.07	0.03
Plin190	44	16	0.82	0.84	0.23*	0.00*
Par12	86	23	0.70	0.92	0.16	0.00*
Par66	86	10	0.35	0.41	0.58*	0.00*
Par83	83	9	0.18	0.42	-0.04	1.00
Pcos03	83	4	0.13	0.13	-0.59*	0.00*

Under polygamy by both sexes, and assuming random mating, the *Nb* estimated in the studied population was 126 fishes (95% CI: 94–171).

DISCUSSION

We conducted kinship reconstruction in *Prochilodus lineatus* based on microsatellites' genotypes of larvae, and found genetic evidence suggesting that polygamy is the most prevalent mating system in both sexes, in agreement with previous field observations of shoals during *P. lineatus* spawning events in nature (Godoy, 1975). Kinship analyses showed that 45.98% of the 87 analyzed larvae were not attributed to any sibship array, *i.e.* they were unrelated to each other. This result was further supported by the fact that a high percentage of males (44.7%) and females (42.2%) contributed with only one descendant each. These results seem to suggest that the analyzed breeding population of *P. lineatus* exhibits a percentage of monogamous mating.

It is worth noting that 47 larvae were produced by two or more females and two or more males (57.8% and 55.3%, respectively) with multiple breeders participating in the same reproductive event. Theoretically, reproductive aggregations in species that release pelagic eggs imply little opportunity to choose mates (Stockley *et al*., 1997). Despite the scarce information about spawning of Neotropical fishes in nature, parentage analyses conducted on *P. lineatus* in semi-natural conditions (reproduction in the laboratory) identified unequal contribution of parents to the progeny, suggesting reproductive advantage (Souza *et al*., 2018). Additionally, some gametes (or breeders) may have

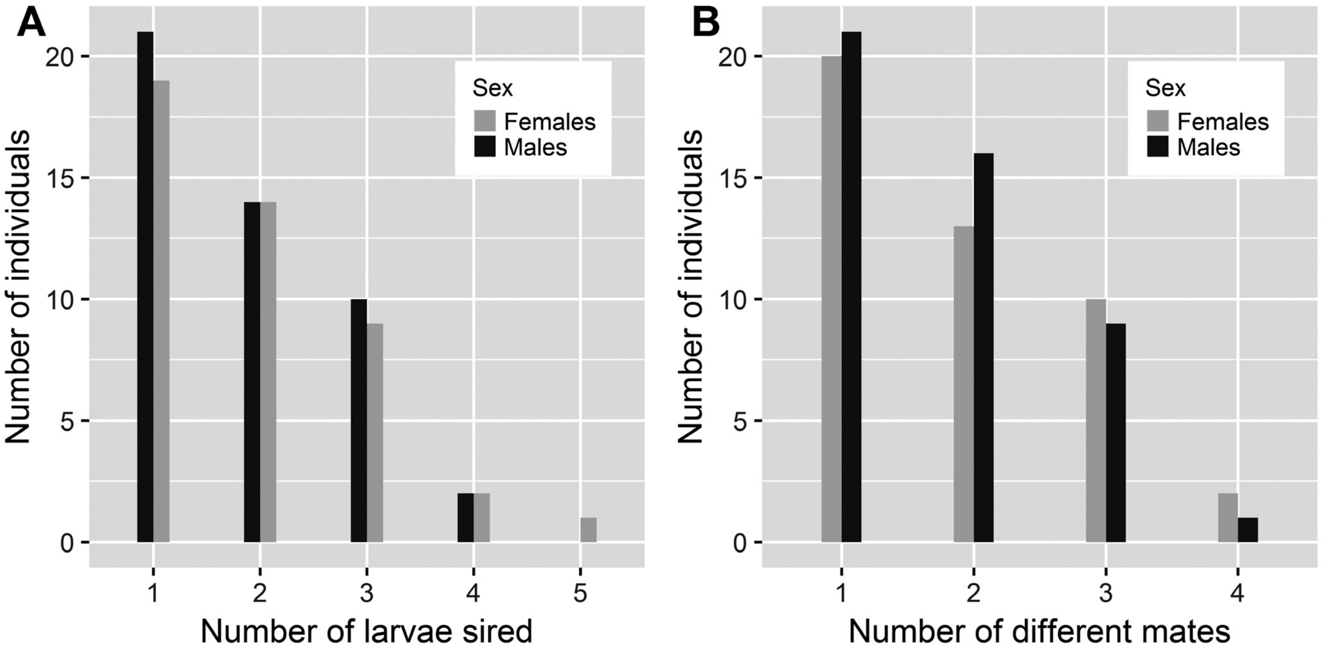

FIGURE 1 | Inferred parentage and mating patterns derived from analysis of 87 *Prochilodus lineatus* larvae sampled at the Middle Uruguay River in Brazil. **A.** Number of adults identified as parents of the sampled larvae; **B.** Number of different mates inferred for males and females.

dominance during fertilization, as observed in pooled-milt fertilizations of *Rhamdia quelen* (Quoy, Gaimard, 1824) in laboratory conditions (Ribolli, Zaniboni-Filho, 2009). It is important to emphasize here that the observed pattern of skewed parental contribution, instead of suggesting male or female dominance, could be reflecting the sampling strategy. For example: considering that each *P. lineatus* female can produce on average 160,000 oocytes per kg of weight (Vazzoler, 1996), the 45 potential females inferred as participating in the spawning event would have produced at least 7,200,000 eggs, of which we randomly sampled 87. Therefore, the single-larvae arrays can be explained as the result of the mixture of eggs from distinct spawning *nuclei* (*i.e.*, sired by different parents) carried out by the river flow.

Another avenue of evidence which could support our findings comes from observations of a peculiar reproductive display in some *Prochilodus* species: in the spawning arenas, some males made 'calls' to attract partners (Godinho *et al*., 2017), similar to the spawning description by Godoy (1975). According to the last author, during the reproduction of *P. lineatus*, a group with thousands of individuals was observed, where males called females by characteristic sounds ("males concentrate in the center of the river and females remain on the margins, waiting for the moment to attack the males"; details in Fig. 2A; Godoy, 1975). Decades after Godoy's (1975) report, Godinho *et al.* (2017) reported a similar reproductive behavior for *P. argenteus* suggesting a "*lek* mating system", stimulated by male calls. On the other hand, direct observation of natural spawning of *P. lineatus* indicated that fishes aggregate in several *nuclei* composed by many shoals of fishes moving the water simultaneously. Likewise, in laboratory conditions, males touch female genitalia and compress the body of females, thus stimulating the release of oocytes (Evoy Zaniboni-Filho, 2019, pers. comm.). In these cases, several males simultaneously stimulated a mature female, which, when exhausted, would move away, allowing males to start the same process with another mature female, and over again (for details see Fig. 2B). The sexual pheromones released by mature females would stimulate reproductive synchrony (Stacey, 2003), and could promote the reproductive aggregation described above. Fonseca *et al*. (2010) also report that artificially induced females of *Prochilodus* sp. do not spawn alone; they naturally spawn when stored in tanks together with males. The sexual pheromones released by mature females in this case would be responsible for attracting males and synchronize the reproduction, as described above. In teleost fish, the pre-spawning release of sex pheromones causes a rapid increase of sexual activity and attraction and excitation of partners to mate (Hoar *et al*., 1983; Chung-Davidson *et al*., 2010). Females' pheromones have been found to modulate males' reproductive behavior in several fish species, including *Salmo trutta* Linnaeus, 1758 (Laberge, Hara, 2003), *Carassius auratus* (Linnaeus, 1758) (Kobayashi *et al*., 2002) and *Misgurnus anguillicaudatus* (Cantor, 1842) (Ogata *et al*., 1994), but have not yet been reported in *P. lineatus*. The descriptions and hypotheses aforementioned of the spawning behavior of *P. lineatus* (Evoy Zaniboni-Filho, 2019, pers. comm.) could explain the polygamous mating by males and females inferred in our study, and complement our understanding of the reproductive behavior of *P. lineatus* in nature.

Our genetic data inferring many males and females as sires of the collected eggs support previous evidence from experimental conditions and field observations in suggesting that reproduction of *P. lineatus* occur in different spawning *nuclei*. Although not investigated in the present study, the formation of such *nuclei* may result of orchestrated calls made by mature males or by attraction and stimulation mediated by sexual pheromones, or both factors acting complementarily. Yet, hormone-behavior studies were not carried out in *P. lineatus* to date, so that this hypothesis remains to be thoroughly investigated.

The studied larvae showed moderate to high levels of neutral genetic diversity, which could be indicative of a stable and large population, as observed in other Neotropical migratory fishes, such as, for example, *Prochilodus costatus* Valenciennes, 1850 (Carvalho-Costa *et al.*, 2008; Braga-Silva, Galetti Jr., 2016) and *P. lineatus* (Rueda *et al.*, 2013). It is worth mentioning that the studied larvae were sampled at an area with low disturbance lying between two areas of high conservation concern, officially protected by Argentina and Brazilian laws. In such preserved habitats, it is possible that *P. lineatus*

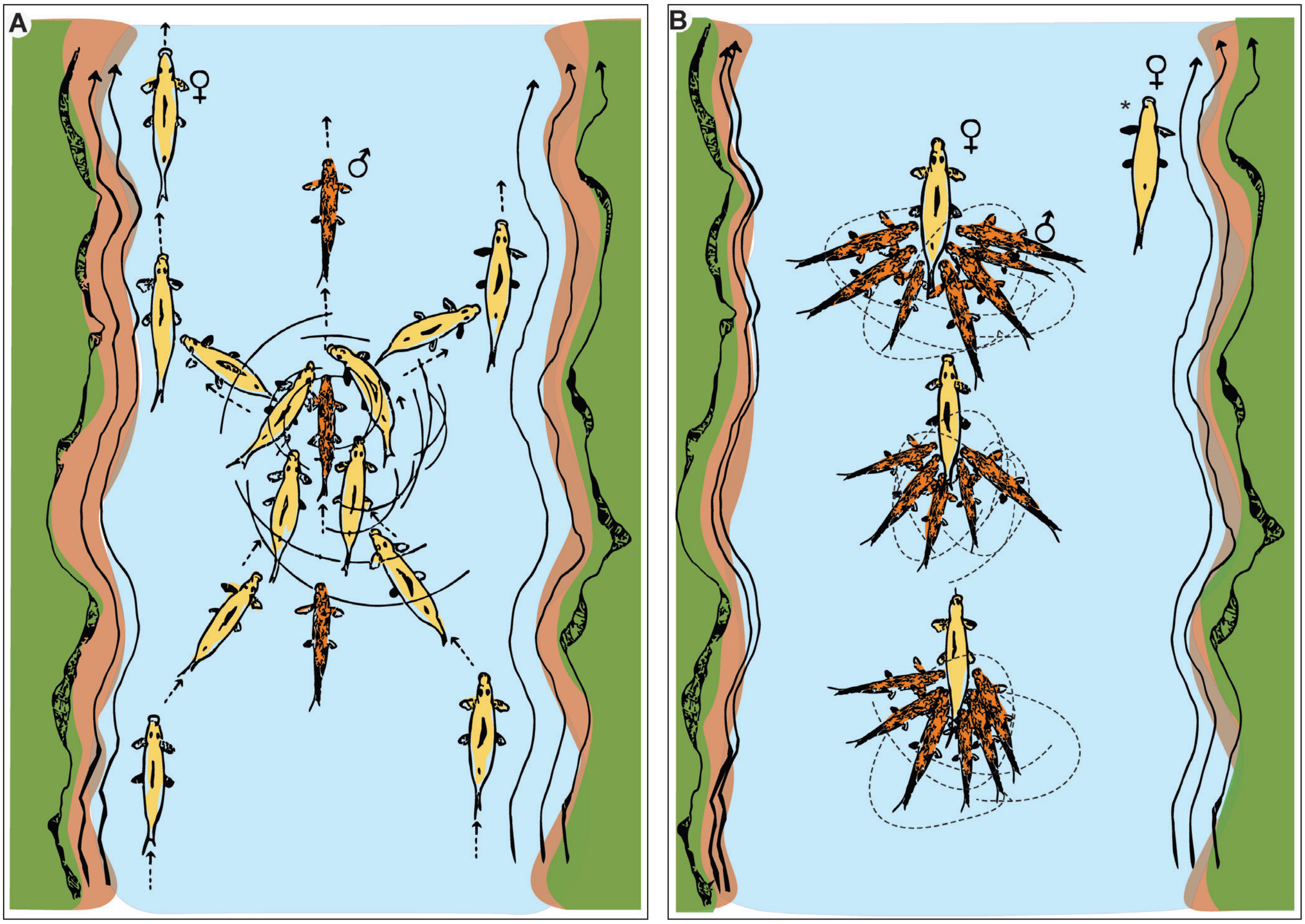

FIGURE 2 | Alternative schematic representations of spawning in *Prochilodus lineatus*. **A.** Schematic drawing of spawning moment of *Prochilodus lineatus* (=*Prochilodus scrofa*), according to Godoy (1975), whom described that males, positioned in the center of the river channel, performed distinctive sounds, possibly attracting females (figure drawn by Josiane Ribolli and Dennis Fernando Moreno, inspired from the original by Godoy, M. P, 1975, fig. 153, p.684); **B.** Hypothetical schematic representation of reproduction and spawning of *P. lineatus* in the wild according to direct observations made by Evoy Zaniboni-Filho (personal communication). The exhausted mature female is marked with an asterisk. Figure drawn by Josiane Ribolli and Dennis Fernando Moreno.

populations remain large and stable, thus maintaining moderate-high levels of genetic diversity. Retention of genetic diversity is important for the conservation of *P. lineatus* which is vulnerable to habitat fragmentation, degradation, pollution, overexploitation, and cascade effects imposed on population dynamics by reduced dispersal, restricted by dams (Smolders *et al*., 2002; Agostinho *et al*., 2004; Pesoa, Schulz, 2010; Rueda *et al*., 2013; Costa *et al*., 2018). The analyzed population could be thus seen as a source of genetic diversity which can enable adaptation to new environmental pressures.

The set of polymorphic microsatellite markers was powerful enough to perform kinship reconstruction of family groups, and the significant deviations from Hardy-Weinberg equilibrium possibly reflect a non-closed population with migration. The *Ne* estimated for the studied population was higher than the number of potential inferred parents; such an increased *Ne* may be reflecting a balanced individual reproductive success during spawning, a low variation in family size, or the reproductive strategy of polygamy by both sexes. In addition, the dispersion of eggs by current-drifting allows the admixture of progeny from several families and different spawning *nuclei*, and might concurrently be contributing to the high genetic diversity of the examined sample, maximizing its effective size.

In sum, this study investigated, for the first time, the genetic mating system in of a wild population of *P. lineatus*, a spawner migratory fish which aggregates in shoals during reproduction. We contribute novel evidence suggesting that this species shows a monogamous genetic mating system in combination with polygamy by males and females, supporting earlier behavioral observations. We suggest that spawning happens with the formation of several *nuclei*, in which the eggs released by a female could be fertilized by several males, which in turn can accompany the spawning of other females and also take part in other fertilizations. The result of this reproductive behavior associated with the environmental characteristics of the site used for spawning (large river flow, which guarantees the dispersal and mixture of the eggs) has allowed the maintenance of a similar sexual proportion of the parents. This study expands the knowledge about the mating strategies and reproductive behavior of Neotropical migratory fishes in nature through the use of molecular approaches. Our results can also support the management and conservation of such an important fishery resource.

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REFERENCES

- **Agostinho AA, Gomes LC, Veríssimo S, Okada EK**. Flood regime, dam regulation and fish in the Upper Paraná River: effects on assemblage attributes, reproduction and recruitment. Rev Fish Biol Fish. 2004; 14:11–19. https://dx.doi.org/10.1007/s11160-004-3551-y
- **Aljanabi SM, Martinez I**. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 1997; 25(22):4692–93. https://dx.doi.org/10.1093/nar/25.22.4692
- **Avigliano E, Fortunato RC, Biole F, Domanico A, Simone S, Neiff JJ, Volpedo AV**. Identification of nurseries areas of juvenile *Prochilodus lineatus* (Valenciennes, 1836) (Characiformes: Prochilodontidae) by scale and otolith morphometry and microchemistry. Neotrop Ichthyol. 2016; 14(3):e160005. http://dx.doi.org/10.1590/1982-0224-20160005
- **Avise JC, Jones AG, Walker D, DeWoody JA**. Genetic mating systems and reproductive natural histories of fishes: lessons for ecology and evolution. Annu Rev Genet. 2002; 36(1):19–45. https://doi.org/10.1146/annurev.genet.36.030602.090831
- **Braga-Silva A, Galetti Júnior PM**. Evidence of isolation by time in freshwater migratory fish *Prochilodus costatus* (Characiformes, Prochilodontidae). Hydrobiologia. 2016; 765:159–67. https://dx.doi.org/10.1007/s10750-015-2409-8
- **Carvalho-Costa LF, Hatanaka T, Galetti Júnior PM**. Evidence of lack of population substructuring in the Brazilian freshwater fish *Prochilodus costatus*. Genet Mol Biol. 2008; 31(S1):377–80. https://doi.org/10.1590/S1415-47572008000200036
- **Chung-Davidson YW, Huertas M, Li W**. A review of research in fish pheromones. Chemical communication in crustaceans. In: Breithaupt T, Thiel M, editors. Chemical Communication in Crustaceans. New York: Springer; 2010. p.467–82.
- **Costa RS, Okada EK, Agostinho AA, Gomes LC**. Variação temporal no rendimento e composição específica da pesca artesanal do alto rio Paraná, PR-Brasil: os efeitos crônicos dos barramentos. Bol Inst Pesca. 2018; 38(3):199–213.
- **de Mestral LG, Herbinger CM, O'Reilly PT**. Mating structure of an endangered population of wild Atlantic salmon (*Salmo salar*) as determined using sibship reconstruction and a novel method of sex inference. Can J Fish Aquat Sci. 2012; 69(8):1352–61. https://dx.doi.org/10.1139/f2012-065
- **DeWoody JA, Avise JC**. Genetic perspectives on the natural history of fish mating systems. J Hered. 2001; 92(2):167–72. https://dx.doi.org/10.1093/jhered/92.2.167
- **Duong TY, Scribner KT, Forsythe PS, Crossman JA, Baker EA**. Interannual variation in effective number of breeders and estimation of effective population size in long-lived iteroparous lake sturgeon (*Acipenser fulvescens*). Mol Ecol. 2013; 22(5):1282–94. https://dx.doi.org/10.1111/mec.12167
- **Espinach Ros A, Fuentes C**. Recursos pesqueros y pesquerías de la cuenca del plata. In: Bezzi S, Akselman R, Boschi E, editors. Síntesis del estado de las pesquerías marítimas Argentinas y de la cuenca del Plata. Mar del Plata: INIDEP; 2000. p.1–31.
- **Flanagan SP, Jones AG**. The future of parentage analysis: from microsatellites to SNPs and beyond. Mol Ecol. 2019; 28(3):544–67. https://dx.doi.org/10.1111/mec.14988
- **Fonseca FAL, Ituassu DR, Cavero BAS, Bordinhon AM**. Cultivo de curimatã (*Prochilodus* spp.). In: Baldisserotto B, Gomes LC, organizers. Espécies nativas para piscicultura no Brasil. Santa Maria: Editora da Universidade Federal de Santa Maria; 2010. p.57–83.
- **Fraser DJ, Duchesne P, Bernatchez L**. Migratory charr schools exhibit population and kin associations beyond juvenile stages. Mol Ecol. 2005; 14(10):3133–46. https://dx.doi.org/10.1111/j.1365-294X.2005.02657.x
- **Godinho AL, Silva CCF, Kynard B**. Spawning calls by zulega, *Prochilodus argenteus*, a Brazilian riverine fish. Environ Biol Fishes. 2017; 100:519–33. https://doi.org/10.1007/s10641-017-0582-5

- **Godoy MP**. Peixes do Brasil, subordem Characoidei: bacia do rio Mogí Guassú. Piracicaba: Editora Franciscana; 1975.
- **Goudet J**. FSTAT: a computer program to calculate F-statistics. J Hered. 2001; 86(6):485–86. Release 2.9. 3.2. https://dx.doi.org/10.1093/oxfordjournals.jhered.a111627
- **Griffith SC, Owens IP, Thuman KA**. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol. 2002; 11(11):2195–12. https://dx.doi.org/10.1046/j.1365-294x.2002.01613.x
- **Haddeland PJ, Junge C, Serbezov D, Vøllestad LA**. Genetic parentage analysis confirms a polygynandrous breeding system in the European grayling (*Thymallus thymallus*). PloS One. 2015; 10(3):e0122032. https://dx.doi.org/10.1371/journal.pone.0122032
- **Harvey B, Carolsfeld J**. Introduction: fishes of the floods. In: Carolsfeld J, Harvey B, Ross C, Baer A, editors. Migratory fishes of South America: biology, fisheries and conservation states. Victoria; Washington (DC); Ottawa: International Development Research Centre; World Bank; World Fisheries Trust; 2003. p.1–18.
- **Hermes-Silva S, Reynalte-Tataje D, Zaniboni-Filho E**. Spatial and temporal distribution of ichthyoplankton in the upper Uruguay river, Brazil. Braz Arch Biol Technol. 2009; 52(4):933–44. http://dx.doi.org/10.1590/S1516-89132009000400017
- **Hoar WS, Randall DJ, Donaldson EM**. Fish Physiology Reproduction: behavior and fertility control. Academic Press; 1983.
- **Jay K, Crossman JA, Scribner KT**. Estimates of effective number of breeding adults and reproductive success for white sturgeon. Trans Am Fish Soc. 2014; 143(5):1204–16. https://dx.doi.org/10.1080/00028487.2014.931301
- **Jones AG, Ardren WR**. Methods of parentage analysis in natural populations. Mol Ecol. 2003; 12(10):2511–23. https://doi.org/10.1046/j.1365-294X.2003.01928.x
- **Jones OR, Wang J**. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour. 2010; 10(3):551–55. https://dx.doi.org/10.1111/j.1755-0998.2009.02787.x
- **Kanno Y, Vokoun CJ, Letcher BH**. Sibship reconstruction for inferring mating systems, dispersal and effective population size in headwater brook trout (*Salvelinus fontinalis*) populations. Conserv Genet. 2011; 12:619–28. https://dx.doi.org/10.1007/s10592-010-0166-9
- **Karageorge KW, Wilson, RR, Jr**. An integrative mating system assessment of a nonmodel, economically important Pacific rockfish (*Sebastes melanops*) reveals nonterritorial polygamy and conservation implications for a large species flock. Ecol Evol. 2017; 7(24):11277–91. https://doi.org/10.1002/ece3.3579
- **Klug H**. Animal mating systems. In: eLS. Chichester: John Wiley & Sons, Ltd:; 2011. https://doi.org/10.1002/9780470015902.a0022553
- **Kobayashi M, Sorensen PW, Stacey NE**. Hormonal and pheromonal control of spawning behavior in the goldfish. Fish Physiol Biochem. 2002; 26:71–84. https://dx.doi.org/10.1023/A:1023375931734
- **Laberge F, Hara TJ**. Behavioural and electrophysiological responses to F-prostaglandins, putative spawning pheromones, in three salmonid fishes. J Fish Biol. 2003; 62(1):206–21. https://dx.doi.org/10.1046/j.1095-8649.2003.00020.x
- **Leberg PL**. Estimating allelic richness: effects of sample size and bottlenecks. Mol Evol. 2002; 11(11):2445–49. https://dx.doi.org/10.1046/j.1365-294x.2002.01612.x
- **Legendre S, Clobert J, Møller AP, Sorci G**. Demographic stochasticity and social mating system in the process of extinction of small populations: the case of passerines introduced to New Zealand. Am Nat. 1999; 153(5):449–63. https://dx.doi.org/10.1086/303195
- **Liley NR, Stacey EN**. Hormones, pheromones, and reproductive behavior in fish. In: Hoar WS, Randall DJ, Donaldson EM, editors. Fish Physiology. Academic Press; 1983. p.1–63.
- **Lowe-McConnell RH**. Estudos ecológicos de comunidades de peixes tropicais. São Paulo: Edusp; 1999.
- **MacCrimmon HR, Gots BL**. World distribution of Atlantic salmon, *Salmo solar*. J Fish Res Board Can. 1979; 36(4):422–57. https://dx.doi.org/10.1139/f79-062

- **Martinez JL, Moran P, Perez, De Gaudemar B, Beall E, Garcia–Vazquez E**. Multiple paternity increases effective size of southern Atlantic salmon populations. Mol Ecol. 2000; 9(3):293–98. https://doi.org/10.1046/j.1365-294x.2000.00857.x
- **Nunney L**. The influence of mating system and overlapping generations on effective population size. Evolution. 1993; 47(5):1329–41. https://doi.org/10.1111/j.1558-5646.1993.tb02158.x
- **Ogata H, Kitamura S, Takashima F**. Release of 13, 14-dihydro-15-ketoprostaglandin F2a, a sex pheromone, to water by cobitid loach following ovulatory stimulation. Fish Sci. 1994; 60(2):143–48.
- **Peakall ROD, Smouse PE**. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 2006; 6(1):288–95. https://doi.org/10.1111/j.1471-8286.2005.01155.x
- **Pesoa NA, Schulz UH**. Diel and seasonal movements of grumatã *Prochilodus lineatus* (Valenciennes 1836) (Characiformes: Prochilodontidae) in the Sinos River, southern Brazil. Braz J Biol. 2010; 70(4):1169–77. http://dx.doi.org/10.1590/S1519-69842010000600006
- **Raymond M, Rousset F**. An exact test for population differentiation. Evolution. 1995; 49(6):1280–83. http://dx.doi.org/10.2307/2410454
- **Reynalte-Tataje DA, Barcellos RP, Hartmann PB, Scherer JB, Martine G, DeVlieger IT, Zaniboni-Filho E, Hermes-Silva S, Pelicice FM**. O médio rio Uruguai como importante área de reprodução do surubim-pintado *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae). Boletim Sociedade Brasileira de Ictiologia. 2017; (122):10–15.
- **Reynalte-Tataje DA, Zaniboni-Filho E**. Biologia e identificação de ovos e larvas de peixes do alto rio Uruguai, Brasil. In: Zaniboni-Filho E, Nuñer APO, editors. Reservatório de Itá: estudos ambientais, desenvolvimento de tecnologias de cultivo e conservação da ictiofauna. Florianópolis: Editora UFSC; 2008. p.157–76.
- **Reynalte-Tataje DA, Zaniboni-Filho E, Bialetzki A, Agostinho AA**. Temporal variability of fish larvae assemblages: influence of natural and anthropogenic disturbances. Neotrop Ichthyol. 2012; 10(4):837–46. http://dx.doi.org/10.1590/S1679-62252012000400017
- **Reynolds JD**. Animal breeding systems. Trends Ecol Evol. 1996; 11(2):68–72. https://dx.doi.org/10.1016/0169-5347(96)81045-7
- **Ribolli J, Miño CI, Zaniboni-Filho E, Souza Guerreiro TCS, Reynalte-Tataje DA, Freitas PD, Galetti Junior PM**. Preliminary insights into the genetic mating system of Neotropical *Salminus brasiliensis*: kinship assignment and parental reconstruction reveal polygynandry. Ichthyol Res. 2016; 63:187–91. https://dx.doi.org/10.1007/s10228-015-0487-2
- **Ribolli J, Zaniboni-Filho E**. Individual contributions to pooled-milt fertilizations of silver catfish *Rhamdia quelen*. Neotrop Ichthyol. 2009; 7(4):629–34. https://doi.org/10.1590/S1679-62252009000400011
- **Ribolli J, Zaniboni-Filho E, Freitas PD, Galetti Junior PM**. Genetic evidences of non-reproductive shoaling in the freshwater fish *Salminus brasiliensis*. Hydrobiologia. 2018; 815:65–72. https://dx.doi.org/10.1007/s10750-018-3550-y
- **Rowe S, Hutchings JA**. Mating systems and the conservation of commercially exploited marine fish. Trends Ecol Evol. 2003; 18(11):567–72. https://dx.doi.org/10.1016/j.tree.2003.09.004
- **Rueda EC, Carriquiriborde P, Monzón AM, Somoza GM, Ortí G**. Seasonal variation in genetic population structure of sábalo (*Prochilodus lineatus*) in the Lower Uruguay River. Genetica. 2013; 141:401–07. https://doi.org/10.1007/s10709-013-9739-0
- **Sato Y, Godinho HP**. Peixes da bacia do rio São Francisco. In: Lowe-McConnel RH, editor. Estudos ecológicos de comunidades de peixes tropicais. São Paulo: Edusp; 1999. p.401–13.
- **Serbezov D, Bernatchez L, Olsen EM, Vøllestad LA**. Mating patterns and determinants of individual reproductive success in brown trout (*Salmo trutta*) revealed by parentage analysis of an entire stream living population. Mol Ecol. 2010; 19(15):3193–205. https://doi.org/10.1111/j.1365-294X.2010.04744.x

- **Smith C, Wootton RJ**. The remarkable reproductive diversity of teleost fishes. Fish Fish. 2016; 17(4):1208–15. https://dx.doi.org/10.1111/faf.12116
- **Smolders AJP, Guerrero Hiza MA, van der Velde G, Roelofs JGM**. Dynamics of discharge, sediment transport, heavy metal pollution and sábalo (*Prochilodus lineatus*) catches in the Lower Pilcomayo River (Bolivia). River Res Appl. 2002; 18(5):415–27. https://dx.doi.org/10.1002/rra.690
- **Souza FPD, Castro PLD, Goes ESDR, Ribeiro RP, Santos SCAD, Lima ECSD, Murari PJF, Lopera-Barrero NM**. Genetic variability of *Prochilodus lineatus* in artificial and semi-natural reproduction. Ital J Anim Sci. 2018; 17(2):321–25. https://doi.org/10.1080/1828051X.2017.1365312
- **Stacey N, Chojnacki A, Narayanan A, Cole T, Murphy C**. Hormonally derived sex pheromones in fish: exogenous cues and signals from gonad to brain. Can J Physiol Pharmacol. 2003; 81(4):329–41. https://dx.doi.org/10.1139/y03-024
- **Stockley P, Gage MJG, Parker GA, Møller AP**. Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. Am Nat. 1997; 149(5):933–54. https://dx.doi.org/10.1086/286031
- **Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P**. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004; 4(3):535–38. https://doi.org/10.1111/j.1471-8286.2004.00684.x
- **Vazzoler AEAM**. Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: Eduem; 1996.
- **Wang J**. Informativeness of genetic markers for pairwise relationship and relatedness inference. Theor Popul Biol. 2006; 70(3):300–21. https://doi.org/10.1016/j.tpb.2005.11.003
- **Weir BS, Cockerham CC**. Estimating F-statistics for the analysis of population structure. Evolution, 1984; 38(6):1358–70. https://dx.doi.org/10.2307/2408641
- **Weir LK, Breau C, Hutchings JA, Cunjak RA**. Multiple paternity and variance in male fertilization success within Atlantic salmon *Salmo salar* redds in a naturally spawning population. J Fish Biol. 2010; 77(3):479–93. https://doi.org/10.1111/j.1095-8649.2010.02690.x
- **Winemiller KO**. Life history strategies, population regulation, and implications for fisheries management. Can J Fish Aquat Sci. 2005; 62(4):872–85. https://dx.doi.org/10.1139/f05-040
- **Wootton RJ, Smith CR**. Reproductive biology of teleost fishes. Oxford: Wiley-Blackwell; 2014.
- **Ziober SR, Reynalte-Tataje DA, Zaniboni-Filho E**. The importance of a conservation unit in a subtropical basin for fish spawning and growth. Environ Biol Fish. 2015; 98:725–37. https://doi.org/10.1007/s10641-014-0307-y

AUTHOR'S CONTRIBUTION

Josiane Ribolli: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing-original draft, Writing-review & editing.

Carolina Isabel Miño: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing-original draft, Writing-review & editing.

Bianca Maria Soares Scaranto: Methodology, Writing-review & editing.

David Augusto Reynalte-Tataje: Data curation, Methodology.

Evoy Zaniboni Filho: Conceptualization, Funding acquisition, Project administration, Writing-original draft, Writing-review & editing.

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The authors declare no competing interests.

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