



ECOSYSTEMS

Reproduction of saguiru *Steindachnerina elegans* (Steindachner, 1874) (Pisces: Curimatidae) in the São Francisco River, downstream from a large reservoir in south-eastern Brazil

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Abstract: The gametogenesis and reproduction of *S. elegans* from the São Francisco River at Três Marias, Minas Gerais, were analyzed in this study. *Steindachnerina elegans* is a species abundant in the São Francisco River basin and an important fish in the food chain. The size at first gonadal maturation (indicated by the total length of the shortest spermatid male and spawned female) was 10.5 cm and 15.0 cm, respectively. Reproduction occurred more frequently from September to April. This period had high water temperature values. A high frequency of females was observed at the mature and spawned stages and height gonadosomatic index (GSI). Meanwhile, in males, the reproductive peak was from November to February. The long spawning period and the histological characteristics of the spawned ovaries that contained oocytes at different stages of development along with post-ovulatory and atretic oocytes indicates that the spawning of *S. elegans* is of the partial type.

Key words: ovary, testes, spawner, gonadal maturation, fecundity, follicles.

INTRODUCTION

The saguiru *S. elegans* is abundant in the Pardo and Jequitinhonha Rivers in Bahia and Minas Gerais, as São Francisco River basin and has a wide geographic distribution (Fowler 1951). They habit in several Brazilian hydrographic basins and belong to the Curimatidae family of the Characiforms order (Buckup et al. 2007). Fish from the Curimatidae family inhabit the bottom of rivers or lentic environments. They feed mainly on debris and decomposing organic matter, being considered important forage species (Sato et al. 1997). The *S. insculpta*, small detritivorous fish species in Furnas reservoir, upper rio Paraná basin, Minas Gerais, Brazil, has a prolonged period of reproductive activity

that extends from September to March, with partial spawning (Ribeiro et al. 2007). The *S. elegans* does not exhibit migratory reproductive behavior and has a large number of oocytes per gram of ovaries with the oocytes showing little adhesiveness (Sato et al. 1997).

Studies on fish breeding provide essential information for the conservation of biodiversity (Rizzo & Bazzoli 2020). The present study analyzes the main parameters of the reproductive biology of the species from the São Francisco River downstream of the Três Marias dam in Minas Gerais, Brazil. There are no known studies on the reproduction of *S. elegans* in lotic environments.

MATERIALS AND METHODS

Samples of *S. elegans* were previously collected between January and December 2012 from the São Francisco River (SFR) in an area that was 34 to 54 km downstream of the Três Marias dam, after the confluence with the Abaeté River (18°00'49" S, 45°10'51" W), Minas Gerais, Brazil (Figure 1). The fish were captured using gill nets, with mesh sizes of 3.0 cm between opposing nodes. The fish, when alive, were sacrificed through the cross-section of their cervical spine, following the ethical principles of animal manipulation established by the Brazilian College of Animal Experimentation (COBEA). This study was submitted to and approved by the Ethics Committee on Animal Use (Protocol n°. 071/2008 - UFMG). The sex proportion was determined as

the ratio of the absolute frequency of females to that of males.

For histological analyses, all fragments of ovaries and testes were previously fixed in Bouin's fluid for 12 hours, embedded in paraffin, cut to a 5 µm thickness, and, finally, stained with hematoxylin-eosin (HE). The gonadal maturation stages were established based on the microscopic and macro (such as size, staining, and vascularization of the ovaries and testes) (Weber et al. 2013, Brandão et al. 2017, Bazzoli et al. 2019). The gonadosomatic index (GSI = $GW \times 100 / BW$) and the Fulton (K) factor ($K = BW \times 100 / TL^3$) per bimester were calculated from the biometric data obtained. The size at the first gonadal maturation was indicated by the total length of the shortest spermatid male and spawned female (Boncompagni-Júnior et al. 2013). The diameter of 50 follicles of each maturation stage and 50 nuclei from cells spermatogenic lineages were measured using the Olympus Cell software from images captured by an Olympus SC30 camera that was connected to an Olympus-BX50 microscope (Santos et al. 2019). The physiochemical variables of the water were temperature, pH, electrical conductivity, dissolved oxygen, and transparency. Each of which was obtained downstream from the Abaeté River, using the Horiba device model U-10.

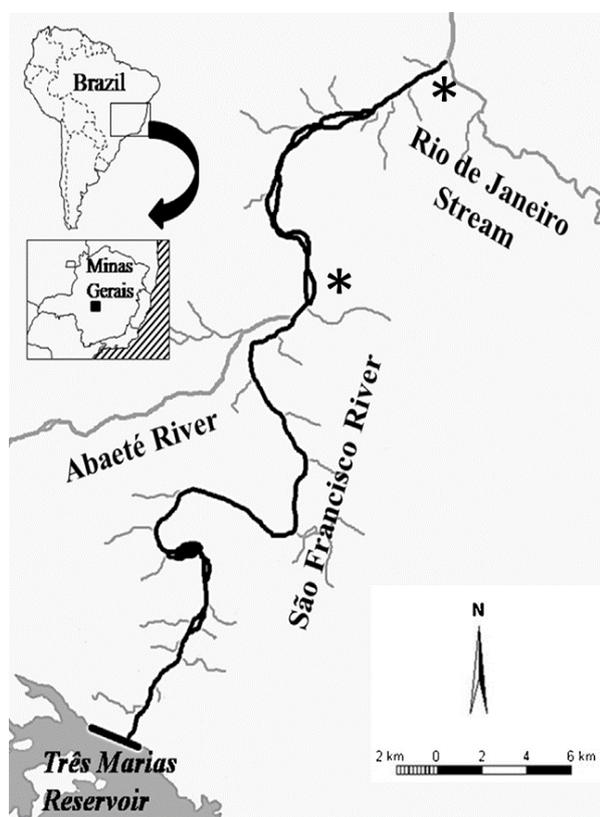


Figure 1. Study area: São Francisco River (SFR) 34 (*) to 54 km (*) downstream of the Três Marias dam after the confluence of the SFR with the Abaeté River.

Statistical analysis

The average values of the biological indices and water parameters for each bimester were assessed by using the Statistica 7.0 software and analysis of variance. After a normal distribution test, the data were compared using a one-way ANOVA analysis of variance followed by a Duncan test. A t-test was used to compare the total length (TL), body weight (BW), gonadosomatic index (GSI), and Fulton condition factor (K) of the males and females. The level of significance

was $p < 0.05$. The chi-square test (χ^2 ; $p < 0.05$) was applied to detect possible differences in the proportions between the sexes.

RESULTS

The water parameters for the São Francisco River (SFR) downstream of the Três Marias dam showed in November, December, January, and February high values of temperature and dissolved oxygen and low values of transparency (Table I).

The ovaries and testes of the *S. elegans* were paired, and elongated organs located in the coelomic cavity were attached to the gaseous bladder by a peritoneal fold. Variations in the volume, vascularization, and color of the gonads were observed in all stages of the gonadal maturation.

A total of 224 specimens were collected: 71 males and 153 females. The mean values of the total length, K ($p > 0.05$), body weight and GSI ($p < 0.05$) of the females were higher than that of the males (Table II). The female reproductive peak occurred from September to April, while, for males, it was from November to February, when the GSI and K had the highest values (Table

III). There were more females than males in the study area (2:1), with differences in proportions between the sexes ($\chi^2=30.02$).

Histologically, folliculogenesis was classified as the initial perinucleolar, advanced perinucleolar, previtellogenic, and vitellogenic oocytes, and the stages were characterized by changes in the ooplasm, follicular cells, and zona radiata (Figure 2). In spermatogenesis, cells of the spermatogenic lineage were also observed. The cells were classified as primary spermatogonia, secondary spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, and spermatozoa (Figure 3). The following stages of gonadal maturation were determined for females based on the macro and microscopic characteristics of the gonads. (Figure 2): F1 = rest, F2 = maturation/mature, and F3 = spawned and for males (Figure 3): M1 = rest, M2 = maturation/mature, and M3 = spent.

The smallest male captured in the spent stage had a total length of 10.5 cm, while the shortest female in the spawning stage measured 15.0 cm in total length. In females, the period with reproductive activity (F2 and F3 stages) occurred from September to April (Figure 4a, Table IV). Whereas, in males, it was from November to

Table I. The bimester values of Temperature (T), dissolved oxygen (DO), pH, electrical conductivity (CE) and transparency (T) between January and December 2012 in the São Francisco River, after the confluence with the Abaeté River, Minas Brazil.

Water parameters	Sample per bimester					
	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec
T (°C)	26.40 ± 0.21 ^C	23.51 ± 0.60 ^B	22.71 ± 0.21 ^A	22.76 ± 0.11 ^A	24.09 ± 0.67 ^B	25.85±0.50 ^C
DO (mg/L ¹)	7.67 ± 0.13 ^{AB}	7.01 ± 0.60 ^B	7.20 ± 0.50 ^{AB}	7.93 ± 0.35 ^A	6.24 ± 0.42 ^B	7.25 ± 0.45 ^{AB}
pH	6.16 ± 0.10 ^{AB}	6.08 ± 0.40 ^B	6.10 ± 0.30 ^B	6.51 ± 0.19 ^A	6.07 ± 0.37 ^B	6.13 ± 0.19 ^{AB}
EC(μS/cm)	75.17 ± 15.82 ^A	73.30 ± 5.90 ^A	72.80 ± 9.0 ^A	78.00 ± 3.46 ^A	77.00 ± 3.20 ^A	72.11 ± 9.71 ^A
T (m)	1.58 ± 0.13 ^B	2.80 ± 0.91 ^A	2.86 ± 0.72 ^A	3.36 ± 0.18 ^A	3.35 ± 0.30 ^A	1.98 ± 0.68 ^B

Values represent mean ± standard deviation of 5–8 measurements. Different letters indicate significant differences between the sampling periods, with $p < 0.05$.

Table II. The amplitude, mean, and standard deviation (SD) of the total length (TL) in centimeters, body weight (BW) in grams, gonadosomatic index (GSI), and Fulton condition factor (K) of *S. elegans* females and males captured in the São Francisco River between January and December 2012.

	Females (n = 153)		Males (n = 71)	
	Mean ± SD	Amplitude	Mean ± SD	Amplitude
TL	12.72 ± 2.12a	10.0 – 15.4	11.5 ± 1.40a	9.0 – 14.1
BW	28.8 ± 12.15a	11.1 – 50.0	20.6 ± 8.89b	11.0 – 41.0
GSI	4.66 ± 4.53a	0.42 – 12.7	0.64 ± 0.84b	0.04 – 2.05
K	1.34 ± 0.70a	1.26 – 1.45	1.28 ± 0.04a	1.22 – 1.34

Data expressed as mean ± standard deviation (SD). Different letters in same line = differences between males and females ($p < .05$).

Table III. The average values per bimester of gonadosomatic índice (GSI) and condition factor (K) of *S. elegans* females and males captured in the São Francisco River between January and December 2012.

Bimester	Females		Males	
	GSI	K	GSI	K
Jan-Feb	12.71 ± 5.49 ^A	1.38 ± 0.15 ^A	2.05 ± 1.56 ^B	1.34 ± 0.17 ^A
Mar-Apr	4.02 ± 3.58 ^{BCD}	1.26 ± 0.35 ^B	0.04 ± 0.01 ^A	1.25 ± 0.14 ^B
May-Jun	0.42 ± 0.25 ^C	1.27 ± 0.07 ^B	0.07 ± 0.01 ^A	1.27 ± 0.17 ^B
Jul-Aug	1.01 ± 0.06 ^{BCD}	1.29 ± 0.24 ^B	0.06 ± 0.01 ^A	1.22 ± 0.12 ^B
Sep-Oct	3.13 ± 0.14 ^{BC}	1.40 ± 0.14 ^A	0.33 ± 0.89 ^A	1.33 ± 0.13 ^A
Nov-Dec	6.71 ± 3.65 ^D	1.45 ± 0.20 ^A	1.30 ± 1.10 ^B	1.32 ± 0.18 ^A

Data expressed as mean ± standard deviation (SD). Different letters in same column = differences between bimesters ($p < .05$).

February (Figure 4b, Table IV). The occurrence of the spawning females with follicles in all stages of development, as well as atretic and post-ovulatory follicles, confirms that *S. elegans* is a partial spawner.

DISCUSSION

The macro- and microscopic morphology of ovaries and testes are similar to other Characiforms (Hojo et al. 2004).

For females, the mean values of the total length, body weight, and GSI were higher than males. This demonstrates sexual dimorphism, a characteristic of fish of the order Characiforms (Hojo et al. 2004, Thomé et al. 2005, Abdo et al. 2018). During the reproductive peak, high

values of the gonadosomatic index and Fulton condition were observed in *S. elegans* females and males, indicating that reproduction did not interfere with the fish's physiological condition or health (Froese 2006, Roriz Lemes et al. 2016). In the present study, we observed a predominance of females, similar to that in other studies (Hojo et al. 2004, Bazzoli et al. 2019). However, the sex ratio may be related to differences between the sexes, the selectivity of the sampling apparatus, the amount of available food, and population stratification (Carvalho et al. 2009).

The follicular development of *S. elegans* is similar to the descriptions of other fish (Grier et al. 2017). It is comprised of the perinucleolar, vitellogenic, and final maturation stages. These phases occur inside follicles, whose walls consist of zona radiata, the follicular cell,

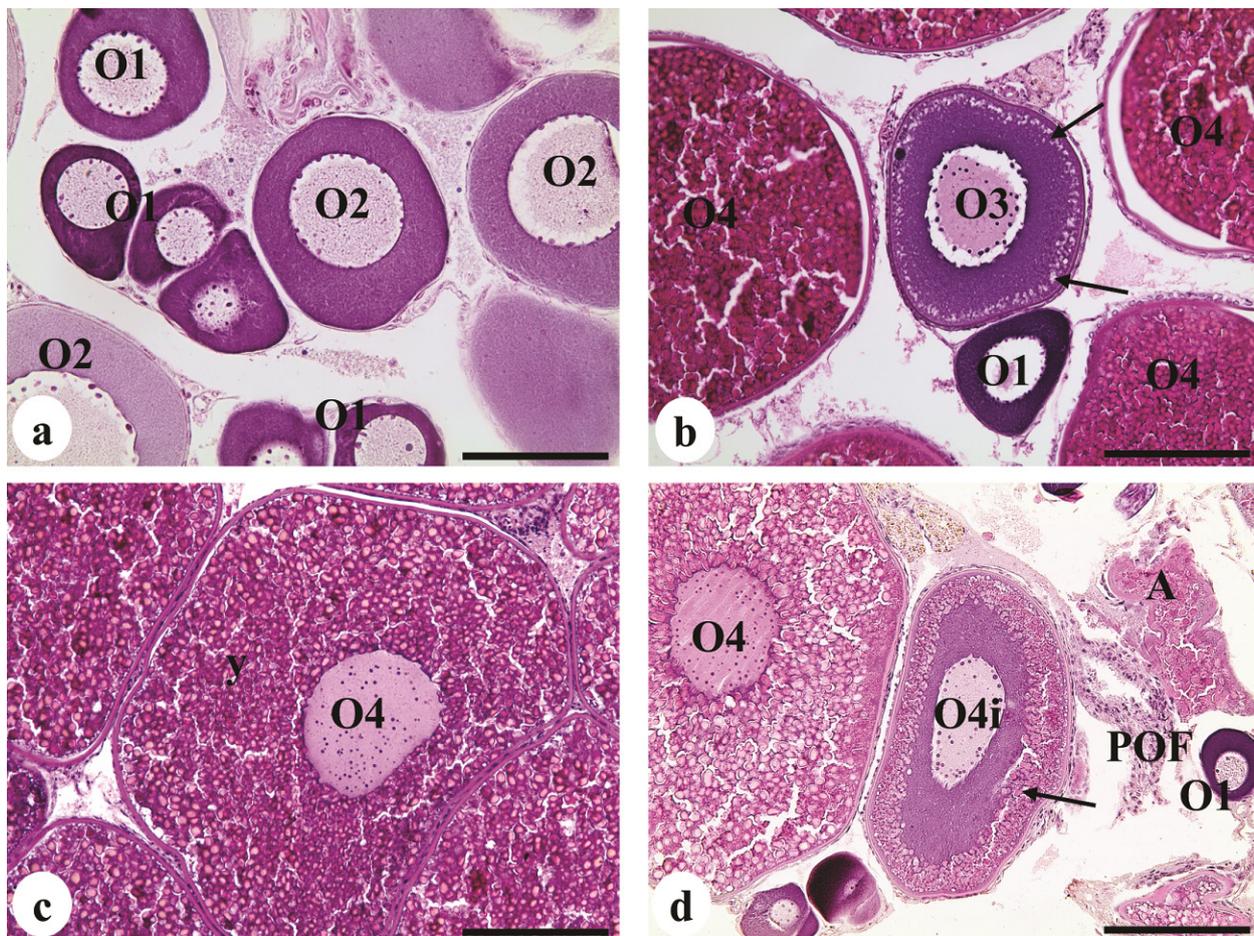


Figure 2. Histological sections of *S. elegans* ovaries in the different stages of the gonadal maturation are stained by HE. a) Stage F1 = rest with initial perinucleolar follicles (O1, diameter = $80.70 \pm 12.43 \mu\text{m}$). It's with basophilic cytoplasm and vesiculous nucleus and advanced perinucleolar follicles (O2 = $149.66 \pm 21.10 \mu\text{m}$), as well as granular cytoplasm and nucleus with nucleoli attached to the nuclear envelope; b) Stage F2 = maturation with previtellogenic oocyte (O3 = $217.10 \pm 36.05 \mu\text{m}$), presenting characteristic cortical alveoli (arrow) and vitellogenic follicles (O4 = $442.01 \pm 51.72 \mu\text{m}$) with ooplasm full of yolk globules; c) Detail of O4 showing squamous follicular cells (FC), thin zona radiata (ZR) and spherical and acidophilus yolk globules (Y); d) Stage F3 = spawned with post-ovulatory follicles (POF) and follicles at all stages of development as well as atretic follicles (A) and previtellogenic follicles (O4i) with early of acidophilus yolk globules (arrow). Bars: a and b = $100 \mu\text{m}$; c and d = $200 \mu\text{m}$.

basement membrane, and theca. They play an important role during folliculogenesis and fertilization. The mature ovaries of *S. elegans* exhibit asynchronous development with oocytes in different growth stages, as has been reported for other fish, (i.e., perinucleolar, previtellogenic, and vitellogenic oocytes) (Honorato-Samapio et al. 2009, Marcon et al. 2017). Spermatogenesis consists of a sequence of morphological changes that occur in germ cells while differentiating

from spermatogonia to spermatozoa. In *S. elegans*, the cells of the spermatogenic lineage were observed in the germinal epithelium, similarly observed in other fish (Grier & Uribe-Aranzábal 2009).

Three gonadal maturation stages for females and males were established, similar to the studies of Brandão et al. (2017) and Bazzoli et al. (2019). The long spawning period and histological characteristics of spawned ovaries

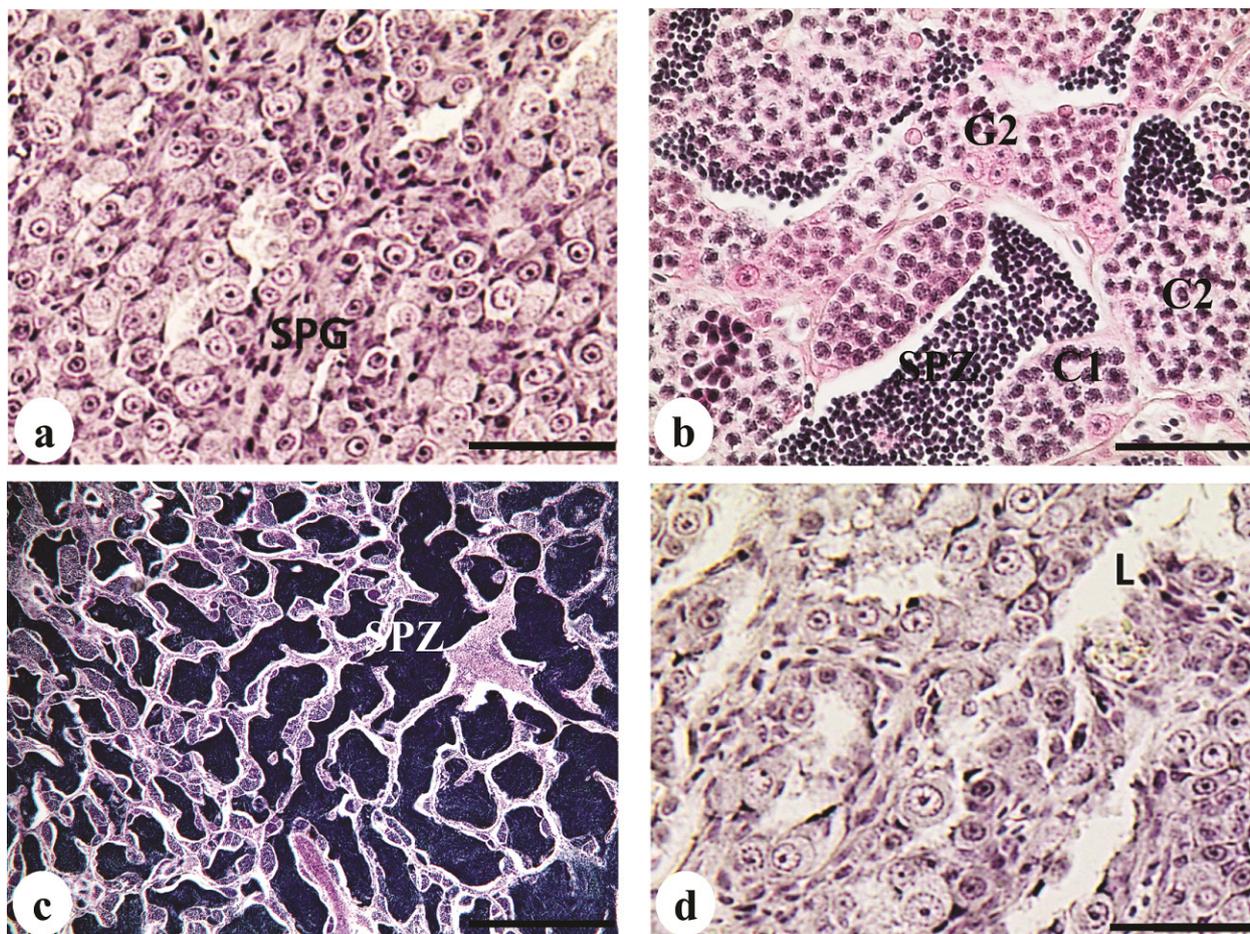


Figure 3. The histological sections of the testes of *S. elegans* are shown in different stages of the gonadal maturation stained by HE. a) Stage M1 = rest containing only spermatogonia (SPG) and lumen of the closed seminiferous tubules. b) The cells of the spermatogenic lineage are shown with primary spermatogonia (G1, diameter = $6.18 \pm 1.01 \mu\text{m}$). An abundant cytoplasm and a spherical central nucleus are displayed, as well as secondary spermatogonia (G2 = $5.19 \pm 0.80 \mu\text{m}$) with little cytoplasmic material and a spherical central nucleus. The primary spermatocyte (C1 = $3.95 \pm 0.51 \mu\text{m}$) is with spherical the central nucleus and granulated chromatin. The secondary spermatocyte (C2 = $2.93 \pm 0.54 \mu\text{m}$) is with the spherical central nucleus and fine chromatin. The spermatid (T = $1.32 \pm 0.17 \mu\text{m}$) with the dense spherical nucleus. The spermatozoa (SPZ = $0.95 \pm 0.15 \mu\text{m}$) presents a spherical head and strongly condensed nucleus. c) Stage M2 = maturation with the lumen of the seminiferous tubules filled with spermatozoa. d) M3 = time spent with the lumen (L) of the seminiferous tubules open and the wall containing only spermatogonia. Bars: a, b, and d = $50 \mu\text{m}$. Bar c = $200 \mu\text{m}$.

containing oocytes at all stages of development, in addition to post-ovulatory oocytes and atretic oocytes, indicate that *S. elegans* presents partial spawning, similar to other Characiforms of the Curimatidae family (Schifino et al. 1998, Ribeiro et al. 2007). Asynchronous folliculogenesis is characteristic of species with long reproductive periods and multiple or partial spawning (Bazzoli et al. 2019), as is the case for *S. elegans* of the

present study. Partial spawning is a strategy that allows several spawns in the same reproductive cycle, the asynchronous development of larvae, and, consequently, the occupancy of distinct niches (Ratton et al. 2003).

Larger size at the first gonadal maturation was observed for females, which may be related to differences in environmental conditions and food availability (Nikolsky 1963, Pawson et al.

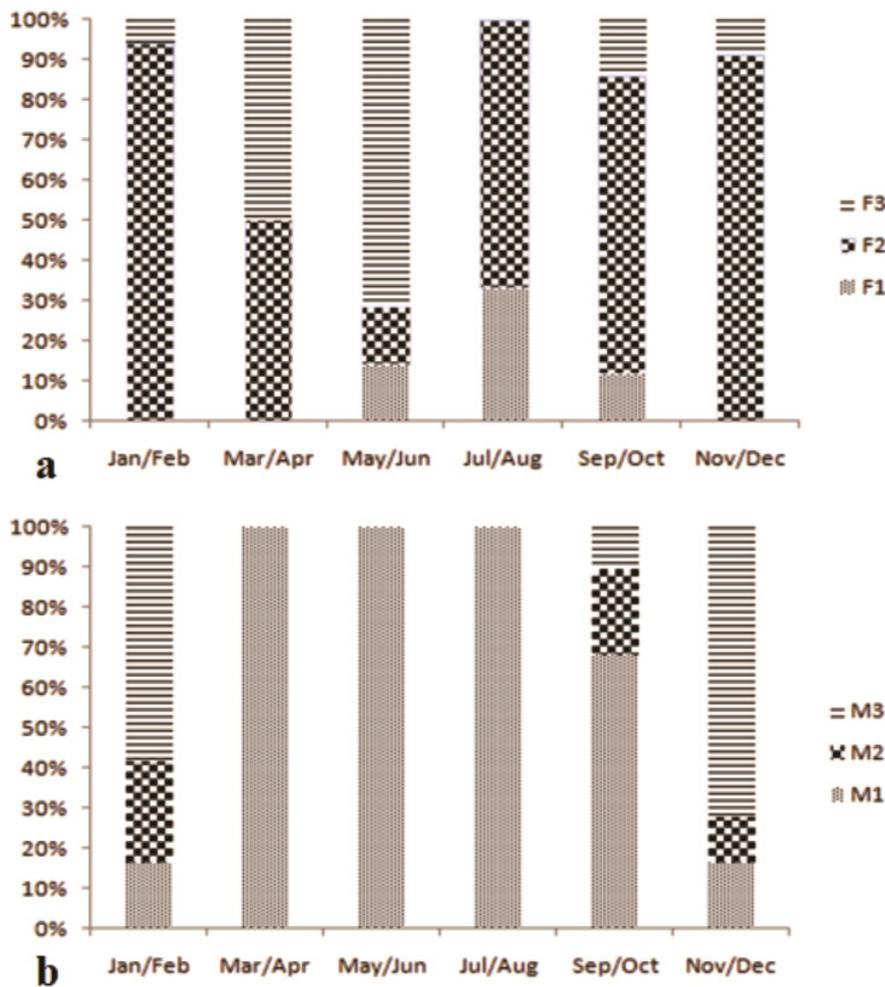


Figure 4. The bi-monthly distribution of the gonadal maturation stages of *S. elegans* (a) females and (b) males caught in the São Francisco River downstream of the Três Marias dam between January and December 2012.

2000). Another explanation could be related to the production of sex hormones (such as 17- β oestradiol), which is responsible for both the somatic growth and gonadal development in females (Arantes et al. 2010).

After spawning, non-released structures of the follicle remain in the ovary, constituting the post-ovulatory follicle (Grier et al. 2017), as seen in the present study. Follicular atresia is common in vertebrate ovaries. It can occur at any stage of folliculogenesis but is more frequent in vitellogenic oocytes (Santos et al. 2008) with a low frequency, as observed in the present study. In this degenerative process, as reported by Marcon et al. (2017), morphophysiological events

are essential in ovarian remodeling for the next reproductive cycle (Marcon et al. 2019).

The reproductive activity of both sexes of *S. elegans* in the São Francisco River was influenced by environmental factors, such as water temperature and rainfall in the region. This was observed in other studies, where the fish found favorable conditions for reproduction (Weber et al. 2013, Brandão et al. 2017, Bazzoli et al. 2019). These peaks occurred from September to February, coinciding with the greatest number of fish in the maturation stage and GSI, with the fish responding to water conditions in tropical environments (Lowe-McConnel 1987), similar in *S. insculpta* that extends from September

Table IV. The bi-monthly absolute distribution (n) of the gonadal maturation stages (GMS) of *S. elegans* females (F) and males (M) caught in the São Francisco River downstream of the Três Marias dam between January and December 2012.

GMS	Sample per bimester						
	Jan/Feb	Mar/Apr	May/Jun	Jul/Aug	Sep/Oct	Nov/Dec	
	n	n	n	n	n	n	
F1	0	0	1	1	7	0	
F2	33	5	1	2	38	41	
F3	2	5	5	0	7	5	
Total	35	10	7	3	52	46	153
M1	2	2	8	3	15	4	
M2	4	0	0	0	5	4	
M3	8	0	0	0	3	15	
Total	14	2	8	3	21	23	73

Females: F1 = rest, F2 = maturation/mature, and F3 = spawned. Males: M1 = rest, maturation/mature, and M3 = spent.

to March (Ribeiro et al. 2007). In Neotropical freshwater teleost, high rainfall triggers the final maturation of the gonads, and high-water temperatures are associated with spawning (Carvalho et al. 2009).

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