

## Research Article

**Cite this article:** Cordeiro JG *et al.* (2019) Reproductive cycle of the tetra *Astyanax bimaculatus* (Characiformes: Characidae) collected in Amazonian streams. *Zygote* page 1 of 8. doi: [10.1017/S0967199419000601](https://doi.org/10.1017/S0967199419000601)

Received: 26 February 2019

Revised: 22 May 2019

Accepted: 5 September 2019


**Keywords:**

Fish; Morphology; Oogenesis; Reproduction; Spermatogenesis

**Author for correspondence:**

Diógenes Henrique de Siqueira-Silva. Research Group on the Reproduction of Amazon fish (GERPA/LANEC), Biology Faculty (FACBIO), University of South and Southern of Pará (Unifesspa), Marabá, Pará, Brazil.  
Tel: +55 94 981135614. Fax: +55 9421015810.  
E-mail: [siqueira.diogenes@gmail.com](mailto:siqueira.diogenes@gmail.com)

# Reproductive cycle of the tetra *Astyanax bimaculatus* (Characiformes: Characidae) collected in Amazonian streams

Jhennifer Gomes Cordeiro<sup>1</sup>, Jeane Rodrigues<sup>1</sup>, Maira da Silva Rodrigues<sup>3</sup>, Rafaela Manchin Bertolini<sup>2</sup>, Rafael Henrique Nóbrega<sup>3</sup>, George Shigueki Yasui<sup>2,4</sup>, Caio Maximino<sup>1,5</sup> and Diógenes Henrique de Siqueira-Silva<sup>1,5</sup> 

<sup>1</sup>Research Group on the Reproduction of Amazon fish (GERPA/LANEC), Biology Faculty (FACBIO), University of South and Southern of Pará (Unifesspa), Marabá, Pará, Brazil; <sup>2</sup>Laboratory of Fish Biotechnology, National Center for Research and Conservation of Continental Fish, Chico Mendes Institute of Biodiversity Conservation, Pirassununga, SP, Brazil; <sup>3</sup>Reproductive and Molecular Biology Group, Department of Morphology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, Brazil; <sup>4</sup>Department of Veterinary Medicine, FZEA, University of São Paulo, Pirassununga, SP, Brazil and <sup>5</sup>PPG in Biodiversity and Biotechnology (BIONORTE)

**Summary**

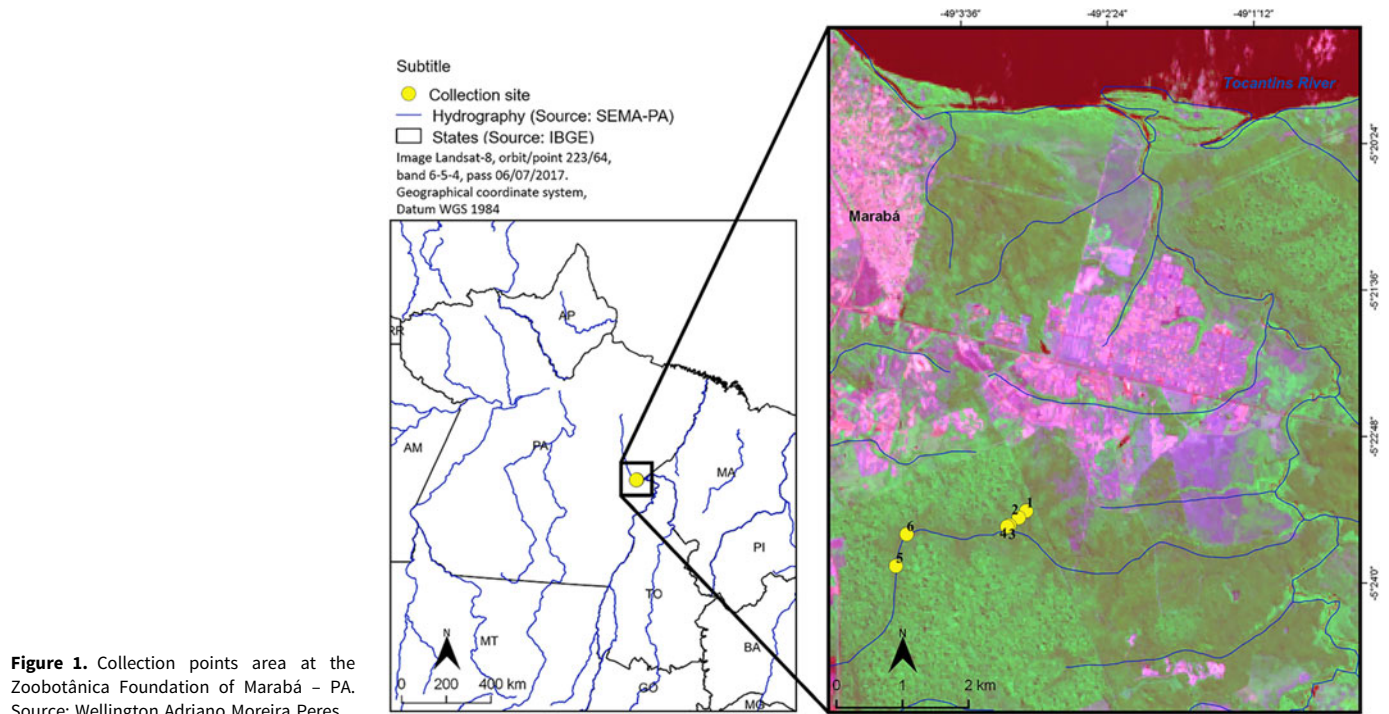
A histological characterization of gonadal development in the tetra *Astyanax bimaculatus* was performed, aimed at determining its reproductive cycle in streams localized inside the Amazonian forest. Collections were carried out monthly from August 2017 to July 2018 at the Zoobotânica Foundation of Marabá, PA. Collected specimens were weighed and measured, and their gonads and liver were removed and weighed to calculate gonadosomatic and hepatosomatic indexes. Gonads were fixed and treated for routine histology for light microscopy. Materials were stained with toluidine blue and haematoxylin and eosin. The Amazonian *A. bimaculatus* species presented two reproductive periods in the year, one at the end of the winter season and another during the summer. Females showed an asynchronous development of their oocytes and only two reproductive phases of development were observed during the whole period ‘developing’ and ‘spawning capable’. Males presented cystic spermatogenesis, with an anastomosing tubular testis containing spermatogonia spread along the germinal epithelium (unrestricted spermatogonial). These morphological characteristics are considered phylogenetically more primitive. Male specimens were observed to have five different phases during the period: immature, initial maturation, mid maturation, final maturation and regression. The huge fluctuations in Amazonian streams was observed, in which water volumes varied considerably across seasons, culminating even in total drought. In spite of this, *A. bimaculatus* could be found during all seasons, showing its impressive reproductive adaptation to its conditions.

**Introduction**

Popularly known as ‘tambuí tetra’, *Astyanax bimaculatus* (Linnaeus 1758) is a fish species belonging to the Characidae family, which is mostly comprised of freshwater fish from Brazil. According to Fricke *et al.* (2018), this family contains approximately 1177 valid species of fish. Among these, *A. bimaculatus* is considered as one of the most important in the *Astyanax* genus (Vilela and Hayashi, 2001). Its ample distribution from the Brazilian northeast region to the Prata basin, large harvests and its importance in the sport of fishing are highlighted (Barbieri *et al.*, 1982). In addition it is the main constituent of some fish diets that include ‘saicanga’ (*Oligosarcus longirostris*), and ‘traíra’ (*Hoplias malabaricus*) (Andrian *et al.*, 2001).

Its adaptive plasticity is probably associated with the reproductive mechanisms and strategies developed during its lifetime that allow reproduction and survival in most varied habitats such as lakes, dams, streams, swamps and rivers. For this reason, *A. bimaculatus* has been used as a model for toxicology (Santos *et al.*, 2015; Marcon *et al.*, 2017), substrate evaluation (Rezende *et al.*, 2005), hormone induction (Felizardo *et al.*, 2011) and in environmental impact studies (Mereles *et al.*, 2017; Normando *et al.*, 2013). Moreover, this species is the subject of many related studies such as embryo development (Weber *et al.*, 2012), larval development (Vilela & Hayashi, 2001), nutrition (Andrian *et al.*, 2001) and reproduction (Barbieri *et al.*, 1982; Santos *et al.*, 1991). None of those studies, however, has described the morphology of gonadal development such as the spermatogenesis, oogenesis and the reproductive cycle.

Histology has been used to study reproduction in many fish species such as the tetra *Astyanax altiparanae* (Rodrigues *et al.*, 2015; Cassel *et al.*, 2017), the Danio *Devario aequipinnatus* (Chagas *et al.*, 2016; De Jesus-Silva *et al.*, 2018) and some Amazonian species including the Amazonian



**Figure 1.** Collection points area at the Zoobotânica Foundation of Marabá – PA. Source: Wellington Adriano Moreira Peres.

whitefish *Plagioscion squamosissimus* (Barbosa *et al.*, 2012) and the ‘piranha’ *Serrasalmus gouldingi* (Da Silveira Prudente *et al.*, 2014). This tool is considered one of the most accurate approaches for analyzing reproductive development as it allows the determination of reproductive phases in males and females, defining, for example, time of first maturation, spawning type, reproductive period (Vazzoler, 1996) and fecundity (Mendes *et al.*, 2018).

This knowledge is important for understanding species population performance, in addition to helping conservation strategies and management of organisms and their environment (Vazzoler, 1996). This is true of species from the Amazonian region, where the rivers and lowlands pass through periodic modification due to flood cycles (Prudente *et al.*, 2015), modifying, subsequently, the reproductive aspects of habitat species, such *A. bimaculatus*, that are in theory physiologically linked to habitat fluctuation (Godinho *et al.*, 2009).

Therefore, considering the economic, tourism, and ecological potentials of this species, this study aimed to characterize histologically gonadal development in male and females of *A. bimaculatus* to determine its reproductive cycle in streams from the Amazonian forest.

## Material and methods

### Place of collection

Collections were authorized by ICMBio (no. 62027-1) in small freshwater streams that opened inside the Amazon forest at the Fundação Zoobotânica de Marabá, PA, which occupies an area of 15,650 m<sup>2</sup> (Fig. 1). The sampling perimeter was demarcated at the following points: 1 (05°23′24.5″S and 049°03′03.9″W); 2 (05°23′27.9″S and 049°03′07.6″W); 3 (05°23′31.6″S and 049°03′12.3″W); 4 (05°23′32.2″S and 049°03′13.3″W); 5 (05°23′51.6″S and 049°04′08.0″W); and 6 (05°23′06.0″S and W 049°04′07.7″W). However, specimens were found only at points 1, 2, and 3. Voucher specimens (voucher number:

25623) were deposited at the Collection of the Fish Biology and Genetics Laboratory at the Institute of Biosciences Universidade Estadual Paulista (UNESP), Botucatu, São Paulo State, Brazil.

Amazonian streams are distinct, some presenting low water circulation (lentic waters) and others with a faster flow of water (lotic waters). In addition, they differ in depth (~0.5–1.5 m), turbidity, solar penetration (1.25–1.45 m, Secch disc) and amount of dissolved organic matter. However, in relation to temperature (25.8 ± 0.87), dissolved oxygen (3.11 ± 1.37), nitrite (0.125 ± 0.3) and water pH (7.06 ± 0.5) streams are very similar. Moreover, due to the water regime of this region with respect to full stoppage of rains between the middle of April and the end of October, many of those streams totally dry up during winter.

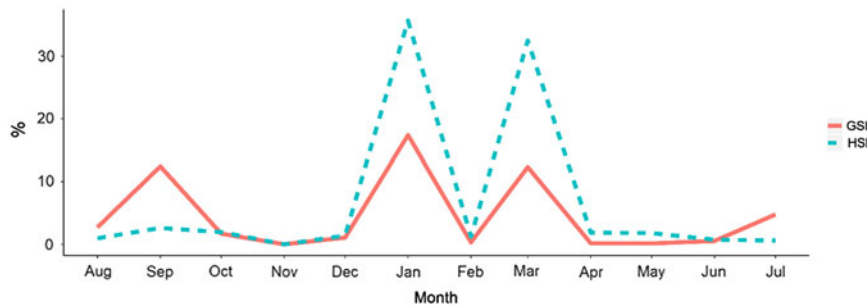
### Sample collection

Collections occurred monthly from August 2017 to July 2018 with the aid of two nets (4.75 × 1.10 m) with a mesh of 1.0 × 1.0 mm that were used for trawling and waiting. Sampled specimens were taken to the laboratory where they were anesthetized in Eugenol (20 ml of Biodynamic Eugenol in 100 ml absolute alcohol) and submitted to euthanasia. Biometric data (total length (TL, cm), standard length (SL, cm), and weight (W, g)) were measured.

A ventro-longitudinal incision was made, and the gonads and liver from each specimen were removed. Livers were weighed to evaluate the hepatosomatic index (HSI) using the formula  $HSI = W_L / W_T \times 100$  ( $W_L$  = weight of the liver;  $W_T$  = total weight of the fish). Gonads were weighed to calculate the gonadosomatic index (GSI) using the formula  $GSI = W_G / W_T \times 100$  ( $W_G$ : weight of the gonad;  $W_T$ : total weight of the fish), and then fixed in Bouin solution (Cinetica) for 24 h for later preservation in 70% alcohol for histology.

### Histological characterization

All materials were processed according to standard protocols for light microscopy analysis (Cordeiro *et al.*, 2019). Gonads were



**Figure 2.** Relationship of the gonadosomatic (GSI) and hepatosomatic (HSI) indexes during the 12 sampling months.

submitted to the inclusion procedure using methacrylate glycol (Historesin, Technovich, 7100). The material was then cut in 5  $\mu\text{m}$  slices in a microtome (Leica, 2200) equipped with a glass razor. Sample slides were stained with toluidine blue and haematoxylin and eosin and then analyzed under an optical microscope (Leica CTR4000). Photodocumentation was performed using a camera (Leica DFC310 FX) and the Leica Application Suite 3.0.0 build 8134 program.

### Statistics

To analyze the proportion of males and females, the chi-square test ( $\chi^2$ ) (at 0.05% significance) was performed, hypothesizing a 1:1 ratio.

### Results

In total, 35 specimens were collected (nine during autumn, seven in spring, four in winter and 15 in summer), comprised of 22 males and 13 females, representing an expected 1:1 sex ratio according to chi-squared test ( $\chi^2 = 2.314$ ;  $P = 0.05$ ). SL and weight of the females were  $5.9 \pm 0.95$  cm and  $4.8 \pm 2.78$  g on average, respectively. In the males, SL and weight were  $5.0 \pm 0.87$  cm and  $2.6 \pm 1.76$  g, respectively.

The GSI showed three peaks during the sampling period. A slight peak in September 2017 and two higher peaks in January and March 2018. These peaks were followed by decreasing values for the GSI in the following months. The hepatosomatic index (HSI) followed the same trend (Fig. 2).

### Females

Definition of the maturation phases in *A. bimaculatus* females was carried out based on De Jesus-Silva *et al.* (2018) for *D. aequipinnatus* species, and using Brown-Peterson *et al.* (2011) as support. The sampled female specimens were identified as having only two maturation phases, developing and spawning capable, as seen in Figs 3–5.

#### Developing

This maturation phase started with the oocytes in primary growth. Most present cells were at the beginning and end of the pre-vitellogenic stage. The initial pre-vitellogenic cells were characterized by an acidophilic round nucleus and the presence of a peripheral nucleolus. The cytoplasm was basophilic, and these cells were smaller than the final pre-vitellogenic cells. Oogonia at different stages of development were also visualized (Fig. 3a, b and 5). The pre-vitellogenic oocytes at the final stage of development were characterized by the appearance of cortical alveoli and the zona radiata. In addition, the cytoplasm became less basophilic

(Figs 3c, d and 5). As the ovaries developed, initial pre-vitellogenic cells were observed less and the final pre-vitellogenic cells became increasingly apparent. Oocytes in secondary growth were now visible on the cortical alveoli and at the beginning of yolk deposition. Oocytes in atresia (Fig. 3d) and the post-ovulatory follicular complex (POC) were observed during this maturation phase (Fig. 3b).

#### Spawning capable

This phase was mainly characterized by the presence of vitellogenic oocytes, which can indicate that individuals are able to reproduce. Migration of the germinal vesicle to the periphery was observed in some oocytes (Figs 3f and 5). Despite the vitellogenic oocytes occupying more volume in the ovaries during this phase, initial and final pre-vitellogenic oocytes were still observed (Figs 3e, f and 5).

Animals were categorized according to their maturation status and subdivided throughout the seasons. Females suitable for spawning during winter and summer periods were observed (Fig. 4).

### Males

Testes of *A. bimaculatus* males were of the anastomosing tubular type, showing spermatogonia spread along the entire length of the germinal epithelium (Fig. 6).

To identify the development phases in *A. bimaculatus* testes, the description used by Siqueira-Silva *et al.* (2013) for the yellow peacock bass *Cichla kelberi* was referenced, using the classification proposed by Grier and Taylor (1998) as basis with necessary adaptations for *A. bimaculatus* made. Five maturation phases for *A. bimaculatus* males were observed.

#### Immature

This phase was characterized by the intense presence of spermatogonia spread along the entire length of the testis and, consequently, corresponded to the absence of lumen. Spermatogonia are the cells that initiate spermatogenesis, other cells were formed as the development of these phases occurred and some spermatocytes could be seen during transition to initial maturation (Fig. 6a, b).

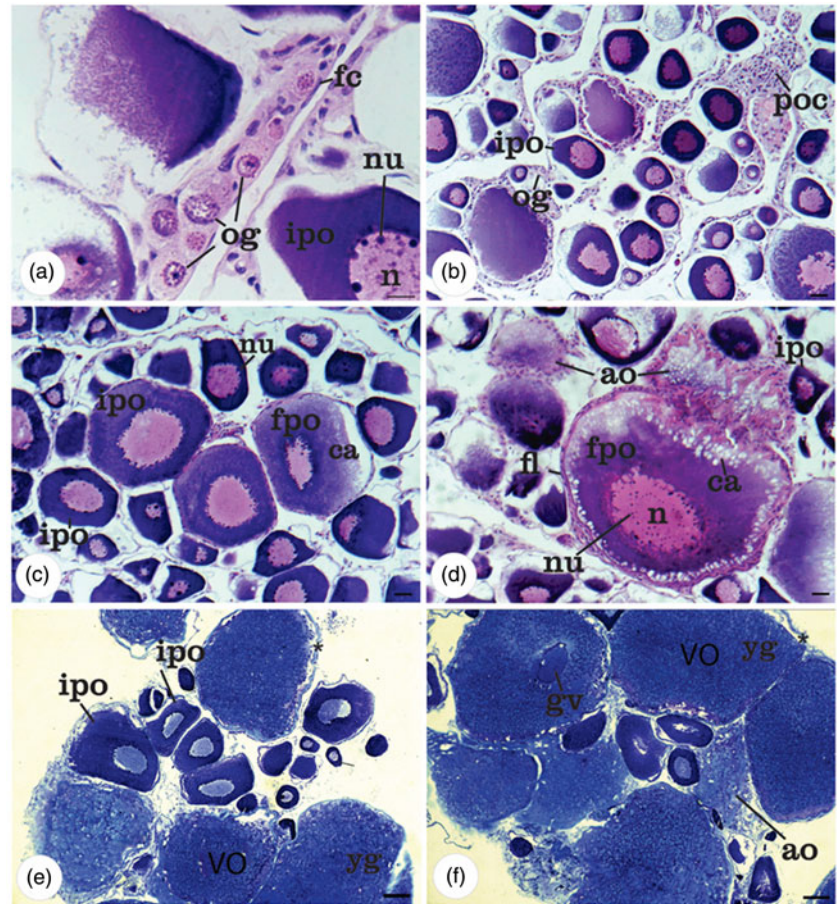
#### Initial maturation

In this phase, testes were characterized by the presence of a continuous germinal epithelium, in which it was possible to find a larger volume of spermatogenic cysts in all development stages, but mainly those of spermatocytes. Many spermatogonia can be visualized, spread along the epithelium and the lumen was already easily observed (Fig. 6c, d).

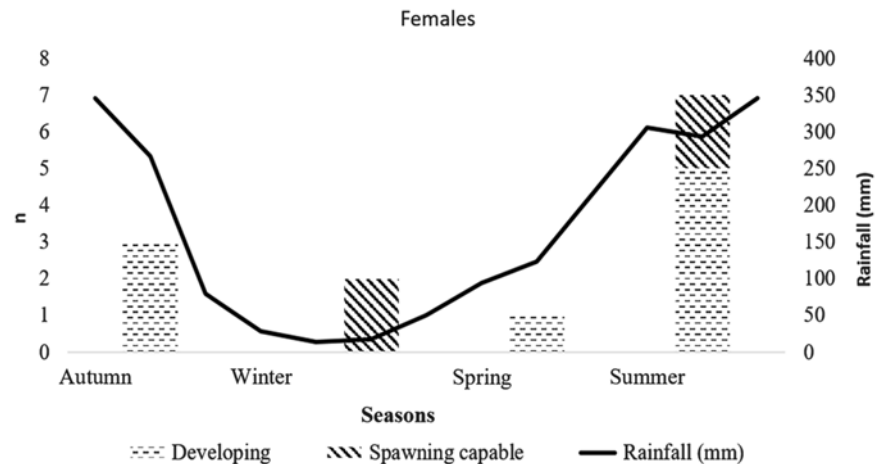
#### Mid maturation

One of the main characteristics of this phase was the beginning of discontinuity in the germinal epithelium, as many spermatocytes





**Figure 3.** Oogenesis in *A. bimaculatus*. Developing: (a, b) initial pre-vitellogenic oocytes (ipo) with perinucleolar nucleoli (nu) and different Oogonia (og). Follicular cells are observed (fc); (c, d) initial pre-vitellogenic oocytes (ipo) and final pre-vitellogenic oocytes (fpo) with follicular layer (fl) and presence of cortical alveolus (ca) at the periphery. Spawning capable: (e, f) vitellogenic oocytes (vo) filled with yolk granules (yg) and germinal vesicular migration (gv). Abbreviations: ao: atresic oocytes; poc: post-ovulatory follicular complex. \*: zona radiate; n: nucleus. Scale bars: (b) 50  $\mu$ m; (a, c–f) 100  $\mu$ m. Staining: (a–d) haematoxylin and eosin; (e, f) toluidine blue.



**Figure 4.** Gonadal development in females of *Astyanax bimaculatus* per season throughout the year. Pluviometry data obtained from [Climate-Data.org](https://climate-data.org).

had started their rupture (Fig. 6f) to release spermatozoa into the lumen. Even with the rupture of some spermatocytes, many cysts and single spermatogonia were still observed. Spermatocytes cysts were still the most frequent in the testes (Fig. 6e, f).

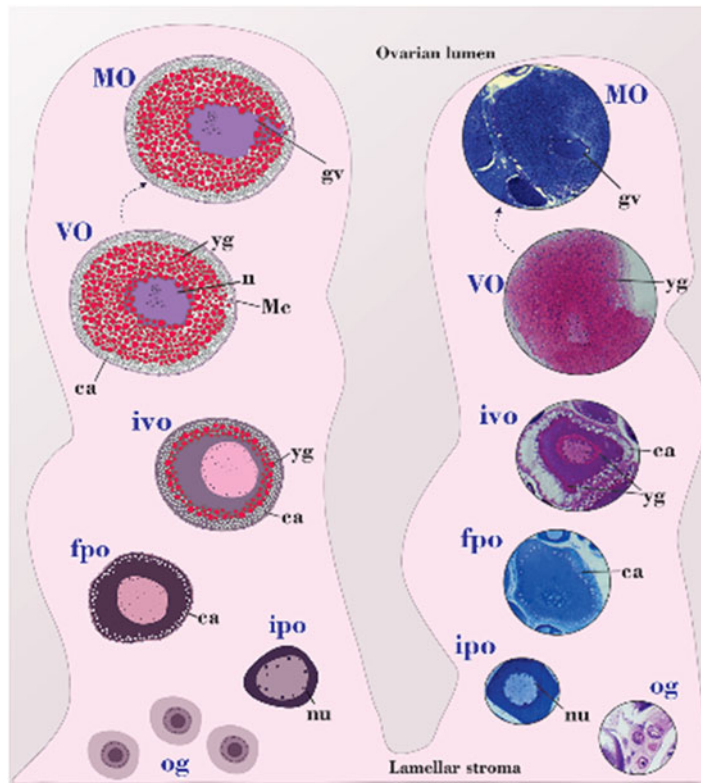
#### Final maturation

In this maturation phase, individuals were ready to release semen into the environment. This was characterized by advancement in discontinuity of the germinal epithelium (Fig. 7a), in which most spermatogenic cysts break, releasing spermatozoa to the testicular

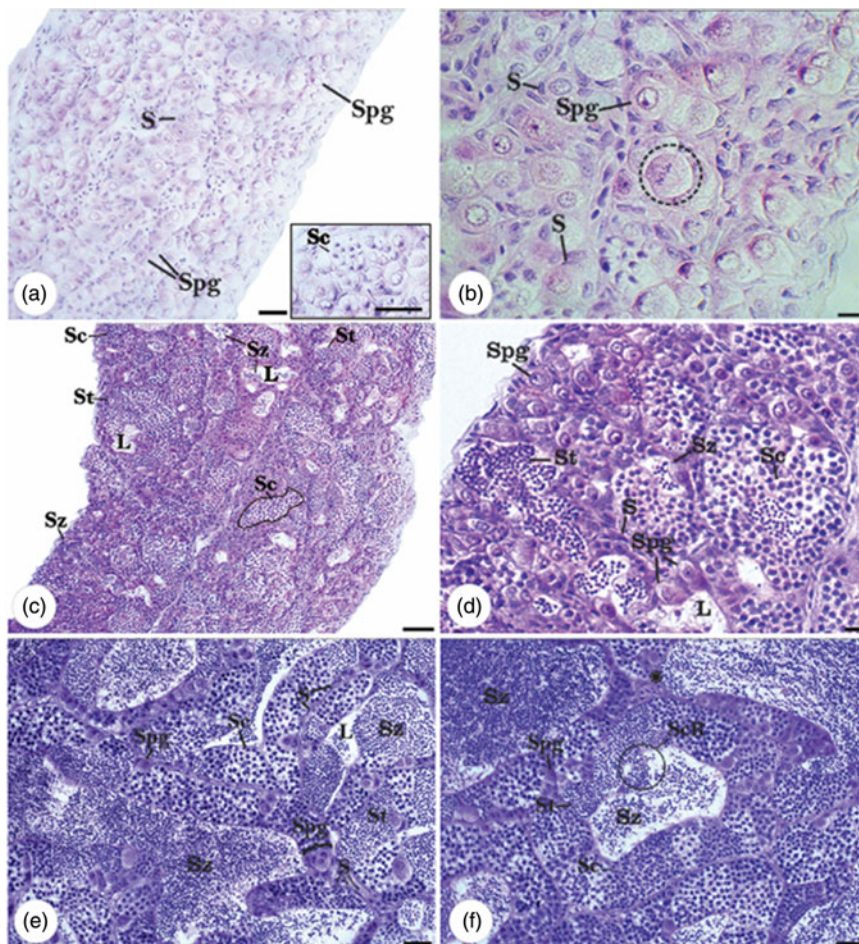
lumen. In the beginning of this phase, it was still possible to visualize cysts of spermatocytes and spermatids, however a reduced number of spermatid cysts were observed and the volume of spermatozoa in the lumen was noted during its progression (Fig. 7a, b). Melano macrophages centres started to be observed in some regions of the testes (Fig. 7c).

#### Regression

The regression phase appeared post-reproduction. It could be identified by testes flaccidity, its length reduction, a totally

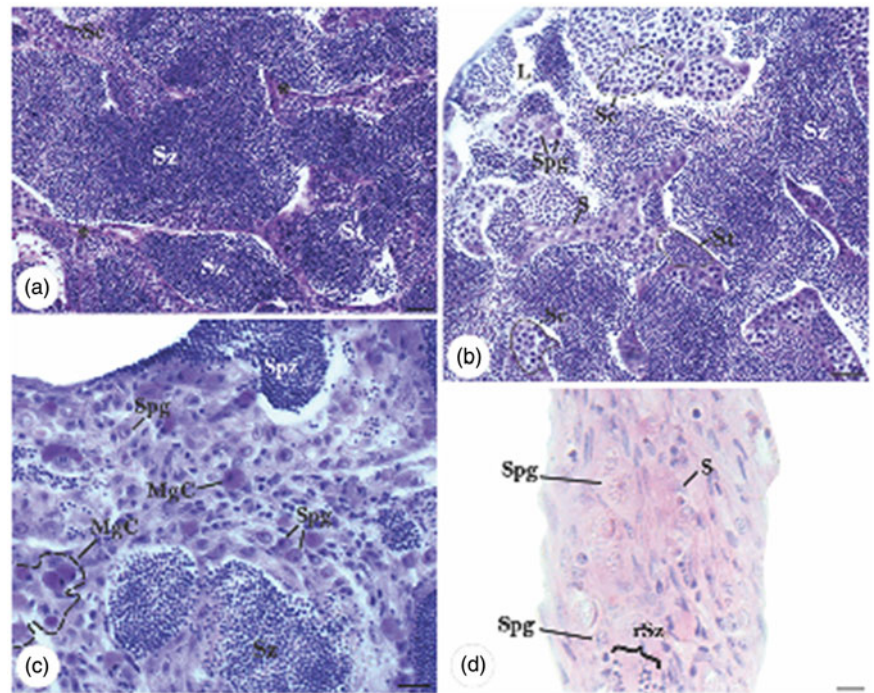


**Figure 5.** Scheme illustrating oocyst development in *Astyanax bimaculatus*. (Og) oogonia; (ipo) initial pre-vitellogenic oocyte; (fpo) final pre-vitellogenic oocyte; (ivo) initial vitellogenic oocyte; (VO) vitellogenic oocyte; (MO) mature oocyte. Abbreviations: (nu) nucleoli; (ca) cortical alveoli; (yg) yolk granules; (n) nucleus; (Mc) micropila; (gv) germinal vesicle.

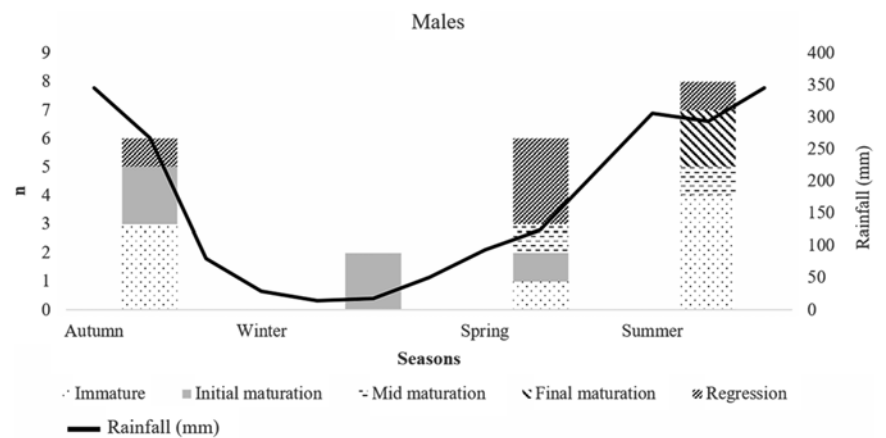


**Figure 6.** Spermatogenesis in *A. bimaculatus*. Immature: (a, b) Proliferation of spermatogonia (Spg) in the beginning of gonadal development. Inset: Spermatocyte cyst (Sc). Initial maturation: (c, d) spermatogonia (Spg), cysts of spermatocytes (Sc) and spermatids (St), and some spermatozoa (Sz) released in the lumen (L) can be observed, and the germinal epithelium is still continuous. Mid maturation: (e, f) Persistence of many cysts of spermatocytes (Sc) and spermatids (St) and spermatocyst rupture (ScR), releasing a large volume of spermatozoa (Sz) into the lumen (L). \*Cases in which discontinuity of the germinal epithelium are known and observed. Abbreviation: S: Sertoli cell. (---): spermatogonia in mitosis. Scale bars: (a, c-f) inset: 25 µm; (b) 10 µm. Staining: haematoxylin and eosin.





**Figure 7.** Spermatogenesis in *A. bimaculatus*. Final maturation: (a, b) Lumen (L) filled with spermatozoa (Sz) and discontinuous germinating epithelium (\*) with some spermatocytes (Sc) and spermatids (St) cysts. Transition from final maturation to regression (c, d) with predominance of spermatozoa and in some regions melanomacrophagic centres (MgC). Regression: Residual spermatozoa (rSz) and spermatogonia (Spg) along the gonad. Scale bars: (a–c) 25  $\mu$ m; (d) 50  $\mu$ m. Staining: haematoxylin and eosin.



**Figure 8.** Gonadal development in males of *Astyanax bimaculatus* per season throughout the year. Pluviometry data obtained from [Climate-Data.org](https://climate-data.org).

discontinuous epithelium, and by the presence of residual spermatozoa. Single Sertoli cells and melanomacrophagic centres were observed. The intertubular compartment (interstitium) occupied a large area of the testis at this period. As the regression progressed, residual spermatozoa were phagocytosed, and stem spermatogonia started to proliferate as the animal prepared to begin a new reproductive cycle (Fig. 7d).

Males were categorized according to their maturation status and subdivided throughout the seasons. The final maturation peak was observed in the summer and other stages of maturation were found throughout the year (Fig. 8).

## Discussion

Compared with previous studies in other populations of the tetra *A. bimaculatus*, in which the number of females was always bigger than males (Santos et al., 1991; Barreto et al., 1998), here the ratio between the sexes was statistically proportional. Moreover, the reproductive strategy of the species was different from the population

studied by Barreto and colleagues (1998) in the Rio Grande basin in São Paulo State as, in the present study, *A. bimaculatus* was capable of spawning in more than a single period during the year. Because of this multiple reproduction, gonadal development occurred rapidly, mainly in females. At the same time as the gonad reached the POC, indicating spawning, new oocyte development also occurred among these presenting oocytes at early stages. As *A. bimaculatus* is considered a 'piracema' species (Agostinho et al., 2003), which means that its reproduction occurs only once in the summer season (the period of the year with highest temperatures and rainfall levels), these results are innovative, showing the high adaptability of this species to the Amazonian streams.

Its reproductive plasticity is evidenced by the presence of spawning capable females in the winter period, specifically at the end of September when the rainfall index increased after the 3 months, following the lowest register of that parameter during the year. As temperatures in this region are constantly high throughout the year (24–34 °C), rain increases may act as a

reproductive trigger, making the species spawn (Winemiller, 1989; Bailly *et al.*, 2008). This capacity is probably related to the asynchronous development of their oocytes, previously reported by Agostinho and colleagues (2003), that allows major reproductive flexibility and enables reproduction as soon as environmental conditions become favourable.

This scenario can help explain why only two maturation phases were observed in all females collected during the sampling period, hypothesizing that *A. bimaculatus* females are always prepared for reproduction, depending only on an environmental trigger. This behaviour has already been observed in other species such as the sardines *Lycenaraulis grossiden* and *Platanichthys platana* (Lopes *et al.*, 2018), which demonstrated the ability to adjust their reproduction based in environmental conditions and to reproduce in any season of the year. Nevertheless, reproduction was mainly triggered by temperature for those species, differing from the present findings. Due to the absence of captured immature females, this difference may be related to low sampling.

The traditional reproductive index used to define maturation phases in fish is GSI, however this index is sometimes considered unable to demonstrate real reproductive status in some species. Because of this caveat, Brown-Peterson and colleagues (2011) contributed valuable research in which one objective was to facilitate the definition of reproductive status using the association between macroscopic (GSI) and microscopic aspects (histology) findings. For *A. bimaculatus*, the GSI fitted well the histological analyses of the gonads, indicating the same period of reproduction. Therefore, using the approach proposed by Brown-Peterson *et al.* (2011), we reinforced the importance of the GSI as a good reproductive indicator, as already stated by Barbieri *et al.* (1982).

As for the GSI, the hepatosomatic index can be very important in understanding the energy expenditure and nutritional status of a species during its reproductive cycle. In most species, HSI is markedly reduced during reproduction (Querol *et al.*, 2002; Zin *et al.*, 2011). According to Lampert *et al.* (2004), food consumption is very intense in non-reproductive periods. This strategy allows obtained energy to be converted into gonadal increases and into nutrients to be stored for some reproductive strategies. This strategy is seen in species showing parental care, such the peacock bass (Kelber, 1999), in which the parent does not feed while taking care of the offspring. *A. bimaculatus* showed a different strategy with higher HSI during the reproductive periods. This finding must be linked to increase in food availability during periods of increased rain and scarcity of food during other periods.

In summary, the structural characteristics of *A. bimaculatus* were similar to those other species of the same family that live in neotropical regions (Siqueira-Silva *et al.*, 2018). Their testes were of the anastomosing tubular type and unrestricted spermatogonia, corroborating the Parenti and Grier (2004) hypothesis that stated that most primitive fish in the phylogenetic scale present this testicular morphology.

The present study showed that a fish species can adopt different strategies of reproduction, being totally dependent on their habitat and, consequently, abiotic factors have an effect in the studied region. Those results emphasize the need for knowledge not only in basic aspects of reproduction biology, but also for crosslinking those characteristics to the surrounding habitat.

**Acknowledgements.** The authors are thankful for the Fundação Zoobotânica de Marabá, where the collections were held. The authors also acknowledge

the Laboratory of Biotechnology/CEPTA – Pirassununga – SP, and the Reproductive and Molecular Biology Group – Institute of Biosciences of Botucatu (IBB–UNESP) for allowing these histological procedures.

**Financial support.** This work was supported by the Conselho Nacional de Pesquisa – CNPq (J.G.C., grant number 24/2017).

**Conflict of interest.** None.

**Ethical standards.** The authors assert that all procedures contributing to this work complied with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

## References

- Agostinho AA, Gomes LC, Suzuki HI and Júlio-Junior HF (2003) Migratory fishes of the upper Paraná River basin, Brazil. In: Carolsfeld J, Harvey B, Ross C and Baer A (eds). *Migratory Fishes of South America: Biology, Fisheries and Conservation Status*. World Bank and IDRC: Victoria, pp. 19–99.
- Andrian IF, Silva HBR and Peretti D (2001) Dieta de *Astyanax bimaculatus* (Linnaeus, 1758) (Characiformes, Characidae), da área de influência do reservatório de Corumbá, Estado de Goiás, Brasil. *Acta Sci* **23**, 435–40.
- Bailly D, Agostinho AA and Suzuki HI (2008) Influence of the flood regime on the reproduction of fish species with different reproductive strategies in the Cuiabá river, upper Pantanal, Brazil. *River Res Applic* **24**, 1218–29.
- Barbieri G, Santos MVR and Santos JM (1982) Época de reprodução e relação peso/comprimento de duas espécies de *Astyanax* (Pisces, Characidae). *Pesq Agropec Bras* **17**, 1057–65.
- Barbosa ND, Rocha RM and Lucena Frédo F (2012) The reproductive biology of *Plagioscion squamosissimus* (Heckel, 1840) in the Pará River estuary (Amazon estuary). *J Appl Ichthyol* **28**, 800–5.
- Barreto BP, Ratton TF, Ricardo MCP, Alves CB M, Vono V, Vieira F, Rizzo E and Bazzoli N (1998) Biologia reprodutiva do lambari *Astyanax bimaculatus* (Pisces, Characidae) no rio do Carmo, bacia do rio Grande, São Paulo. *BIOS, Cadernos do Departamento de Ciências Biológicas da PUC Minas* **6**, 121–30.
- Brown-Peterson NJ, Wyanski D, Saborido-Rey MF, Macewicz BJ and Lowerre-Barbieri SK (2011) A standardized terminology for describing reproductive development in fishes. *Mar Coast Fish* **3**, 52–70.
- Cassel M, Chehade C, Branco GS, Caneppele D, Romagosa E and Borella MI (2017) Ovarian development and the reproductive profile of *Astyanax altiparanae* (Teleostei, Characidae) over one year: applications in fish farming. *Theriogenology* **98**, 1–15.
- Chagas JMA, Ninhaus-Silveira A and Veríssimo-Silveira R (2016) Ciclo testicular de *Devario aequipinnatus* (Teleostei, Cyprinidae): Um potencial modelo biológico em experimentação animal. *Bol. Inst. Pesca* **42**, 765–79.
- Climate-Data.org Clima Marabá. Available at <https://pt.climate-data.org/americas-do-sul/brasil/para/marabá-715120/>. Accessed 26 November 2018.
- Cordeiro JG, Maximino C and Siqueira-Silva DH (2019). Histological staining of fish gonadal tissue. *Protocols.io*. Available at <https://dx.doi.org/10.17504/protocols.io.ygvftw6>.
- Da Silveira Prudente B, Ferreira MAP, Da Rocha RM and Assis Montag LF (2015) Reproductive biology of the piranha *Serrasalmus gouldingi* (Fink and Machado-Allison 1992) (Characiformes: Serrasalminae) in "drowned" rivers of the Eastern Amazon. *Environ Biol Fish* **98**, 11–22.
- De Jesus-Silva LM, Oliveira PV, Ribeiro CO, Ninhaus-Silveira A and Veríssimo-Silveira R (2018) Ovarian cycle in *Devario aequipinnatus* with emphasis on oogenesis. *Zygote* **26**, 168–76.
- Felizardo VO, Murgas LDS, Andrade ES, López PA, Freitas RTF and Ferreira MR (2011) Effect of timing of hormonal induction on reproductive activity in lambari (*Astyanax bimaculatus*). *Theriogenology* **77**, 1570–4.
- Fricke R, Eschmeyer WN and Fong JD (2018) Species by family/subfamily. Available at: <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>. Accessed 11 November 2018.
- Godinho AL, Lamas IR and Godinho HP (2009) Reproductive ecology of Brazilian freshwater fishes. *Environ Biol Fish* **87**, 143–62.
- Grier HJ and Taylor RG (1998) Testicular maturation and regression in the common Snook. *J Fish Biol* **53**, 521–42.

- Kelber D** (1999) *Tucunaré uma paixão internacional*. Arte & Ciência: São Paulo.
- Lampert VR, Azevedo MA and Fialho CB** (2004) Reproductive biology of *Bryconamericus iheringii* (Ostariophysi: Characidae) from rio Vacacaí, RS, Brazil. *Neotrop Ichthyol* 2, 209–15.
- Lopes CA, Reynalte-Tanaje DA and Nuñez APO** (2018) Reproductive dynamics of *Lycengraulis grossidens* (Clupeiformes: Engraulidae) and *Platanichthys platana* (Clupeiformes: Clupeidae) in a subtropical coastal lagoon. *Braz J Biol* 78, 477–86.
- Marcon L, Thomé RG, Munteer AH, Bazzoli N, Rizzo E and Benjamin LA** (2017) Immunohistochemical, morphological and histometrical analyses of follicular development in *Astyanax bimaculatus* (Teleostei: Characidae) exposed to an organochlorine insecticide. *Ecotoxicol Environ Saf* 143, 249–58.
- Mendes YA, Lee JT, Viana IKS, Rocha RM and Ferreira MAP** (2018) Reproductive biology of the tiger pleco *Panaqolus tankei* (Loricariidae) in a lentic system of the Amazon basin. *J Fish Biol* 93, 711–4.
- Mereles MA, Piñeyro JIG, Marshall BG and Sousa RGC** (2017) Impacts of fish farm dams on temporal and spatial distribution of *Astyanax* cf. *bimaculatus* in microbasins of Machado River (Rondônia, Brazil). *Biota Amazônia* 7, 4–7.
- Normando FT, Santiago KB, Gomes MVT, Rizzo E and Bazzoli N** (2013) Impact of the Três Marias dam on the reproduction of the forage fish *Astyanax bimaculatus* and *A. fasciatus* from the São Francisco River, downstream from the dam, southeastern Brazil. *Environ Biol Fish* 97, 309–19.
- Parenti LR and Grier HJ** (2004) Evolution and phylogeny of gonad morphology in bony fishes. *Integr Comp Biol* 41, 333–48.
- Prudente BS, Ferreira MAP, Rocha RM and Montag LFA** (2015) Reproductive biology of the piranha *Serrasalmus gouldingi* (Fink and Machado-Allison 1992) (Characiformes: Serrasalminae) in 'drowned' rivers of the eastern Amazon. *Environ Biol Fish* 98, 11–22.
- Querol MVM, Querol E and Gomes NNA** (2002) Fator de condição gonadal, índice hepatossomático e recrutamento como indicadores do período de reprodução de *Loricariichthys platymetopon* (OSTEICHTHYES, LORICARIIDAE), Bacia do rio Uruguai médio, sul do Brasil. *Iheringia, Sér Zool* 92, 79–84.
- Rezende FP, Filho OPR, Pereira MM, Takabatake ÉY, Navarro RD, Santos LC, Silva RF and Filho CBC** (2005) Eficiência de Diferentes Substratos na Desova de Lambari Tambiú (*Astyanax bimaculatus* Linnaeus, 1758). *Rev Ceres* 52, 527–33.
- Rodrigues MS, Siqueira-Silva DH, Quirino PP, Ninhaus-Silveira A and Verissimo-Silveira R** (2015) Spermatogenesis in the yellowtail tetra *Astyanax altiparanae*: a histological analyses with emphasis to spermatogonial and spermatid types. *Bol Inst Pesca* 41, 697–705.
- Santos DCM, Cupertino MC, Matta SLP, Oliveira JA and Santos JAD** (2015) Histological alterations in liver and testis of *Astyanax* aff. *bimaculatus* caused by acute exposition to zinc. *Rev Ceres* 62, 133–41.
- Santos RA, Campos EC, Camara JJC and Mandelli Jr J** (1991) Curvas de maturação gonadal e crescimento de fêmeas de tambiú, *Astyanax bimaculatus* Linnaeus, 1758 (Characiformes, Characidae), na represa de Ibitinga, Estado de São Paulo, Brasil. *Bol Inst Pesca* 18, 1–11.
- Siqueira-Silva DH, Vicentini CA, Ninhaus-Silveira A and Verissimo-Silveira R** (2013) Reproductive cycle of the neotropical cichlid yellow peacock bass *Cichla kelberi*: a novel pattern of testicular development. *Neotrop Ichthyol* 11, 587–96.
- Siqueira-Silva DH, Rodrigues MS and Nóbrega RH** (2018) Testis structure, spermatogonial niche and Sertoli cell efficiency in neotropical fish. *Gen Comp Endocrinol* 273, 218–26.
- Vazzoler AE** (1996) *Biologia da reprodução de peixes teleosteos: teoria e prática*. São Paulo: EDUEM – Editora da Universidade Estadual de Maringá 1, 146 pp.
- Vilela C and Hayashi C** (2001) Desenvolvimento de juvenis de lambari *Astyanax bimaculatus* (Linnaeus, 1758), sob diferentes densidades de estocagem em tanques-rede. *Acta Sci* 23, 491–6.
- Weber AA, Arantes FP, Sato Y, Rizzo E and Bazzoli N** (2012) Oocyte adhesiveness and embryonic development of *Astyanax bimaculatus* (Linnaeus, 1758) (Pisces: Characidae) *Zygote* 21, 198–202.
- Winemiller KO** (1989) Patterns of variation in life history among South American fishes in seasonal environments. *Oecologia* 81, 225–241.
- Zin T, Than AA and Naing TT** (2011) Fecundity (F), gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K) and length–weight relationship (LWR) in *Channa orientalis* Bloch & Schneider, 1801. *Univ Res J* 4, 47–62.