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Reproductive biology of the lambari *Hyphessobrycon santae* (Eigenmann, 1907) (Pisces: Characidae) in the Fazenda Lagoa do Nado Urban Park, São Francisco river basin, Minas Gerais/Brazil.

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Abstract. In order to study the lambari *Hyphessobrycon santae* reproductice cycle, GSI, spawning, sex ratio and first maturation, a total of 223 individuals (124 females and 99 males) were captured, from November /2006 to October/2007 in the Fazenda Lagoa do Nado urban park, Belo Horizonte/Minas Gerais. Through macro and microscopic gonad features and variations in the GSI values, stages of the reproductive cycle were determined: 1: resting, 2: maturing/mature and 3: spawned for females and spent for males. An analysis of the stages of the reproductive cycle showed reproductively active fish year around, with an GSI peaks in March/April and September/ October. Histological characteristics of ovaries containing post-ovulatory follicles next to oocytes in all phases of development indicate that *H. santae* presents multiple spawning. The smallest spawned females were in the 2.5 to 3.0 cm length class, while the smallest spent males were in the 3.0 to 3.5 cm class. This study can be used as a tool for the conservation of small characids in artificial environments that predominate in the São Francisco river basin, in Minas Gerais state.

Keywords: *Hyphessobrycon santae*, Minas Gerais, Reproduction, Spawning type, Sex ratio

Resumo. Biologia reprodutiva do lambari Hyphessobrycon santae (Eigenmann, 1907) (Pisces: Characidae) no Parque Urbano Fazenda Lagoa do Nado, bacia do rio São Francisco, Minas Gerais/Brasil. Para estudar o ciclo reprodutivo, IGS, tipo de desova, proporção sexual e tamanho da primeira maturação sexual do lambari *Hyphessobrycon santae,* capturou-se um total de 223 exemplares (124 fêmeas e 99 machos), no período de novembro/2006 a outubro/2007 no parque urbano Fazenda Lagoa do Nado, Belo Horizonte/Minas Gerais. Através de características macro e microscópicas das gônadas e as variações dos valores do IGS determinou-se os estádios do ciclo reprodutivos: 1:repouso, 2:em maturação/maduro e 3: desovado para fêmeas e espermeado para machos. A análise dos estádios do ciclo reprodutivo mostrou peixes em atividade reprodutiva o ano todo, com picos de IGS em março/abril e setembro/outubro. Características histológicas de ovários contendo folículos pós-ovulatórios ao lado de ovócitos em todas as fases de desenvolvimento, indicam que *H. santae* apresenta desova do tipo parcelada. As menores fêmeas desovadas estavam na classe de comprimento 2,5 a 3,0, já os menores machos espermeados estavam na classe 3,0 a 3,5. Esse estudo pode ser usado como ferramenta para a preservação de pequenos caracídeos em ambientes artificiais que predominam na bacia do rio São Francisco no estado de Minas Gerais.

Palavras-chave: Hyphessobrycon santae, Minas Gerais, Reprodução, tipo de desova , razão sexual

Introduction

Neotropical fish of inland waters are distinguished by having an immense diversity of species and behavioral patterns. Each species possesses a unique reproductive strategy, in addition to numerous morphophysiological adaptations, directly associated to their evolutionary history (Agostinho *et al.* 2007).

Reproduction contributes to the continuation of populations thusly, learning about specie's reproductive strategies helps establish goals for the administration, management and preservation of ichthyofauna (Lourenço *et al.*, 2008).

Characidae is the largest and most complex family within the order Characiformes with nearly 250 genera in South America (Britski *et al.* 1988). The genus *Hyphessobrycon* is native to South America and is one of the several genera listed as *incertae sedis*. The species, *Hyphessobrycon santae* commonly known as lambari or piaba, is found in the São Francisco basin (Reis *et al*., 2003). Similar to other foraging fishes, it possesses an important role in the maintenance of the food chain, acting as prey for a wide array of piscivore of commercial interest (Bazzoli *et al.* 1997). According to Pompeu & Godinho (2006), this species is considered to be invertivore and does not tolerate low oxygen levels.

The reproductive strategies of foraging fishes have been studied by several authors (Braga & Gennari Filho, 1990; Bazzoli *et al.* 1997; Mazzoni & Silva, 2006); yet, studies focusing on small Characiformes and specially *Hyphessobrycon* are still rare in Brazil. Therefore our objective was to study aspects of the reproduction of this species in an effort to provide additional knowledge concerning its reproductive biology in São Francisco river basin, state of Minas Gerais, southeastern Brazil.

Materials and Methods

Study area

The Fazenda Lagoa do Nado urban park contains the largest contiguous green area in the Córrego do Nado sub-basin, São Francisco basin**,** which is situated in the northern region of Belo Horizonte, Minas Gerais (19° 49' 56'' S, 43° 57 34''W) at an altitude of 77 m. In all, it is an area spanning 300.00 m², of which 1 hectare is inundated (Figure 1) (Bezerra-Neto & Pinto-Coelho 2002).

The damming of three springs located within the confines of the park has led to the formation of a very small reservoir with irregular margins and a conical shape, its average and maximum depth are 2.7 m and 7.6 m respectively (Bezerra-Neto & Pinto-Coelho 2002). The fish fauna is composed by natives *Hyphessobrycon santae* (Eigenmann, 1907), *Hasemania nana* (Reinhardt, 1874), *Callichthys callichthys* (Linnaeus, 1758)*, Gymnotus carapo* Linnaeus, 1758, *Hypostomus* sp., *Australoheros facetus* (Jenyns, 1842), *Geophagus brasiliensis* (Quoy & Gaimard, 1824) and non-natives *Hoplias lacerdae* (Ribeiro, 1908), *Tilapia* sp. and *Poecilia reticulata* (Peters, 1859).

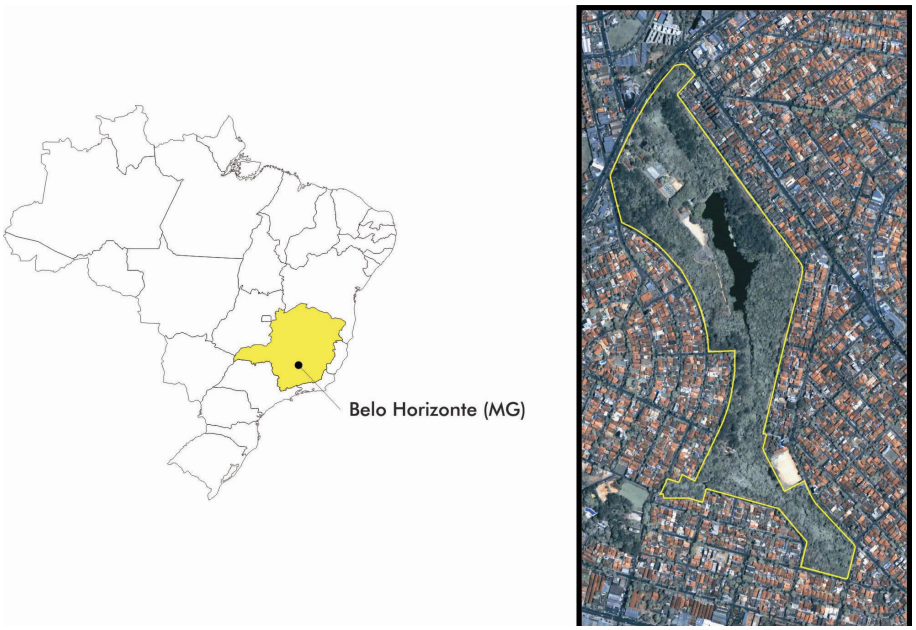

Figura 1. Location of the study site in Belo Horizonte city, Minas Gerais state.

Data collection

Sampling was conducted bi-monthly from November of 2006 to October of 2007 with the aid of two sieves (35x75cm, mesh 0.03mm) and a three hour sampling effort.

A total of 223 species (124 female and 99 males) were collected and fixed in Bouin's fluid or in 10% formaldehyde solution and subsequently transferred to a solution of 70% alcohol for preservation. Voucher specimens were deposited in the fish collection at the Museu de Ciências e Tecnologia PUC-RS (MCP41311). In the laboratory the following biometric data were obtained from each individual: standard length (SL, in cm), body weight and gonad weight (BW e GW respectively, in g), with which the gonadosomatic index was calculated (GSI = GW x 100 / BW).

Gonad fragments were submitted to routine histological techniques, namely encased in paraffin wax, cut at a thickness of between 5 to 7 µm and stained with hematoxylin and eosin.

Based on features at the macroscopic (coloration, volume, flaccidness); and microscopic (distribution of cells of lineage of oogenesis and spermatogenesis) level and the variation of GSI, gonad maturation was evaluated. Spawning was determined based on histological characteristics of spawned ovaries and on analysis of the stages of the reproductive cycle (Bazzoli, 2003).

Males and females were divided into standard length classes (SL, in cm) at 0.5 cm intervals. In these classes, individuals were further separated according to gonad maturation stage in this mannerthe smallest class of reproducing individual was obtained.

Results

Ovaries and testes of *H. santae* are elongated, fusiform, paired organs, located in the lateral-dorsal section of the abdominal cavity, forming a common duct at its tail end which opens up at the urogenital papilla.

Through macro and microscopic gonad features and variations in the GSI values, stages of the reproductive cycle were determined: 1: resting, 2: maturing/mature and 3: spawning for females and spent for males (Table 1).

Reproductive males and females (stage 2) were sampled during the entire study period (Figures 2 and 3) showing the greatest peaks in GSI during the March/April and September/October period (Figure 4). These characteristics showed that the species reproduces throughout the year. The occurrence of reproductively active males (stage 2) in all sampling periods indicates that *H. santae* possesses continuous spermatogenesis (Figure 3).

The greater frequency of reproducing females associated with histological characteristics of spawned ovaries containing post-ovulatory follicles, and also young perinucleolar oocytes (O1), advanced perinucleolar (02), previtellogenic (O3) and vitellogenic oocytes (O4) indicate that the fish possesses asynchronous ovarian development and a multiple spawning type (Figure 5). No totally spawned females and totally spent males were collected.

A greater number of "maturing/mature" individuals were found in the 4.5 to 5.0 cm length class for females and 4.0 to 4.5 cm for males. The smallest spawned females were in 2.5 to 3.0 cm length class, while spent males displayed sizes ranging from 3.0 a 3.5 cm (Figures 6 and 7).

Table 1. Gonadosomatic indexes (GSI), macro and microscopic features of gonadal maturation stages of females and males of H. santae in Lagoa do Nado, Minas Gerais, Brazil, from November/December 2006 to September/October 2007.

Stages	Females	Male
1 Resting	GSI = 0.60 ± 0.38 Fusiform, translucent and this ovaries containing only oogonias, initial perinucleolar oocytes (O1) and advanced perinucleolar oocytes (O2).	GSI = 1.12±0.67 Fusiform and transparent testes. Lumen of closed seminiferous tubules and with spermatogonia.
2 Maturing / mature	GSI = 5.62±2.90 Yellowish and voluminous ovaries with oocytes visible. Ovaries containing O1, O2, previtellogenic (O3) and vitellogenic (O4) oocytes.	GSI = 1.79±1.01 Whitish and voluminous testes. Lumen seminiferous tubules with large quantity of spermatozoa.

Table 1. Continuação.

3 Spawned / spent	GSI = 5.41±2.17 Flaccid and hemorrhagic, with some oocytes visible. Presence of oogonias, O1, O2, post-ovulatory follicles, in addition to rare O3 and O4 in follicular atresia.	GSI = 1.63±0.83 Reduced volume, flaccid and hemorrhagic testes. Lumen of open seminiferous tubules with residual spermatozoa and wall made up of spermatogonias.

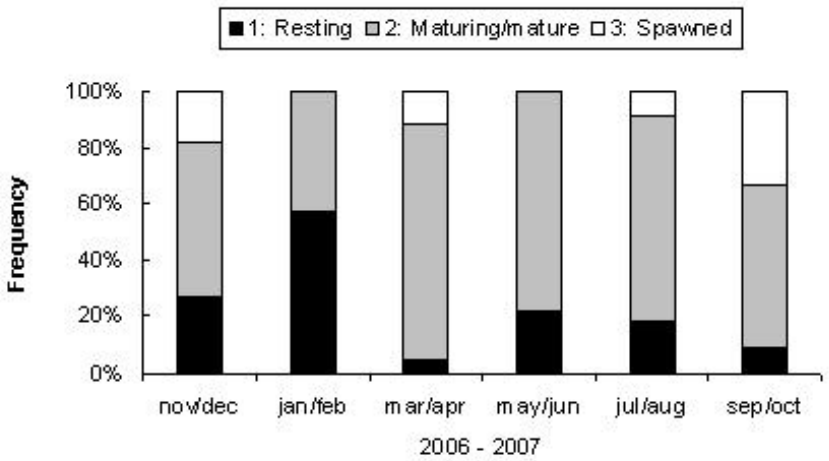

Figura 2. Frequency of the gonadal maturation stages of H. santae females in the Lagoa do Nado, Minas Gerais, from November 2006 to October 2007.

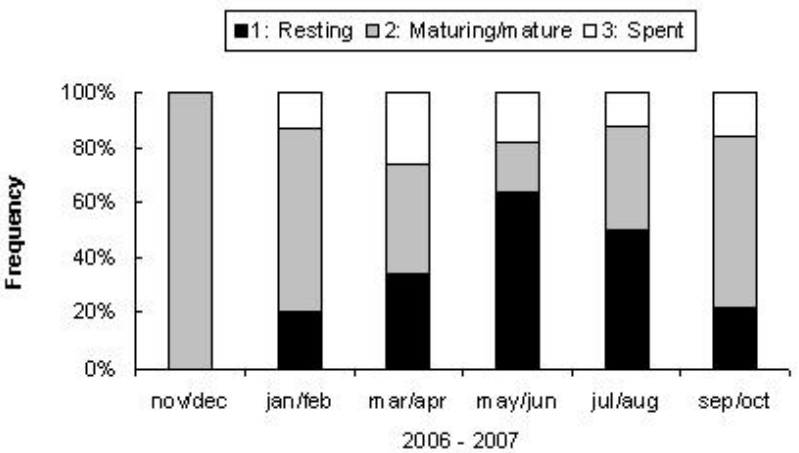

Figura 3. Frequency of the gonadal maturation stages of H. santae males in the Lagoa do Nado, Minas Gerais, from November 2006 to October 2007.

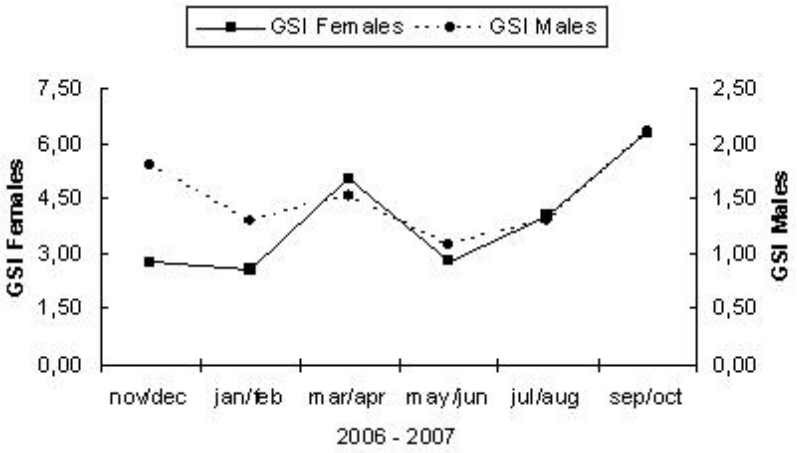

Figura 4. Bimonthly variation of gonadosomatic index (GSI) values on females and males of H. santae of Lagoa do Nado, Minas Gerais between November 2006 and October 2007.

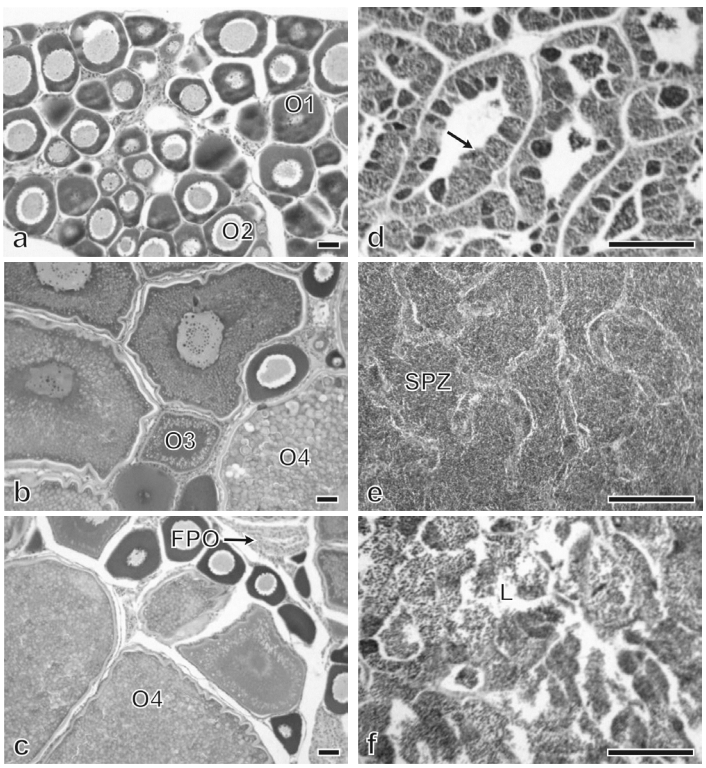

Figura 5. Histological slides if gonads of H. santae females (a - c) and male (d - f) in Lagoa do Nado (hematoxylin and eosin). a – Resting ovary with initial perinucleolar oocytes (O1) and advanced perinucleolar oocytes (O2); b – Maturing / mature ovary containing previtellogenic (O3) and vitellogenic oocytes (O4); c – Spawned ovary showing post-ovulatory follicles (FPO) and vitellogenic oocytes (O4); d – Resting testes with spermatogonias in the walls (arrows); e – Maturing / mature testicle showing seminiferous tubules with lumen full of spermatozoa (SPZ); f – Spent testicle with emptied seminiferous tubules with opened lumen (L), presenting some residual spermatozoa. Bar = 200µm

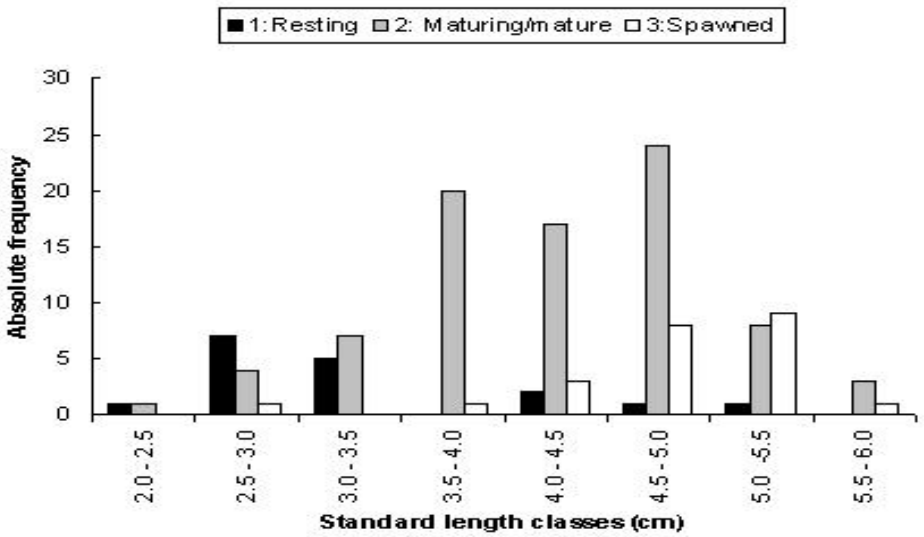

Figura 6. Distribution of standard length (SL) classes by reproductive cycle stage (RCS) of females of H. santae the Lagoa do Nado, Minas Gerais, from November 2006 to October 2007.

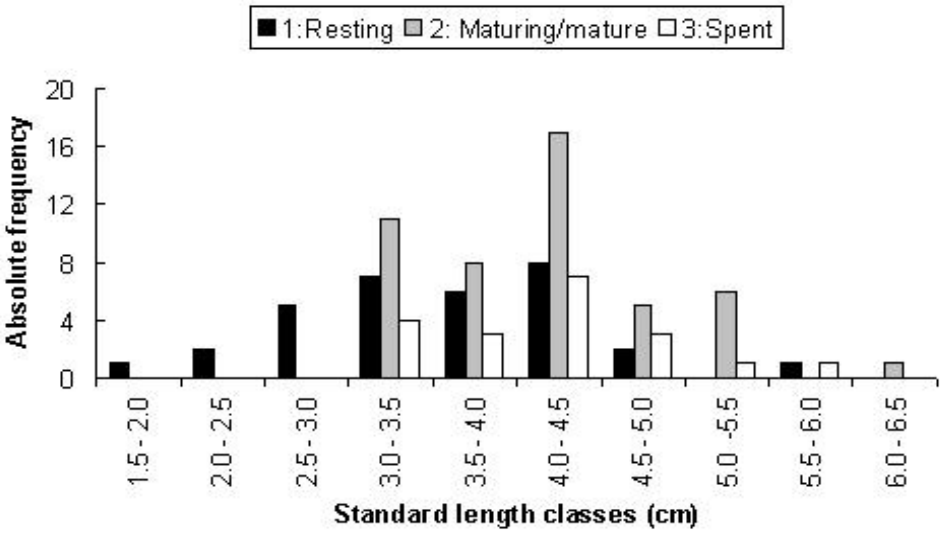

Figura 7 Distribution of standard length (SL) classes by reproductive cycle stage (RCS) of males of H. santae the Lagoa do Nado, Minas Gerais, from November 2006 to October 2007.

Discussion

Ovaries and testicles of *H. santae* possess characteristics similar to those described for other Characiformes according to Bazzoli (2003). Determining the stages of the reproductive cycle is an essential parameter to comprehending a species behavior within its native habitats (Barreto *et al.*, 1998). The stages of the reproductive cycle were similar to those found by Thomé *et al.* (2005) for *Leporinus taeniatus* in Juramento Reservoir, São Francisco River basin.

Reproducing males and females of *H. santae* were found in every sampling period, a characteristic similar to that found for other foraging fish such as *Hemigrammus marginatus*, *Moenkhausia costae*, *Roeboides xenodon* and *Astyanax bimaculatus* (Bazzoli *et al*., 1997; Bazzoli *et al*., 1998). The reproduction of the species is prolonged probably in response to tropical conditions of Southern Hemisphere like absence of well-defined seasons (Lowe-McConnell, 1987).

The variation of the GSI for males was discreet, if compared to that of the females, this is due to the difference between testicles and ovaries (in terms of volume, mass and the energy required during the production of gametes) (Ribeiro *et al.*, 2007). According to Vazzoler (1996), the GSI provides indications about the reproductive period of a species or population. The increase of the GSI together with gonad modifications in the March/April and September/October period showed that *H. santae* was at its reproductive peaks, similar to that found for various species of Characiformes (Bazzoli *et al.*, 1997; Ricardo *et al.*, 1998; Lampert *et al.*, 2004; Carvalho *et al*., 2009). The small variation of the GSI with the microscopic characteristics showed that *H. santae* appears to have continuous spermatogenesis. Most likely, following sperm, the testicles recover rapidly and reinitiate a new cycle (Barbieri, 1992). These characteristics have been observed for other Characiformes such as *Acestrorhynchus lacustris* (Bazzoli & Godinho, 1991) and *Tetragonopterus chalceus* (Ricardo *et al.*, 1998).

Reproductive strategies seek to increase the success of the reproduction of the species. Fractionated spawning is frequent in fish species living in lentic environments, represents a mechanism through which certain species increase the number of oocytes they produce in a reproductive period, to a much greater level than would be predicted based on their size (Vazzoler, 1996; Ratton *et al*., 2003). According to Barbieri (1992), multiple spawning permits the asynchronous development of the larvae and, congruently, the occupation of distinct niches between larger and smaller individuals, avoiding competition for spawning locations and food for larvae. Long and multiple spawning is an efficient strategy observed in *H. santae* in the production of future offspring, it has also been shown by Kramer (1978) and Burt *et al.* (1988) for *Hyphessobrycon panamensis* and *Hyphessobrycon pulchripinnis* which both spawn several times during mating.

The difficulty in separating young, immature individuals and adults at rest made it impossible to use the calculation of first sexual maturation described by Vazzoler (1996). Therefore, we determined first sexual maturation based on the smallest male and female spent or spawned, respectively, a procedure similarly used by Bazzoli (2003) and Gonçalves *et al.* (2006). In the presente study, females spawned early compared to the length found for the species of *Astyanax* (Barreto *et al*., 1998; Veregue & Orsi, 2003) and *Bryconamericus* (Mazzoni & Silva, 2006) suggests that is related to an adaptive behavior probably due to the small artificial environment and massive presence of non-native fishes *Hoplias lacerdae*, *Tilapia* sp. and *Poecilia reticulata* (Sales, personal observation).

Knowledge of reproductive tactics in forage fish species such as *H. santae* is fundamental to comprehend the species life cycle strategies, which are crucial to direct administration, management, and preservation measures from certain human actions, such as urbanization, elimination of reproductive areas by constructing dams and introduction of non-native fish (Gomiero & Braga, 2007).

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