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Astyanax altiparanae OVARIAN MATURATION AFTER SPAWNING IN WATER RECYCLING SYSTEMS*

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ABSTRACT

In this study, we evaluated the possibility of obtaining successive *Astyanax altiparanae* spawns under laboratory conditions. In order to do so, 104 specimens were randomly distributed into four boxes (10 females and 16 males each) and kept in a recirculation system at an average temperature of 29.24 ± 0.42 °C, under natural photoperiod, for 30 days. On the onset of the experiment, males and females were induced for reproduction with a 6 mg kg^{-1} carp pituitary extract dose. After that, ovary (for gonadosomatic index and stereological assessment) and blood samples (for steroid evaluation) were collected from eight females (two per box) at the following moments: immediately after hormonal induction (day 0) and on days 1, 6, 16 and 30 after spawning. On day 6, spawned females presented complete mature ovaries similar to those on day 0 and, in this period, we did not observe postovulatory complexes, indicating that their resorption happened very fast. Concomitantly, the steroid levels increased gradually up to day 6, which corroborated an intense vitellogenic activity in this period. This study has demonstrated that *A. altiparanae* females are suitable for induced spawning within six days after spawning, when kept at 29.24 ± 0.42 °C, in a system maintained with recirculated water.

Key words: successive spawning; vitellogenesis; estradiol; 17α-hydroxyprogesterone.

MATURAÇÃO OVARIANA DE *Astyanax altiparanae* APÓS DESOVA EM SISTEMA DE RECIRCULAÇÃO DE ÁGUA

RESUMO

Neste estudo, avaliamos a possibilidade de obtenção de desovas sucessivas de *Astyanax altiparanae* mantidos em biotério. Para isso, 104 indivíduos foram distribuídos aleatoriamente em quatro caixas (10 fêmeas e 16 machos em cada) e mantidos a uma temperatura média de 29,24 ± 0,42 °C, sob fotoperíodo natural, por 30 dias. No início do experimento, machos e fêmeas foram induzidos à reprodução com uma dose de extrato de hipófise de carpa (6 mg kg^{-1}). Posteriormente, amostras de ovário (para cálculo do índice gonadosomático e avaliação estereológica) e de sangue (para avaliação de esteroides) foram coletadas de oito fêmeas (duas por caixa) nos seguintes períodos: imediatamente após a indução hormonal (dia 0) e 1, 6, 16 e 30 dias após a desova. No dia 6, as fêmeas desovadas já apresentavam ovários maduros completos similares aos descritos no dia 0. No dia 6 não foram mais observados complexos pós-ovulatórios, indicando que a sua reabsorção foi relativamente rápida. Concomitantemente, os níveis de esteroides aumentaram gradativamente até o dia 6, corroborando uma atividade vitelogênica intensa neste período. Neste estudo demonstramos que fêmeas de *A. altiparanae* apresentam-se aptas para indução hormonal seis dias após a reprodução, quando mantida a 29,24 ± 0,42 °C, em um sistema mantido com água recirculada.

Palavras-chave: desovas sucessivas; vitelogênese; estradiol; 17α-hydroxiprogesterona.

INTRODUCTION

Lambari (*Astyanax altiparanae*) is a small-sized South American fish, whose farming aims mainly at the market of live bait sport fishing. Even with a significant increase in its annual production (6% between 2013 and 2014 (IBGE, 2014)), currently it does not meet the existing demand (ROSA and SIMÕES, 2016). This species is also important for fishing, as well as for restocking programs (HASHIMOTO *et al*., 2015), and has been widely used as a model in studies concerning fish reproduction (SILVA *et al*., 2011; WEBER *et al*., 2013; GOMES *et al*., 2013; ADOLFI *et al*., 2015; CHEHADE *et al*., 2015; YASUI *et al*., 2015; SANTANA *et al*., 2015; KIDA *et al*., 2016).

A. altiparanae attains full maturity relatively fast (only four months) and spawns easily with the slightest variation in water levels in captivity (CHEHADE *et al*., 2015), but in order to ensure the production of a sufficient number of fingerlings, hormonal induction protocols are applied for farming purposes (FELIZARDO *et al*., 2012). *A. altiparanae* is an egg laying without parental care (SUZUKI *et al*., 2005), multiple spawning fish (SATO *et al*., 2006; CHEHADE *et al*., 2015) which can spawn over the year; however, the peak of its spawning activity happens in the warmer months - spring and summer (PRADO *et al*., 2011; CASSEL *et al*., 2017; JESUS *et al*., 2017). Differently from most tropical egg layers (ZANIBONI-FILHO and WEINGARTNER, 2007; PORTO-FORESTI *et al*., 2010; SCHORER *et al*., 2016; PEREIRA *et al*., 2017), *A. altiparanae*'s final maturation and ovulation are achieved through a single carp pituitary extract (CPE) dose (SATO *et al*., 2006).

Due to an increased demand and consequent interest in farming this species, we have observed that farmers find it necessary to have tools in order to increase productivity and make *A. altiparanae*'s reproduction more productive and organized. In this context, obtaining successive ovarian maturation and spawning in laboratories, with recirculated water, allows them to reduce both the costs of *A. altiparanae*'s breeding management and the typical waste of water of earthen ponds (OZÓRIO *et al*., 2004). Moreover, the maintenance of breeders in small boxes in laboratory under controlled conditions promotes a higher efficiency, control and organization for directed crosses for fish stocking and for genetic improving programs (HASHIMOTO *et al*., 2015). From a physiological point of view, the standardization of protocols for successive breedings allows a continuous production of fingerlings, thus avoiding long ovarian resting and regression periods, which are common in tropical migratory species (ABREU *et al*., 2015; MORAIS *et al*., 2016; PEREIRA *et al*., 2013; KURADOMI *et al*., 2017), such as *A. altiparanae* (CASSEL *et al*., 2017).

Recently, it has demonstrated that this species could be manipulated to spawn out of the breeding season by keeping breeders under some specific conditions. It has been shown an increased spawning performance by keeping breeders under controlled conditions when compared to fish maintained in external earthen ponds (DOMICIANO, 2015). Therefore, since the species can be manipulated to improve reproductive performance, the main objective of this study was to investigate if the same *A. altiparanae* females could be manipulated to spawn successive times under controlled conditions with water constantly maintained at about 29 °C.

METHODS

Animals and culture conditions

For this study, we used male and female adult *Astyanax altiparanae* specimens (12 months old, mean ± SE total length 9.75 ± 0.60 cm, and weight 14.32 ± 3.11 g), reared in 200 m^3 outdoor earthen ponds at nearly 500 fish m^{-3} density, with constant running water at 30 L min^{-1} flow at the Aquaculture Center, Sao Paulo State University (Jaboticabal, SP, BR) (CAUNESP). Fish were fed six days a week, in two portions at 9:00 h and 16:00 h, with a commercial extruded diet for omnivores [crude protein (28.0% (maximum)), ethereal extract (3.5% (minimum)), fibrous matter (9.0% (maximum)), ash (12.0% (maximum)), calcium (1.2% (maximum)), phosphorus (0.6% (minimum))], until apparent satiety. Fish were obtained by induced spawning at CAUNESP.

Water parameters were measured weekly at 8:00 h: dissolved oxygen (oximeter 550A (Yellow Spring Incorporation, with an accuracy of ± 0.3 mg L^{-1})), pH (pH meter pH10A (Yellow Spring Incorporation - within ± 0.2 units)), electrical conductivity and temperature of the water (HI 98311 (Hanna Instruments - accuracy 0.1 µS cm^{-1} conductivity and 0.15 °C for temperature)).

Experimental design and analysis

In September (Spring), one hundred and four adult specimens (40 females and 64 males) were selected for their external characteristics of mature individuals: bulging belly and hemorrhagic urogenital papilla (for females) and the presence of spikes on the anal fin (for males). After the selection, fish were acclimated to the laboratory conditions, equipped with a water recirculation system and set at a constant temperature of 29.24 ± 0.42 °C, under a natural photoperiod. After that, fish were randomly distributed into four rectangular acrylic water boxes (91 × 25 × 10 cm) with the following composition: 10 females and 16 males in each box. Each box was considered as an experimental unit. The beginning of the experiment (day 0) was characterized by the time of the hormonal induction, when all females and all males received a 6 mg CPE kg^{-1} dose diluted in 0.2 mL saline solution (0.9%) injected in the dorsal region of the body. Hormonal induction started at 21:30 h (day 0). Fish were sampled at five different times over the 30-day experimental period: one sampling immediately after the hormonal induction (day 0) and four post-hatching samplings (day 1, 6, 16 and 30).

Spawning occurred in a "semi-natural" system. The period between the hormonal induction and ovulation was determined through a visual record of the first eggs in the box. Immediate 1 day after spawning, males were removed from the boxes to prevent further spawning. All females were evaluated during their post spawning periods and those that did not spawn (remained with bulging belly and did not eliminate residual oocytes) were removed from the experiment to avoid mistaken interpretations. Ovulated females of this species are easily recognized for releasing oocytes by gentle abdominal compression, even after ovulation.

In each sample, eight females were randomly chosen (two per box) and euthanized with an overdose of benzocaine (500 mg L^{-1}). However, among the 32 induced females, only two did not spawn and were removed from the experimental units to avoid misinterpretations. So, in the last analysis period (day 30) only one female (but not two as initially proposed) from two boxes were analyzed (totalizing 6 females in this sampling).

The following biometric data were recorded: body weight (g), total length (cm), standard length (cm), and weight of gonads (g). Subsequently, the gonadosomatic index (GSI) was calculated through the following formula: [(weight of gonads / body weight) x 100]. From the same females, blood samples were collected using disposable syringes and needles previously washed with anticoagulant (sodium heparin 5000 U.I. mL^{-1}). The blood was transferred into micro tubes (2 mL) and centrifuged at 2640 rpm for 10 minutes to obtain the plasma, this plasma was stored in liquid nitrogen. From the same females, ovarian samples (cranial, middle and caudal portions), in duplicate, were fixed in modified Karnovsky's solution (4% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M PBS buffer pH 7.2) for 24 hours and then stored at 4 °C.

Reproductive performance

After fertilization, all the eggs from each one of the experimental units were collected to determine the ova volume. From each experimental unit, four samples of 0.5 mL eggs were fixed in Karnovsky's solution for subsequent counting the number off eggs in a stereomicroscope (Leica M50) and the total number of eggs was estimated by extrapolating the average number of eggs in the samples. The average fecundity rate (AFR = total number of oocytes released per unit / sum of body weight of spawned females per unit). After that, 4 mL of eggs (from each experimental unit) were transferred to a 7 L conic shape incubator (three replicates per experimental unit = 12 incubators). Fertilization rates (FR = percentage of fertilized oocytes, 4-7 hours post-fertilization at gastrula stage) and hatching rates (HR = percentage of larvae that hatched, 15-20 hours post fertilization) were estimated by evaluating approximately 100 randomly collected eggs per incubator. In order to do so, the following formulae were used: FR = the number of viable eggs / total number of eggs counted per sample (approximately 100) * 100, and HR = viable eggs (with embryonic development) / total number of eggs counted per sample (approximately 100) * 100.

Stereological analysis of the ovaries

Ovary samples were embedded using the kit resin (Technovit 7100, Kulzer Histo-Technik), in 3 µm thick sections. The histological sections were stained with hematoxylin-eosin. For the volume density analysis, we applied the same methodology used by CRISCUOLO-URBINATI *et al.* (2012) with some adaptations. Briefly, we considered the following types of structures: previtellogenic oocytes (PVO), cortical alveoli oocytes (CAO), incomplete vitellogenic oocytes (IVO), complete vitellogenic oocytes (CVO), GVBD oocytes that were not ovulated (NO), post-ovulatory complex (POC), atretic oocytes (AO) and interstitial tissue (IT). The artefacts were rare and were not included. The volume density occupied by each structure was determined. To do so, we used three microscopic fields (5 x objective) of three ovarian regions (totalizing nine ovarian microscopic fields). Counts were performed on a 352 points grid. The number of points from each of the aforementioned structures was used to determine their frequencies (n. points / total points * 100).

Steroid hormones

Plasma levels of estradiol (E_2) and 17α-hydroxyprogesterone (17α-OHP) were quantified in plasma through ELISA, using commercial kits (DRG Instruments GmbH, Inc. Frauenbergstr, Marburg, Germany), according to the manufacturer's instructions. The absorbance rates were read at 450 nm using a microplate reader (Epoch2, Bio Tek Instruments, Inc., Highland Park, Winooski, USA). All samples were analyzed in duplicate, and the validation of the kits was determined by calculating the inter- and intra-assay coefficients of variation. The acceptable limit for the intra- and inter- assay was ≤ 20.

Statistical analysis

Statistical analyses were performed using the STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA) and Excel (Microsoft, Redmond, USA). We used the Kruskal-Wallis one-way analysis of variance followed by multiple comparisons ($P < 0.05$); therefore, the results were expressed as a mean followed by the interquartile range and maximum and minimum values.

Study certification

Fish were treated and euthanized according the protocols approved by the Ethics Committee on Animal Use (CEUA), Universidade Estadual Paulista "Julio de Mesquita Filho" - Jaboticabal, SP (5726/15).

RESULTS

Concerning the physical and chemical characteristics of water, average values obtained for water characteristics were: 29.24 ± 0.42 °C; 7.66 ± 0.08 pH; 5.74 ± 0.14 mg L^{-1} of dissolved O_2; and 182.38 ± 5.53 µS for water conductivity.

Hormonal induction started at 21:30 h (day 0), the first and last spawning were respectively observed at five and nine hours after that.

Among the 32 induced females, only two did not spawn and were removed from the experimental units. The four experimental units together gave raise to ~ 100 mL of eggs, containing approximately 457.00 ± 17.47 eggs in each 0.5 mL of egg. Therefore, according to our estimate, a total of ~ 91,466 eggs were obtained. The relative fecundity was 216.71 ± 56 eggs per gram of female; thus, we concluded by extrapolation that a female of average size weighing 20 g would release ~ 4320 eggs. The average fertilization and hatching rates were 56.26. ± 23.55% and 54.42 ± 20.49%, respectively.

The mean values observed during the experiments for GSI are shown in Figure 1. GSI values for day 1 were lower than all other

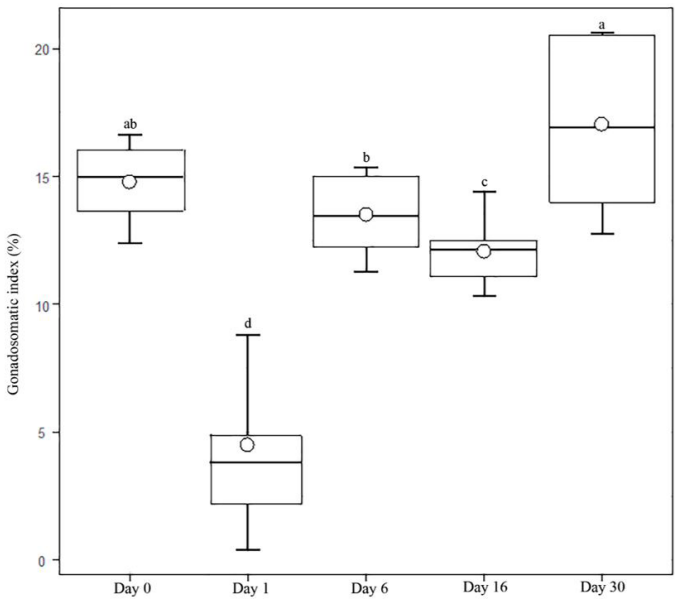

Figure 1. Gonadosomatic index (GSI) over time of *A. altiparanae* females. Different letters indicate a significant difference ($P < 0.05$). Kruskal Wallis test shows the maximum and minimum values (extreme vertical line), the 1st and 3rd values quartile (ends of the rectangle), median (line segment inside the rectangle) and mean (circle inside the rectangle).

periods. Values for day 0 and day 6 were similar, but higher than day 16. Values for day 30 were similar to day 0, but higher than all other periods. The morphological aspects of oocytes and ovarian structures considered in this study are respectively shown in Figures 2 and 3.

On day 0, immediately before the induced spawning, control females presented their ovaries which were predominantly occupied by CVO (~ 80%). Immediately after spawning, on day 1, due to ovulation, the volume density of CVO significantly decreased to ~ 6%

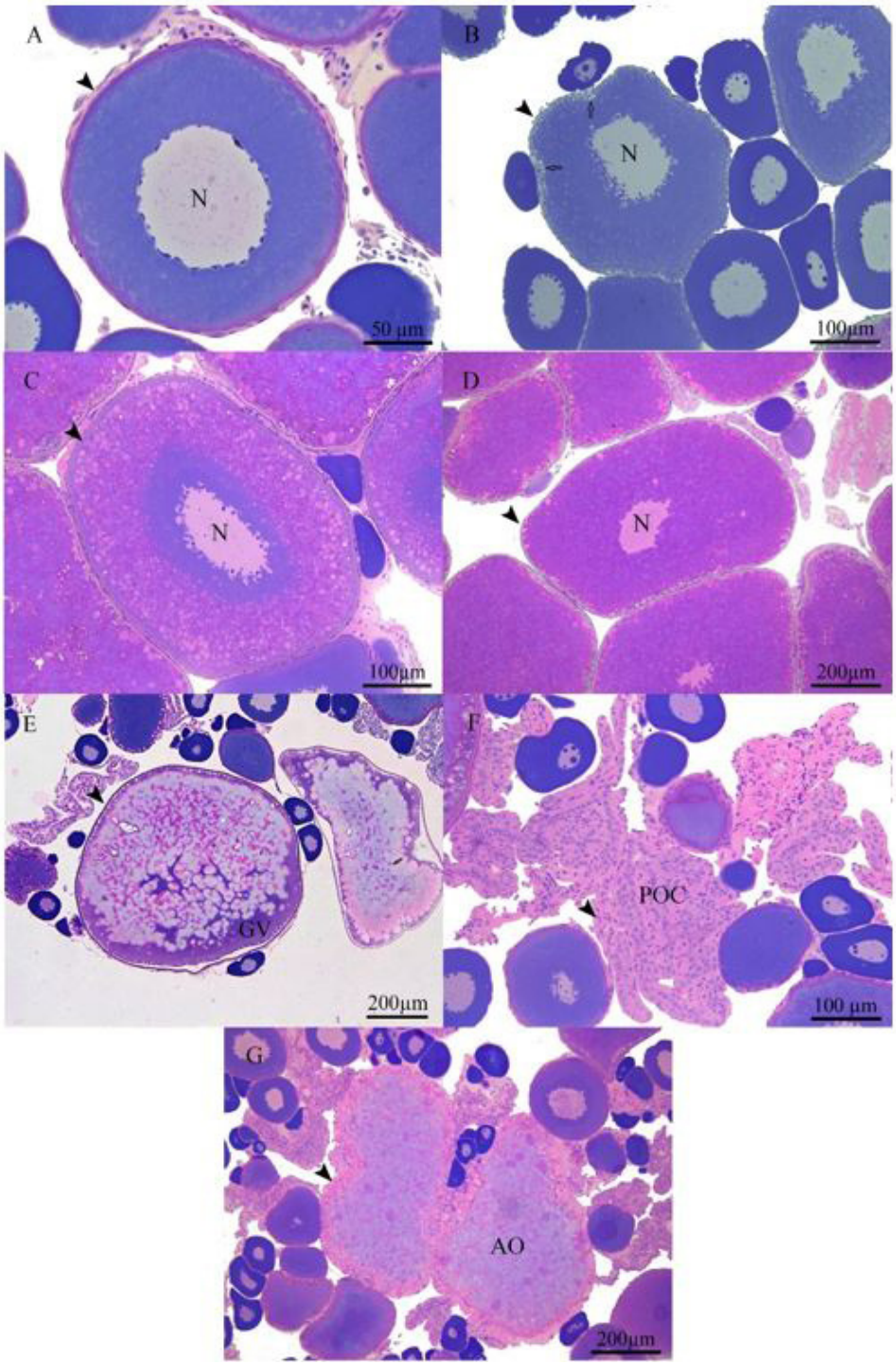

Figure 2. Photomicrographs of histological sections of *A. altiparanae* structures considered in this study. (A) Previtellogenic oocyte; (B) Cortical alveoli oocyte (Arrowhead). Open arrow: cortical alveoli; (C) Incomplete vitellogenic oocyte (Arrowhead); (D) Complete vitellogenic oocytes (Arrowhead); (E) GVBD oocytes that were not ovulated (Arrowhead); (F) Post-ovulatory complex (Arrowhead); (G) Atretic oocytes (Arrowhead). Staining with hematoxylin-eosin. AO = Atretic oocyte; GV: Germinal vesicle; N = Nucleus; POC = Post-ovulatory complex.

(Figure 4D). On day 6, similar to day 0, ovaries were again mature and predominantly filled by CVO (~ 70%) (Figures 3A, C and 4D). On the other hand, after day 6, GSI values kept on varying with a subsequent slight decrease and increase, respectively, on days 16 and 30.

Concerning the gonadal steroid levels, except for day 6, mean values of E_2 and 17α-OHP were similar over the period of analysis (Figures 5A and B). On day 6, the mean for plasma levels of E_2 (1.99 ng mL^{-1}) was superior to day 0 (0.62 ng mL^{-1}), but similar in other periods (Figure 5A). In the same period, the mean values for

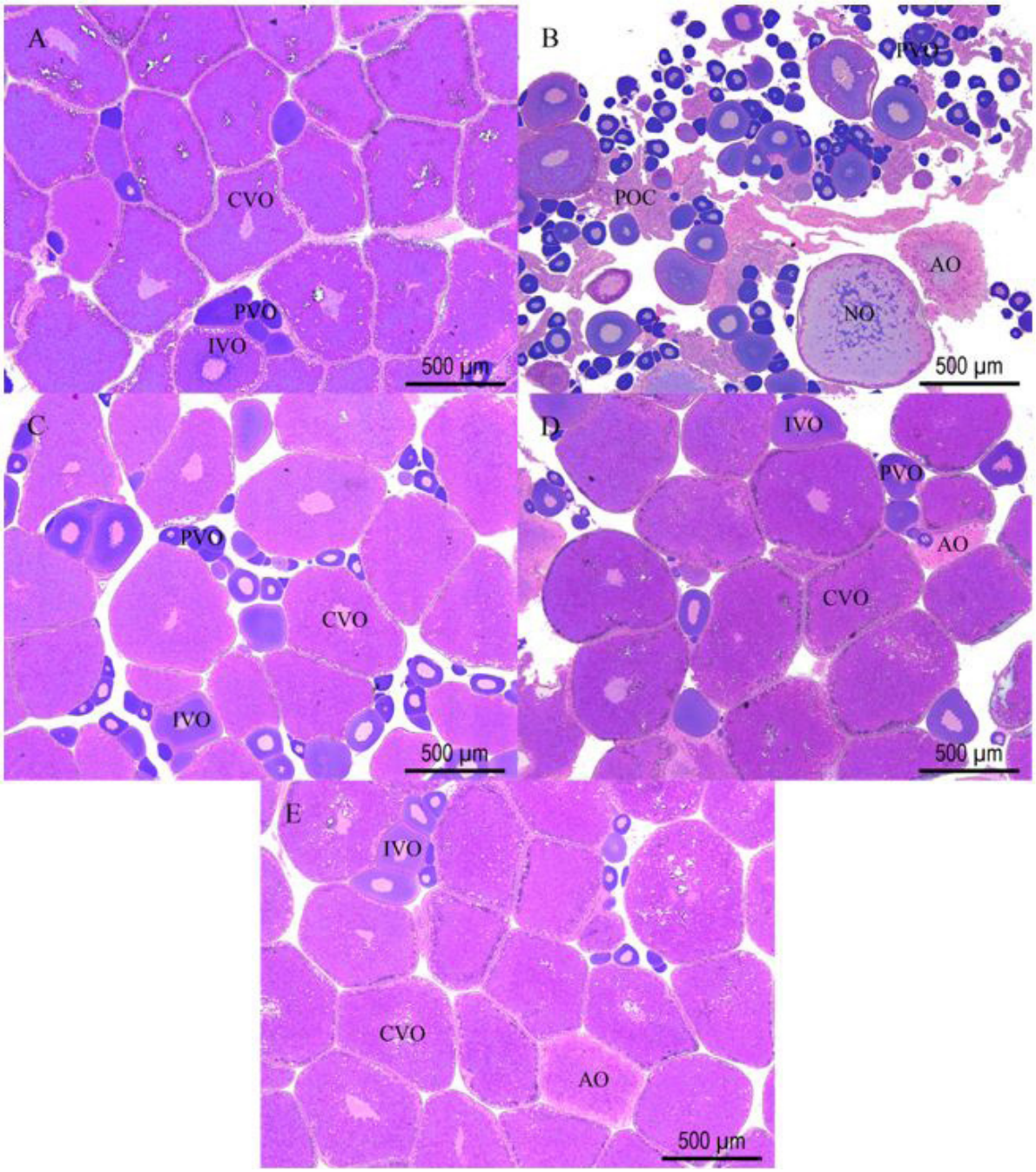

Figure 3. Photomicrographs of representative histological sections of ovarian *A. altiparanae* in each sampling of this study. (A) Day 0: ovaries predominantly occupied by CVO, but also containing PVO and IVO; (B) Day 1: ovaries predominantly occupied by PVO and POC. CAO, CVO, AO and NO were present in reduced amounts; (C) Day 6: ovaries predominantly occupied by CVO, but with a relatively high frequency of PVO and IVO; (D) Day 16; and (E) Day 30: Ovaries with similar aspect to Day 0 and Day 6, with a typical appearance of mature ovaries predominantly occupied by CVO. 5x objective. Staining with hematoxylin-eosin. PVO = Previtellogenic oocytes; IVO: Incomplete vitellogenic oocytes; CVO = Complete vitellogenic oocytes; NO = GVBD oocytes that were not ovulated; POC = Post-ovulatory complex; AO = Atretic oocytes.

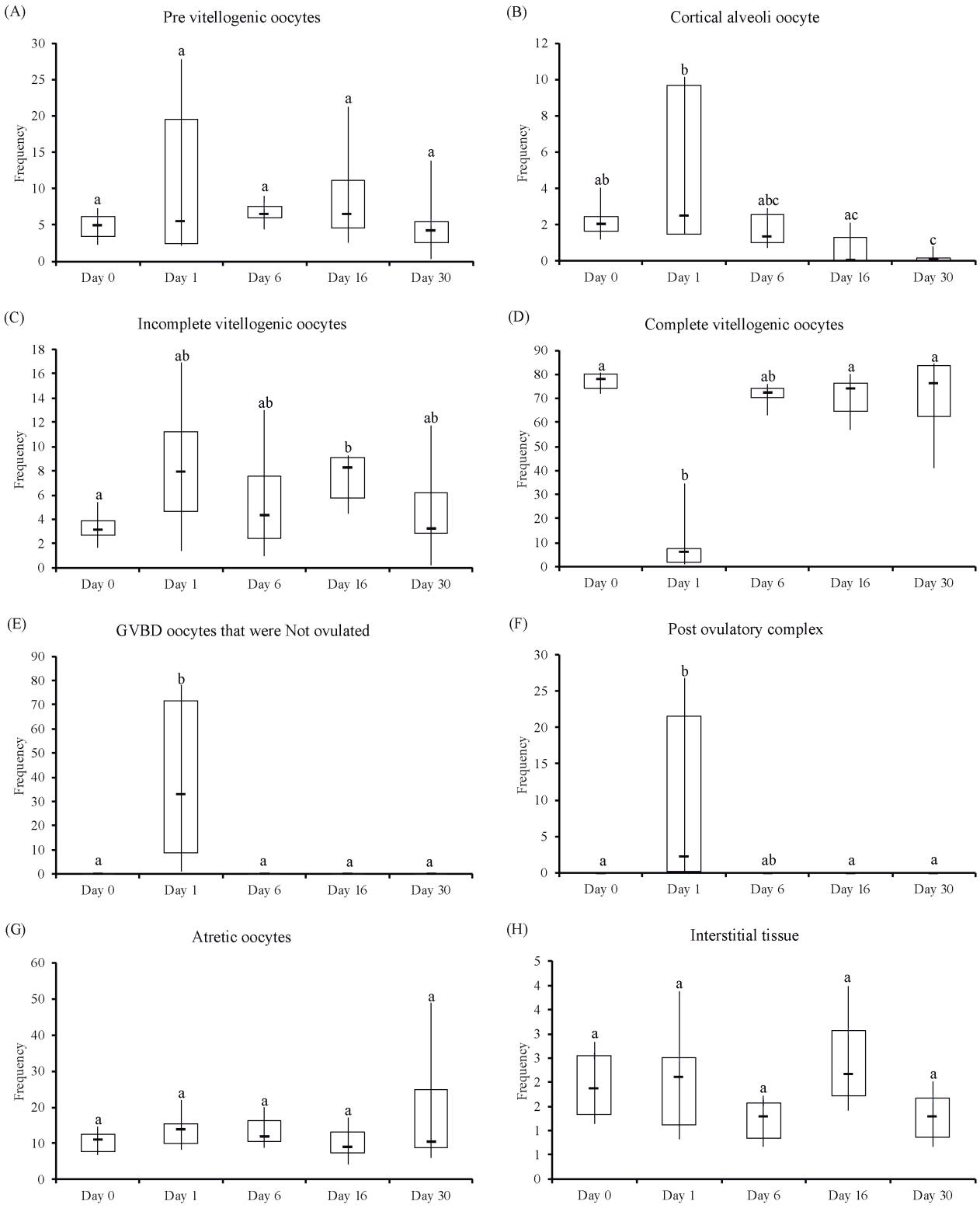

Figure 4. Comparison of the frequency (volume densities (%)) of *A. altiparanae* ovarian structures at different experimental moments. (A) Previtellogenic oocytes (PVO); (B) cortical alveoli oocytes (CAO); (C) incomplete vitellogenic oocytes (IVO); (D) complete vitellogenic oocytes (CVO); (E) GVBD oocytes that were not ovulated (NO); (F) post-ovulatory complex (POC); (G) atretic oocytes (AO); and (H) interstitial tissue (IT). Different letters indicate significant difference ($P < 0.05$). Kruskal Wallis test shows the maximum and minimum values (extreme vertical line), values for the 1st and 3rd quartile (ends of the rectangle) and median (line segment inside the rectangle).

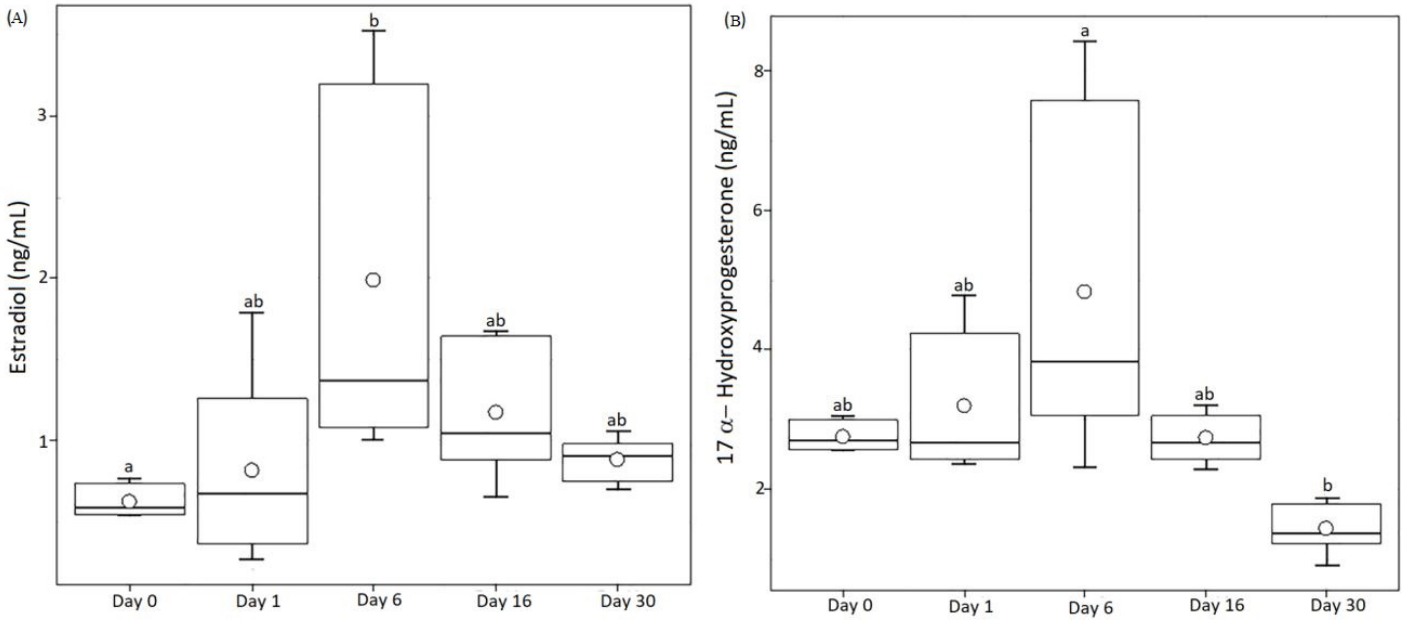

Figure 5. Plasma levels of E_2 (ng mL^{-1}) (A) and 17 α-OHP (ng mL^{-1}) (B) of *A. altiparanae* at different experimental moments. Different letters indicate significant difference (P <0.05). Kruskal Wallis test shows the maximum and minimum values (extreme vertical line), values of the 1st and 3rd quartile (ends of the rectangle), median (line segment inside the rectangle) and mean (circle inside the rectangle).

17α- OHP (4.83 ng mL^{-1}) were respectively higher than on day 30 (1.44 ng mL^{-1}), but similar in other periods (Figure 5B).

DISCUSSION

The interval here observed, between the hormonal induction and the first spawns, was shorter than those described by FELIZARDO *et al*. (2012) and SATO *et al*. (2006) for the same species, which were respectively 11 and 12 hours after hormonal induction. Such differences are probably related to the lower temperature of water used in those studies (27 and 26 °C, respectively) compared to the temperature used in this one (29 °C). This hypothesis is supported by JALABERT (2005), who mentioned the fact that the modulation of physiological processes and endocrine regulation of spawning, in various species, are regulated by water temperature.

On day 0, immediately before the induced spawning, control females presented their ovaries with typical characteristics of mature females (BROWN-PETERSON *et al*., 2011), which were predominantly occupied by CVO (~ 80%) and the same characteristics was observed on ovaries on day 6. The high GSI values confirm this result. In this regard, both GSI and the stereological evaluation confirmed that females had mature ovaries and were theoretically able for a new spawning on day 6.

The six-day interval found in this study for a potential successive spawning induction can be considered very short, comparing to similar data from other fresh water farmed fish species. Successive spawns under captive conditions have been reported, but the gap between spawns is usually longer, e.g., for *Oreochromis niloticus* (9-12 times per year, LAZARD and LEGENDRE, 1996) and *Astronotus ocellatus* (3-4 times per year, SILVA *et al*., 1993), even though those intervals were obtained without controlled temperature conditions in their studies. However, the aim of the present study was not to evaluate the effect of temperature in promoting ovary maturation or under reproductive parameters, but only to provide recirculated water with a similar temperature to that observed in the area of the study during the spawning season. On the other hand, due to the expressive aforementioned results concerning the relatively short periods for both the interval between the hormonal induction and spawn and for the period necessary for an ovarian maturation after spawning, it seems promising for a new approach to study the effect of submitting *A. altiparanae* to different temperatures on this aspect.

A characteristic associated with the short vitellogenic period was the short resorption time of POC, which occurred in 6 days (or even less), since on day 6 these structures were no longer seen in ovaries (Figures 3B, 3C and 4F). This quite short time for POC resorption enabled PVO and CAO to develop, thus providing vitellogenic oocytes with a new spawning. The relatively short period observed here for an egg laying fish without parental care (*A. altiparanae*) was, however, similar to the period described for POC resorption in other multiple spawning farmed species. In a congener, *A. bimaculatus lacustris*, a significant decrease in POC occurs four days after spawning (DRUMMOND *et al*., 2000) and in *Oreochromis mossambicus*, five days post-spawning POCs are hardly seen (SMITH and HALEY, 1987). WANG *et al*. (2014) documented that in *Hemiculter leucisculus,* POCs are in late resorption process 48 hours post-spawning. All species aforementioned present fractional spawning, suggesting that for such species, including (*A. altiparanae*), POC resorption usually happens fast and thus may be associated with a need to quickly provide conditions for the development of a new batch of vitellogenic oocytes.

We believe that the intense process of POC resorption may have influenced the GSI reduction between days 6 and 16 due to decreased ovarian mass. After that, with a continuous process of vitellogenesis and yolk incorporation to the CVO, on day 30, oocytes reached their maximum size and consequently generated increased GSI values. To what the differences observed regarding the GSI, but not a stereological evaluation, between days 6 and 30, are concerned, a further study with more focus on the reproductive performance for a successive spawning among the three different periods is suggested.

The relatively short period for the herein observed ovary maturation was concomitant to - and may be explained by - a rapid rise in E_2 levels (between day 0 and day 6), which is known to be responsible for the synthesis of vitellogenin in the liver (GAZOLA and BORELLA, 1997; MIURA *et al*., 2007; NAGAHAMA and YAMASHITA, 2008; LUBZENS *et al*., 2010; ARANTES *et al*., 2010; NELSON and HABIBI, 2013). Therefore, these findings indicate that the species is characterized by presenting a quick E_2 peak after spawning, which guarantees a theoretical reproducibility of these results if the same conditions are offered to *A. altiparanae* by fish farmers.

Fish oocytes increase in volume due to the yolk accumulation (HAINFELLNER *et al*., 2012; PEREIRA *et al*., 2013; QUAGIO-GRASSIOTTO *et al*., 2013; HIRAMATSU *et al*., 2015). When vitellogenesis is complete, oocytes become larger cells, of about 1 mm (in tropical species), which contain all the information of maternal mRNAs, proteins, lipids, carbohydrates, vitamins, and hormones necessary for the embryo's development (LUBZENS *et al*., 2010). Full vitellogenic oocytes remain dormant in the ovaries during the spawning season (QUAGIO-GRASSIOTTO *et al*., 2013) and are responsible for hormonal induction in this period (BUGEL *et al*., 2011; KUMAR *et al*., 2015). In this context, similar densities occupied by vitellogenic oocytes between days 0, 6, 16 and 30 (Figure 4D) suggest that females remained mature and could theoretically be induced at any time over this period. Nevertheless, it is possible that, along the experimental period, vitellogenic follicles might have different levels of maturity specially concerning gonadotrophin and gonadal steroid receptors (NAGAHAMA and YAMASHITA, 2008; LISTER and VAN DER KRAAK, 2008; LEVAVI-SIVAN *et al*., 2010; ZOHAR *et al*., 2010; LUBZENS *et al*., 2010; MYLONAS *et al*., 2010; YARON and LEVAVI-SIVAN, 2011). On the other hand, the tendency of a 17α-OHP peak on day 6, followed by a gradual decrease until day 30, suggests a potential for higher reproductive performance on day 6. ARANTES *et al*. (2010), working with *Prochilodus argenteus,* obtained a rapid increase in 17α-OHP at the moment of spawning, and a quick post-spawning decrease. 17α-OHP is the precursor of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) and the main inducer of oocyte maturation and ovulation in fish (NAGAHAMA and YAMASHITA, 2008; TOKARZ *et al*., 2015). Further studies, which focus on the ideal time for a second hormonal induction, will need to be undertaken.

These findings further highlight the potential of this species for farming, since it had been previously shown that *A. altiparanae* could be manipulated to have mature ovaries outside the breeding season (EVANGELISTA, 2015). Here we have shown that females may be submitted to successive spawning at 6-day intervals; these findings open a new paradigm, which is to keep females in laboratory during the breeding season (or over the year) in order to obtain successive spawns and fingerlings in a predictable way. It is possible to hypothesize that these conditions enable the production of this species in a reduced area, with lower water consumption (especially considering the recent global problems with water supply), with reduced costs of laboratories, and reduced costs of maintenance and selection of large broodstocks. Moreover, the easy control and handling of *A. altiparanae* broodstocks may be useful for genetic improvement programs and for directed crosses used for restocking programs. Hence, it may be possible to make the production of this native species even more competitive and to be in accordance with the sustainability precepts linked to a modern and competitive aquaculture.

Concerning the physical and chemical characteristics of water, these values, except for conductivity, have been satisfactory, being within the standard recommended for the proper culture of *A. altiparanae* (GARUTTI and BRITSKI, 2000). High conductivity values may occur in recirculated systems through the accumulation of ions due to the lack of water exchange and mineralization of the feed waste and fish excreta (RAFIEE and SAAD, 2005). In spite of the quite high conductivity value observed in this study, the water parameters apparently did not interfere with fish welfare, but the reasons for such values (filters efficiency, excess of fish and others) remain an issue for future research.

CONCLUSIONS

This study has demonstrated that *A. altiparanae* females are suitable for induced spawning within six days after spawning, when kept at 29.24 ± 0.42 °C, in a system maintained with recirculated water. The species presented both POC resorption and an E_2 peak six days after spawning, with conditions to have mature ovaries in this period.

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