Latin American Journal of Aquatic Research, 47(2): 292-302, 2019
DOI: 10.3856/vol47-issue2-fulltext-9

Research Article

Environmental manipulation on *Astyanax altiparanae* out-of-season spawning

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ABSTRACT. This study aimed to evaluate the effect of manipulating hours of light and water temperature in some important reproductive parameters for *Astyanax altiparanae* fish farming during winter. The experiment was conducted from July 3rd to August 28th 2013 (57 days), and two groups of 32 couples of fish (G1 and G2) were used. On G1, hours of light and water temperature were not controlled; on G2, otherwise, these variables were manipulated in order to achieve similar conditions to those observed in spring. Every 14 days, eight couples of each group were hormonally induced with carp pituitary gland extract, and eggs were collected after semi-natural spawning. The percentage of females from G2 that spawned was higher than G1 (81.25 *vs.* 9.38%), the same trend was observed for a number of eggs produced per female (G1: 2,976.57 ± 1,085.71; G2: 8,471.14 ± 860.08). The G2 ovaries presented a higher incidence of primary growth oocytes and post-ovulatory follicles whereas G1 ovaries had more atretic follicles. Economic analysis showed that operational profit from eggs and larvae production on G2 was higher than on G1, as well as the gross margin. In conclusion, the results showed that environmental manipulation might improve reproduction management practices extending eggs and larvae production during the natural non-breeding season. Further studies are necessary to determine more appropriate facilities to be used by farmers in large scale, as well as management protocols to ensure the survival of post-larvae.

Keywords: economic analysis; egg production; environmental manipulation; lambari-do-rabo-amarelo; larvae

INTRODUCTION

In most fish species, reproduction occurs during limited periods of the year, when environmental conditions are more suitable for offspring survival (Vazzoler *et al.*, 1997; Martínez, 2000; Bromage *et al*., 2001). On the other hand, one of the prerequisites to produce farm animals is the capacity to control all the phases of their life cycle, especially reproduction (Mylonas *et al.*, 2010). Thus, the restriction on the production of eggs and larvae to the natural breeding season is a major problem for fish farmers, since it results in disruption of fingerlings production (Bairwa *et al.*, 2013). In this context, the adjustment of the reproductive period through environmental manipulation is a highly applicable tool in getting out-of-season eggs, providing greater control of fingerling production and reducing off-season (Ramos *et al.*, 2002; Duncan *et al.*, 2013).

Many environmental variables are involved in the control of fish reproduction, such as rainfall, lunar phase and food availability (Pankhurst & Potter, 2003). Moreover, in temperate fish species, hours of light and water temperature are the major parameters that can be manipulated in order to achieve the advance or delay of gonadal development (Duncan *et al.*, 2013). For Neotropical fish species, however, there are few studies associating manipulation of environmental variables to the control of the reproductive cycle.

In this study it was chosen *Astyanax altiparanae* Garutti & Britski, 2000, popularly known as "lambari-do-rabo-amarelo", species of high potential for Brazilian aquaculture (Gonçalves *et al.*, 2014; Valladão *et al.*, 2016). Its current production is primarily destined for commercialization as live bait and human consumption (Ferreira *et al.*, 2014). Besides, it has also been used as a fish model (Gomes *et al.*, 2013; Yasui *et al.*, 2014; Adamov *et al.*, 2017; Nascimento *et al.*, 2017) due to characteristics as rapid growth, simple handling, acceptance of artificial feeding and high prolificacy (Gonçalves *et al.*, 2014). *A. altiparanae* females and males reach gonadal maturation at the fifth month of life, approximately (Garutti, 2003), and show partial spawning from September to March (Porto-

Corresponding editor: Enrique Dupré

Foresti *et al.*, 2010); this restriction of spawning season limits the product offer throughout the year.

Given this scenario, the study aimed to evaluate the effect of light and water temperature manipulation hours on the egg production of *Astyanax altiparanae* during the winter period (out-of-season) and to compare the reproductive parameters and economic indices that were checked during the spring (natural reproductive). The reproductive parameters evaluated were the percentage of females that spawned, absolute fecundity, egg diameter, fertilization and survival rates, and histologic characteristics of ovaries. Additionally, costs, incoming, and profit of the production were also estimated.

MATERIALS AND METHODS

The experiment was conducted at the São Paulo State Agribusiness Technology Agency, Pirassununga, São Paulo, Brazil (21°55'37.4"S, 47°22'10.0"W) from July 3rd to August 28th, 2013 (57 days), and has obtained approval from the Ethics Committee for Animal Experimentation of the Fishery Institute (Protocol Nº07/2013).

In the beginning of the experimental period, an amount of 64 couples of *Astyanax altiparanae* were randomly selected (mean standard length ± standard deviation of females and males: 9.05 ± 0.48 and 6.90 ± 1.43 cm, respectively; mean body mass ± standard deviation of females and male: 11.72 ± 2.07 and 7.80 ± 0.47 g, respectively) and then divided into two groups (G1 and G2). The identification of males and females was made considering the presence of spicules on the caudal fin, which is present only in males (Garutti & Britski, 2000). Each group was allocated in an experimental tank (3×1×1 m) coated with geomembrane. Both tanks were maintained by the same water recirculation systems provided with a mechanical and biological filter, and aeration was provided by an air compressor.

In the tank where fish of G1 group were reared, hours of light and water temperature were not controlled and reflected the natural winter conditions. This tank was allocated indoor, but the room was provided with windows that allowed natural illumination to reach the water surface, and the water temperature was comparable to the temperature of outdoor tanks. On the other hand, on the G2 group, these variables were weekly raised in order to achieve similar conditions to those observed during spring, as shown in Table 1.

In this study, hours of light have comprised both photoperiod and civil twilight. Photoperiod has been defined as the number of hours between sunrise and sunset (Lanna *et al*., 2014), and civil twilight as the intervals after sunset and before sunrise when the sun is below the horizon but not more than 6° below the horizon. The inclusion of civil twilight was based on the results obtained by Priede & Young (1977). Those authors studied changes in cardiac rhythms of *Salmo trutta* and concluded that civil twilight is the point of transition between day and night for the species. Time and Date AS© (1995-2012) was used as a reference for determination of the number of hours of light to be used for G2 group and to verify the values obtained for G1 group during the experimental period (Table 1). The manipulation of water temperature considered values obtained in previous years (September to December 2009-2012) on the same location where the experiment was conducted (Table 1).

On the G2 tank, the light was supplied by fluorescent lamps controlled by timers. During the photoperiod interval, the light intensity on the water surface was 1000 lux. In order to avoid stress caused by sudden changes in light intensity (Bromage *et al.*, 2001), this was reduced to 150 lux during civil twilight. Water temperature was manipulated using a temperature controller and two heaters (Minjiang, 500 W). Minimum and maximum temperatures were monitored daily in G1 and G2 tanks.

Dissolved O_2 (G1 group: 5.81 ± 0.67 mg L^{-1}; G2 group: 5.42 ± 0.45 mg L^{-1}) was monitored daily; water pH (G1 group: 6.95 ± 0.30; G2 group: 6.72 ± 0.35) and non-ionized ammonia (G1 group: 1.44×10^{-3} ± 5.27×10^{-4} mg L^{-1}; G2 group: 1.56×10^{-3} ± 5.27×10^{-4} mg L^{-1}) were measured weekly. During the experimental period, fish were fed twice a day to apparent satiation and received a commercial feed with 36% crude protein (3000 kcal kg^{-1} ED).

Every 14 days (July 16th and 30th, August 13th and 28th, 2013) eight couples from each group were randomly selected and hormonally induced with crude carp pituitary extract (CCPE), totalizing 32 females and 32 males per group. Females received two doses: the first one of 0.50 mg CPG kg^{-1} at 20:00 h, and the second one, of 4.50 mg CPG kg^{-1}, was applied six hours later. Males received a single dose of 5.0 mg CPG kg^{-1} along with the second application in the females. The CPG was applied via intraperitoneal injection to the pectoral region. After hormonal induction, each couple was allocated in individualized aquariums (0.31×0.50×0.34 m) where the temperature was kept the same of the original tanks (Table 1). Next, semi-natural spawning, *i.e*., hormonal induction followed by the release of naturally occurring oocytes.

After oocyte releasing and fertilization, the eggs were collected and transferred to a sieve in order to remove external water; then, the eggs were put in a petri dish and weighed. Subsequently, three sub-samples of 0.10 g were taken and counted. Thus, it was possible to estimate the total number of eggs produced by each female. These sub-samples were stored in Gilson solution (Simpson, 1951), and egg diameter (average length of longitudinal and latitudinal axis) of 50 eggs per female was analyzed afterward. The measurements were performed in a stereo-

Table 1. Conditions of hours of light and water temperature used to keep *Astyanax altiparanae* breeders from G1 and G2 at each week, for 57 days. Weeks in which fish were hormonally induced are highlighted in bold.

Week	Water temperature (°C)		Photoperiod		Twilight		Hours of light	
	G1	G2	G1	G2	G1	G2	G1	G2
1 (Jul 3rd to 7th)	19.5	23.9	10:50	12:26	00:48	00:45	11:38	12:23
2 (Jul 8th to 14th)	19.5	24.4	10:52	12:35	00:48	00:45	11:40	12:26
3 (Jul 15th to 21st)	20.2	25.0	10:56	12:44	00:47	00:45	11:44	12:30
4 (Jul 22th to 28th)	18.3	25.1	11:01	12:52	00:47	00:46	11:49	12:35
5 (Jul 29th to Aug 4th)	19.8	26.2	11:07	13:00	00:46	00:46	11:54	12:41
6 (Aug 5th to 11th)	20.7	26.2	11:14	13:07	00:46	00:47	12:00	12:48
7 (Aug 12th to 18th)	20.3	26.3	11:21	13:13	00:45	00:48	12:07	12:55
8 (Aug 19th to 25th)	21.8	27.2	11:29	13:19	00:45	00:48	12:14	13:03
9 (Aug 26th to 28th)	21.8	27.2	11:38	13:23	00:45	00:49	12:23	13:13

microscope with an attached camera (BEL, Stmpro-T-Led; software BEL View).

After a period of 120 degree-hours from fertilization (blastopore closure, Weber *et al.*, 2012) 100 eggs of each female were separated to estimate the fertilization rates (RF = number of eggs × 100 / total number of eggs, Romagosa *et al.*, 1990). The translucent eggs were considered fertilized, while the opaque eggs were the unfertilized eggs (Woynarovich & Horvath, 1989).

Then 100 fertilized eggs were selected from each female (G1 group: n = 32; G2 group: n = 32) were transferred to aquariums (0.31×0.50×0.34 m) with constant water flow (2.32×10^{-2} L s^{-1}). Eggs produced by the G1 group were kept in room temperature water, and eggs produced by G2 group were kept in heated water, in the same temperature that spawning occurred (Table 1). After three days, the survival rate was estimated by counting the number of larvae.

After oocyte releasing, females were anesthetized on benzocaine solution for 10 min (2 g benzocaine: 5 mL alcohol: 1 L water, Felizardo *et al.*, 2012), euthanized by spinal transection, and their ovaries were removed. Sections from the mid-region of the ovaries were fixed in 4% buffered formalin (24 h), embedded in historesin (LKB Leica), cut into 5 μm thick sections and stained with toluidine blue, for histological examinations (Romagosa, 2010). In order to access the percentage of cells in each developmental stage (primary growth, pre-vitellogenic, vitellogenic and atretic follicles, and post-ovulatory follicles), a stereological analysis was performed. For each female three fields were photographed on a microscope (Nikon, Eclipse50i) with an attached camera (BEL, Stmpro-T-Led; software BEL View) under 200x magnification and analyzed using a 165-intersection grid (ImageJ 1.39p). Developmental stages of oocytes were identified based on the study of Garcia *et al.* (2001).

All results are presented as the mean ± standard deviation (SD) and were primarily tested for normality (Shapiro-Wilk test) and homogeneity of variance (F test). Parametric data were tested by Student's *t*-test, while nonparametric data were tested by Mann-Whitney test. The percentage of females that released oocytes was analyzed by the test for difference between two proportions. All analyses were performed using the software Past 2.17c and Statistica® 8.0, and the level of significance applied was $P \leq 0.05$.

In addition to reproductive parameters, economic indicators were also evaluated. Thus, it was performed an analysis of costs, income and profit of the production of *A. altiparanae* larvae obtained during winter from breeders reared on G1 and G2 conditions of hours of light and water temperature. Only expenses with the production were considered; investments and depreciation of the building construction and equipment were not considered. It was assumed a situation in which facilities similar to those used in this study would be already available on the property, stocking 125 couples of fish. In the hypothetical scenario, fish would be allocated on these tanks since the first week of July, as done on the experiment. Besides, fish would be induced in two groups: the first one 15 days after the beginning of environmental manipulation, and the second one 15 days after the first. In order to estimate monthly production costs, we adopted the methodology from the Institute of Agribusiness from São Paulo State (Matsunaga *et al.*, 1976; Martin *et al.*, 1994). Effective operating costs (EOC) were composed of costs of labor, energy, feed and hormonal induction (CCPE, syringe, needle and saline solution) (Table 2). This index did not comprise expenses with packaging, marketing, and sale taxes. Total operating costs (TOC) were the sum of EOC and social burdens, which were considered 43% of labor force cost (Ayroza *et al.*, 2011) (Table 2). Data on the percentage of spawning, absolute fecundity, fertilization rate, and larval survival

were calculated based on the results obtained in this study. Gross income (GI) was calculated as the product of the number of larvae and the price of commercialization of *A. altiparanae* larvae (1,000 units: US$ 4,636, thus, GI = n larvae × 4.636/1,000). Operational profit (OP) was the difference between GI and TOC (OP = GI - TOC), and gross margin (GM) was the margin about TOC, so MB = (LO/COT) ×100).

RESULTS

There were a higher percentage of spawned females from G2 group than from the G1 group (81.25 and 9.38, respectively, $P < 0.001$; Table 3). The same pattern was also observed on the number of eggs produced (G2 group: 220,250 and G1 group: 8,930; Table 3).

The absolute fecundity was also higher in females of G2 group than the ones in the G1 group (8,471.14 ± 860.08 and 2,976.57 ± 1,085.71, respectively; Mann-Whitney test, $P = 0.0181$; Table 3). Concerning egg size, females of G1 group produced larger eggs than the ones in G2 group (608.16 ± 0.01 *vs*. 603.20 ± 0.01 μm diameter, respectively; $P = 0.0042$, Student's *t*-test; Table 3).

There were no differences between the groups when considering the values of fertilization rate (G1 group: 58.33 ± 22.18%, G2 group: 84.85 ± 3.60%; $P = 0.0853$, Mann-Whitney test; Table 3) and larvae survival rate (G1 group: 22.00 ± 22.00%, G2 group: 38.75 ± 6.97%; $P = 0.2461$, Mann-Whitney test; Table 3).

Morphologically, oocytes from G1 and G2 groups showed no structural differences. Although primary growth oocytes (Fig. 1a) were found in both groups, the percentage found in ovaries from females of G2 group was higher than in G1 group (G1 group: 20.08 ± 3.08%, G2 group: 34.94 ± 3.84%; $P = 0.0018$, Mann-Whitney test; Table 4). On the other hand, there was no significant difference on the percentage of pre-vitellogenic oocytes (Fig. 1a; G1 group: 11.91 ± 2.54%, G2 group: 13.14 ± 2.07%; $P = 0.3143$, Mann-Whitney test; Table 4) and vitellogenic oocytes (Fig. 1b; G1 group: 2.65 ± 1.58%, G2 group: 6.01 ± 2.78%; $P = 0.3242$, Mann-Whitney test; Table 4) between females of both groups. Furthermore, in females from G2 group rare vitellogenic oocytes were in mature stage (Fig. 1c), and in some of them, it was possible to observe the micropylar apparatus in the animal pole, consisted by the vestibule and micropylar channel (Fig. 1d).

The incidence of post-ovulatory follicles (Fig. 2) was highest in ovaries from females of G2 group than in the ones from G1 group (G1 group: 4.30 ± 1.74%, G2 group: 10.40 ± 2.16%; $P = 0.0027$, Mann-Whitney test; Table 4). These richly vascularized structures composed of hypertrophied granulosa cells, basement membrane and theca cells protrude irregularly toward the lumen indicating that ovulation has occurred. On the other hand, these structures were less found in females from the G1 group reflecting its absolute fecundity, which was 64.86% lower than that observed in the G2 group. In Figure 2a, it is shown the micropylar cell within the post-ovulatory follicle. The atretic follicles were more abundant in females from G1 group than in females from G2 group (G1 group: 61.06 ± 5.24%, G2 group: 35.50 ± 5.03%; $P = 0.0005$, Mann-Whitney test; Table 4), being found on initial, intermediate and final atretic stages (Figs. 1e-1g, respectively).

The economic analysis of *A. altiparanae* out-of-season spawning revealed that gross income from commercialization of larvae obtained from breeders kept in conditions of luminosity and water temperature like G1 and G2 groups were US$ 21.483 and 1.153,418, respectively (Table 5). G2 system has shown higher total operating costs (Table 2), even with the gross margin 5,268.98% higher than that observed in the G1 system. Thus, G2 system operational profit and gross margin were positive (US$ 976,558 and 552.16%, respectively), contrasting to the values observed for G1 system (US$ -75.033% and -77.74, respectively).

DISCUSSION

In this study, it was found that the percentage of spawned females, the number of eggs produced, and the absolute fecundity were higher in G2 group (81.25; 220,250; 8,471.14 ± 860.08%, respectively) than in G1 group (9.38; 8,930; 2,976.57 ± 1,085.71%, respectively). Although *A. altiparanae* only reproduce in captivity after reproductive management and many management techniques have already been used and standardized for this species (Felizardo *et al*., 2012; Chehade *et al*., 2015; Cassel *et al.*, 2017), hypophysation has been widely used since it leads to greater egg production and allows controlling the breeders and the time of spawning (Felizardo *et al.*, 2012). Also, hormonal induction with CCPE has shown great results in inducing reproduction of native fish (Arantes *et al.*, 2011; Sanches *et al.*, 2011; Freitas *et al.*, 2013).

About egg size (diameter), females from the G1 group (608.16 ± 0.01 μm) produced larger eggs than the ones from the G2 group (603.20 ± 0.01 μm). The smaller diameter of G2 eggs can be related to the higher absolute fecundity of females from this group since, according to Yousefian (2011), there is a negative correlation between these parameters. There were differences between the groups for the values of egg fertilization rate and larvae survival rate (G1 group: 58.33 ± 22.18%; 22.00 ± 22.00% and G2 group: 84.85 ± 3.60%; 38.75 ± 6.97%, respectively). Those parameters are important in the evaluation of egg production and are directly related to the number of larvae produced (Mylonas & Zohar, 2007). Traditionally it has

Table 2. Items and values of effective operating costs (EOC) and total operating costs (TOC) of the production of *Astyanax altiparanae* larvae during winter from breeders kept on different conditions of hours of light and water temperature (57 days). *Expenses with an employee of 6 h of weekly dedication, considering the minimum salary in São Paulo State (US$ 375,522).

EOC items	Monthly consumption	Unit cost (US$)	Monthly cost (US$)
Employee*	-	-	48,981
Electricity (G1 system)	88.00 kWh	0.213	18,744
Electricity (G2 system)	465.20 kWh	0.213	99,088
Feed	6.69×10^{-1} kg	1.052	0,704
Hormonal induction (pituitary, syringe, needle and saline solution)			7,025
EOC (US$)			
G1 system			75,454
G2 system			155,798
TOC (US$)			
G1 system			96,516
G2 system			176,860

Table 3. Reproductive parameters of *Astyanax altiparanae* kept in different conditions of hours of light and water temperature. *Indicates a significant difference between G1 and G2 groups ($P \leq 0.05$).

Reproductive parameters	G1	G2
Spawned females (%)	9.38	81.25*
Absolute fecundity	2,976.57 ± 1085.71	8,471.14 ± 860.08*
Total egg production (n)	8,930	220,250*
Fertilization rate (%)	58.33 ± 22.18	84.85 ± 3.60
Egg diameter (µm)	608.16 ± 0.01*	603.20 ± 0.01
Larvae survival rate (%)	22.00 ± 22.00	38.75 ± 6.97

been suggested that larger eggs would be more likely to complete embryonic development, yielding larvae with greater survival chances (Lahnsteiner *et al.*, 2008; Migaud *et al.*, 2010). On the other hand, for *A. altiparanae* this relationship has not been confirmed and corroborates reports from Bromage *et al.* (1992) for *Oncorhynchus mykiss* and Régnier *et al.* (2013) for *Salmo trutta*.

About the influence of water temperature during incubation of eggs and larvae, there is disagreement in the literature. Longo & Nuñer (2010) and Sulis-Costa *et al.* (2013) found no differences in the values of survival rates of *Rhamdia quelen* larvae kept at different temperatures (19, 25 and 30°C; 19, 24 and 29°C, respectively). On the other hand, for *Heterobranchus longifilis* it was observed higher survival rate (59.2 to 80.4%) when the eggs were kept at 25 and 27°C than at 20, 23, 29 and 32°C (<21.2%) (Nwosu & Holzlöhner, 2000). The thermal tolerance in *A. altiparanae* eggs has been completed at temperatures from 20.2 to 27.2°C. The same trend was reported by Pereira-Santos *et al.* (2016), who observed no significant difference on hatching and normal larvae rates of *A. altiparanae* embryos kept at 22, 26, and 30°C. Which may be a clue leading to that production of *A. altiparanae* larvae during winter can be feasible in natural conditions of temperature, although more studies are required.

Morphologically, the percentage of primary growth oocytes of females from the G1 group did not show structural differences when compared to those of the G2 group. Besides, a few mature oocytes were found in the G2 group, and in some of them, it was possible to observe in the animal pole the presence of a micropylar apparatus, as described by Drummond *et al.* (2000) to *Astyanax bimaculatus lacustris*. Changes in the development of oocytes that lead to the process of atresia may be associated with stressful situations as inadequate management (Scherek *et al.*, 2001), food restriction (Corriero *et al.*, 2011), exposure to toxic substances (Gaber *et al.*, 2013; Magar & Bias, 2013; Narayanaswamy & Mohan, 2014), and also conditions of hours of light and water temperature that are unfavorable to maturation and spawning (Linares-Casenave *et al.*, 2002; Migaud *et al.*, 2003; Báez *et al.*, 2011). The last two factors may have resulted in the high incidence of atretic follicles in the G1 group and were probably responsible for the interruption of vitellogenesis. The high incidence of post-ovulatory follicles in the G2 group projecting towards the lumen (empty cavity) showed

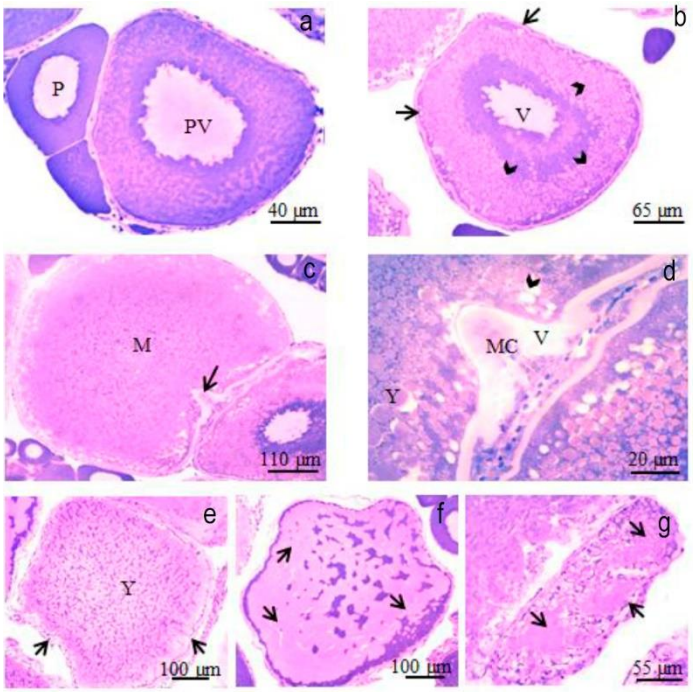

Figure 1. Oocyte development stages in *Astyanax altiparanae*. a) Primary growth oocyte (P - small, spherical or triangular shaped, basophilic cytoplasm, a prominent and central nucleus with many peripheral spherical nucleoli, surrounded by pre-follicle cells) and pre-vitellogenic oocyte (PV - less basophilic cytoplasm, a central nucleus with an irregular border and peripheral spherical nucleoli, zona pellucida and follicle cells present), b) vitellogenic oocyte (V - cytoplasm with a remarkable increase in volume, peripheral cortical alveoli (thick arrow), deposition of yolk granules (arrowhead), central nucleus, zona pellucida is evident.), c,d) Mature oocyte (M-Cytoplasm full of spherical yolk granules (Y), cortical alveoli are present (arrowhead), in general, flattened follicular cells, thick zona pellucida with little apparent striations, the presence of micropylar apparatus (arrow) consisted by vestibule (V) and micropylar channel (MC), d) detail of micropylar apparatus, e) initial atretic follicle (irregular shape, yolk granules (Y) begin to merge, thick and folded zona radiata (arrow)), f) intermediate atretic follicle (wavy shaped, yolk granules merged forming a mass (arrow), hypertrophied follicle cells losing contour), and g) final atretic follicle (few yolk granules (arrows), follicle cell layer invaginates, zona pellucida disappears).

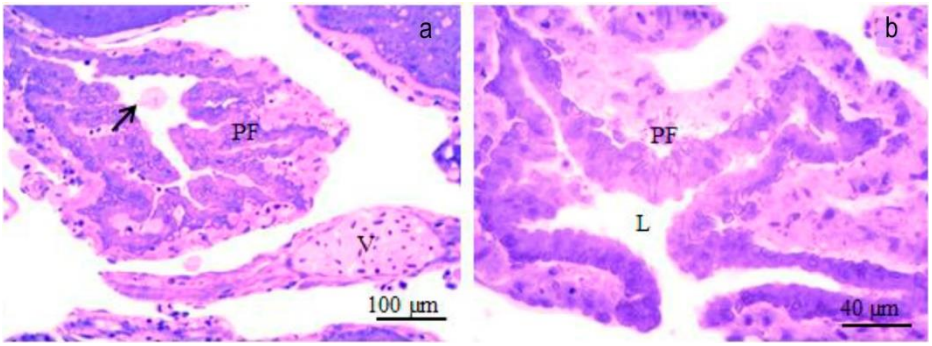

Figure 2. a) Postovulatory follicle (PF), micropylar cell inside the lumen (arrow), and adjacent blood vessel (V), b) detail of postovulatory follicle (PF) showing hollow lumen (L).

that ovulation in *A. altiparanae* occurred. These same observations were confirmed in later works (Romagosa *et al.*, 2005; McMillan, 2007; Wildner *et al.*, 2013). In *A. altiparanae*, the reabsorption of the post-ovulatory follicles in the G2 group occurred rapidly, which according to Lira *et al.* (2018) females of this species are suitable for hormonal induction six days after reproduction, when maintained at 29.24 ± 0.42 in a system maintained with recirculated water. Should be associated with the need to rapidly provide conditions for the development of a new batch of vitellogenic oocytes.

Table 4. Percentage of oocytes in each developmental stage in ovaries of *Astyanax altiparanae* kept in different conditions of hours of light and water temperature. *Indicates a significant difference between G1 and G2 groups ($P \leq 0.05$).

Oocyte developmental stages	G1	G2
Primary growth	20.08 ± 3.08	34.94 ± 3.84*
Pre-vitellogenic	11.91 ± 2.54	13.14 ± 2.07
Vitellogenic	2.65 ± 1.58	6.01 ± 2.78
Post-ovulatory follicles	4.30 ± 1.74	10.40 ± 2.16*
Atretic follicles	61.06 ± 5.24*	35.50 ± 5.03

It is described in the literature that hours of light and temperature of the water influence in different moments of the reproductive cycle, also observed for *A. altiparanae* kept in captivity, where the temperature is considered a crucial factor for the gamete is releasing after hormonal induction. So the influence of hours of light and water temperature manipulation on out-of-season reproduction has been observed in this study. According to Migaud *et al.* (2010), changes in conditions of hours of light and water temperature may be the primary factors in determining the fish breeding period. In tropical species, spawning season coincides with periods of high temperatures and long days (Vazzoler *et al.*, 1997), while atresia process is associated with short days and lower temperatures (Leonardo *et al.*, 2006).

Bromage *et al.* (2001), Carrillo *et al.* (2009) and Mañanós *et al.* (2008) described that variations in hours of light are responsible for synchronizing the endogenous clock of fish and environmental changes, acting as signs for animals to get ready for the breeding season and determining the annual cycles. Thus, hours of light stimulate the reproductive activity between fish of the same species simultaneously, as well as the adjustment of gonadal development pace according to the natural external conditions (Chemineau *et al.*, 2007). On the other hand, water temperature seems to be responsible for restricting reproduction to a limited time of year (Carrillo *et al*., 2009).

The influence of hours of light and water temperature manipulation on out-of-season reproduction has been described for marine tropical fish species. Guerrero-Tortorelo *et al.* (2010) obtained *Lutjanus argentiventris* spawning during winter after stocking breeders in a controlled system where the water temperature was kept at 27°C and the natural cycle of hours of light was condensed in a cycle of three months. *Epinephelus merra* females reared in conditions of long days (14 h light - L: 10 h dark - D) and high-water temperature (27°C) showed vitellogenic oocytes out of natural breeding season (Kanemaru *et al.*, 2012). Likewise, in tropical freshwater fishes, Sundararaj & Sehgal (1970) observed the early onset of vitellogenic oocytes on *Heteropneustes fossilis* exposed to long (14L: 10D) or increasing days (rangingfrom 11L: 13E to 14L: 10D), and constant water temperature (25°C). Giannecchini *et al.* (2012) found that constant temperature (28°C) and long days (16L: 8E; 12L: 12D) promoted the satisfactory reproductive performance of *Betta splendens*, considering the number of spawning's and the number of eggs (g female)$^{-1}$.

Table 5. Estimated number of *Astyanax altiparanae* eggs and larvae produced from breeders kept in different conditions of hours of light and water temperature, and economic indicators. G1 System: without control of hours of light and water temperature. G2 System: hours of light and water temperature manipulated in order to achieve similar conditions to those observed during spring.

Data	G1 System	G2 System
Estimated number of eggs (n)	36,115	846,230
Estimated number of larvae (n)	4,634	248,796
Gross income (US$)	21.483	1,153.418
Operational profit (US$)	-75.033	976.558
Gross margin (%)	-77.74	552.16

Despite the success in controlling the reproductive period of fish by manipulating environmental conditions, only a few studies consider economic aspects in the out-of-season production of eggs and larvae. According to Miao & Tang (2002), for the development of more efficient aquaculture technics, it is always important to consider both biological and financial aspects. The economic analysis of *A. altiparanae* out-of-season spawning revealed that gross income from commercialization of larvae obtained from breeders kept in conditions of luminosity and water temperature like G1 and G2 systems were US$ 21.483 and 1,153.418, respectively (Table 5). Even though the G2 system has shown higher total operating costs (Table 2), its system was advantageous in experimental conditions, since the gross margin was 5,268.98% higher than that observed in the G1 system. Thus, G2 system operational profit and gross margin were positive (US$ 976.558 and 552.16%, respectively), contrasting to the values observed for G1 system (US$ -75.033% and -77.74, respectively). Besides providing extra income, out-of-season production of eggs and larvae can also help farmers planning the use of facilities, avoiding that these remain idle (Duncan *et al.*, 2013).

CONCLUSIONS

The differences observed between G1 and G2 groups regarding reproductive parameters (percentage of spawned females, absolute fecundity, fertilization rate, egg size, and larvae survival) associated with economic analysis showed the applicability of our results. The strategy adopted

(manipulation of hours of light and water temperature) can be associated with current management techniques and thus help to extend eggs and larvae production during the natural non-breeding season. Further studies are necessary to determine more appropriate facilities to be used by farmers in large scale, as well as management protocols to ensure the survival of post-larvae (after mouth opening).

ACKNOWLEDGMENTS

The authors are grateful to Coordination for the Improvement of Higher Education Personnel (CAPES) and to São Paulo Research Foundation (FAPESP, 2013/13715-8) for Dr. Maria Letizia master's scholarship. To Dr. Maria Letizia Petesse (postdoctoral student from Fishery Institute) for helping with statistical analysis, and to Dr. João Donato Scorvo Filho (scientific researcher from São Paulo State Agribusiness Technology Agency) for helping with the economic analysis.

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Received: 23 March 2018; Accepted: 3 December 2018

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