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Original Article

Induced reproduction of yellow-tailed lambari (*Astyanax lacustris*) with Ovopel® and buserelin acetate as alternatives to the protocol with carp pituitary extract

Reprodução induzida do lambari-de-cauda-amarela (*Astyanax lacustris*) com Ovopel® e acetato de buserelina como alternativas ao protocolo com extrato de hipófise de carpa

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Abstract

The objective of this study was to evaluate reproductive traits in adults of *Astyanax lacustris* subjected to different spawning inducers. The study involved 240 females (12.54 g ± 2.33 and 7.66 cm ± 0.63 cm) and 240 males (5.83 g ± 0.39 g and 6.14 cm ± 0.64 cm), all at reproductive age. Three different inducers were evaluated: (i) 0.4 pellets of Ovopel®/kg of body weight; (ii) 0.5 ml of buserelin acetate/kg of body weight; and (iii) carp pituitary extract (CPE) (5.5 mg CPE/kg body weight for females and 2.5 mg CPE/kg body weight for males), as well as saline solution (without hormone). The degree-hours for spawning were greater (P<0.05) for the Ovopel® treatment (with 204.93) than in the treatment with CPE (183.2). Ovary weight and gonadosomatic index were higher (P<0.05) in CPE and Ovopel® treatments when compared to buserelin acetate. The number of oocytes per female, absolute and relative fecundity were greater (P<0.05) for Ovopel® and CPE treatments. Fertilization rate was higher (P<0.05) in treatment with buserelin acetate (82.3%) in relation to Ovopel® (72.33%) and CPE (62.40%) treatments, and the highest (P<0.05) hatching rates were achieved with buserelin acetate and Ovopel®. The number of larvae per female body weight was greater (P<0.05) when Ovopel® was used. In conclusion, Ovopel® proves to be a more effective reproductive inducer for induced reproduction of *A. lacustris* when compared to CPE and buserelin acetate.

Keywords: reproductive traits, hormonal induction, buserelin acetate, carp pituitary extract.

Resumo

O objetivo deste estudo foi avaliar as características reprodutivas em adultos de *Astyanax lacustris* submetidos a diferentes indutores da desova. Foram utilizadas 240 fêmeas (12,54 g ± 2,33 e 7,66 cm ± 0,63 cm) e 240 machos (5,83 g ± 0,39 g e 6,14 cm ± 0,64 cm), todos em idade reprodutiva. Três diferentes indutores foram avaliados: (i) 0,4 pellets de Ovopel®/kg de peso corporal; (ii) 0,5 ml de acetato de buserelina/kg de peso corporal; e (iii) Extrato de Pituitária de Carpa (CPE) (5,5 mg CPE/kg de peso corporal para fêmeas e 2,5 mg de CPE/kg de peso corporal para machos) e solução salina (sem hormônio). Horas-grau para desova foi maior (P<0,05) no tratamento com Ovopel® (204,93) do que no tratamento com CPE (183,2). O peso das gônadas e o índice gonadossomático foram maiores (P<0,05) nos tratamentos com CPE e Ovopel® quando comparados ao acetato de buserelina. O número de oócitos por fêmea, a fecundidade absoluta e relativa foram maiores (P<0,05) para os tratamentos com Ovopel® e CPE. A taxa de fertilização foi maior (P<0,05) no tratamento com acetato de buserelina (82,3%) em relação aos tratamentos com Ovopel® (72,33%) e CPE (62,40%); e maior (P<0,05) taxa de eclosão foi obtida nos tratamentos com acetato de buserelina e Ovopel®. O número de larvas por peso corporal da fêmea foi maior (P<0,05) quando utilizado Ovopel®. Conclui-se que Ovopel® é um indutor reprodutivo mais eficaz para a reprodução induzida de *A. lacustris* quando comparado ao CPE e ao acetato de buserelina.

Palavras-chave: características reprodutivas, indução hormonal, acetato de buserelina, extrato de hipófise de carpa.

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1. Introduction

The yellow-tailed lambari, *Astyanax lacustris*, is a small-sized species native to South America that has gained significant attention in recent years, with a production of 599.4 tons in 2021 (IBGE, 2022). This species is early-developing, reaching sexual maturity around four months of age (Porto-Foresti et al., 2001). It is utilized for human consumption, particularly as a snack, and is also widely employed as bait for sport fishing (Valladão et al., 2018). The yellow-tailed lambari engages in split spawning, readily accepts commercial feed, and holds great ecological importance as a forage fish for several piscivorous species (Santos and Novaes, 2008; Gonçalves et al., 2014).

Induced reproduction has been implemented for lambari species to enhance planning, optimize production, and ensure a standard size for commercialization, typically around three months of age (Porto-Foresti et al., 2005; Gonçalves et al., 2014). The most commonly used reproductive inducer in fish reproduction is carp pituitary extract, but some concerns have been raised in recent years (Martins et al., 2017; Souza et al., 2018; Konzen-Freitas et al., 2020), primarily due to the lack of standardization in hormone concentration, leading to inconsistent results (Martins et al., 2017). Such standardization could also support better planning for selective breeding activities.

Studies have been carried out to evaluate the use of synthetic hormones for fish reproduction with the aim of refining induction protocols and minimizing reproduction-related costs (Andrade and Yasui, 2003; Carneiro and Mikos, 2008; Andrade et al., 2014; Abreu et al., 2021; Abreu et al., 2022). Some hormones have proven effective when compared to carp pituitary extract, such as Ovopel® for *Colossoma macropomum* (Souza et al., 2018) and *Brycon henni* (Lenis et al., 2009), and buserelin acetate for *Piaractus brachypomus* (Chaves-Moreno et al., 2012) and *Colossoma macropomum* (Konzen-Freitas et al., 2020). However, the effectiveness of Ovopel® and buserelin acetate as inducers compared to the traditional protocol in induced reproduction in various fish species remains unclear. In this context, the objective of this study was to evaluate reproductive traits in adults of yellow-tailed lambari (*Astyanax lacustris*) induced with Ovopel® and buserelin acetate as alternative protocols to carp pituitary extract.

2. Material and Methods

2.1. Location and animals

The experiment took place at Piraí Fish Farm, situated in the city of Terenos-MS, Brazil (20°26'31" S, 54°51'36" W). The methodology employed in the experiment was approved by the Animal Ethics Committee (CEUA) of the Federal University of Mato Grosso do Sul (approval no. 1083/2019).

A total of 240 females (weight 11.54-13.77 g; standard length 7.30-8.00 cm) and 240 males (weight 5.05-6.46 g; standard length 5.80-6.50 cm) of *A. lacustris* were utilized in the study. Females were selected when their abdomens were dilated and soft, with a swollen and reddish urogenital papilla (Woynarovich and Horváth, 1983); for males, selection was based on the presence of spicules on the anal fin (Garutti, 2003).

In the preparation of the breeder batch for the experiment, the fish were housed in a 1,000-m² earthen fish pond with 10% daily water replacement. They were fed a daily commercial feed equivalent to 1% of the total biomass (kg), consisting of a 2 mm extruded feed (Supra – dry matter 88%, crude protein 40%, ether extract 8%, fiber matter 3%, mineral matter 13%, calcium 1.5%, phosphorus 1%, digestible energy 3400 kcal/kg).

2.2. Hormonal induction

The experiment involved the evaluation of four groups: (i) control (with the application of saline solution); (ii) carp pituitary extract (CPE), administered at a dose of 5.5 mg/kg body weight, divided into two applications (10% initially and 90% after 12 h) for females, and a single dose of 2.5 mg/kg body weight for males (Woynarovich and Horvath, 1983); (iii) Ovopel® (18-20 µg GnRH and 8-10 mg metoclopramide; Das, 2004) at a dosage of 0.4 pellets/kg body weight in a single application for both males and females, following the protocol by Souza et al. (2018); and (iv) buserelin acetate (Sincroforte® - 0.0042 mg/ml of buserelin acetate), administered at a dose of 0.5 ml/kg body weight in a single application for both males and females, as per the protocol of Konzen-Freitas et al. (2020). The inducers were applied intramuscularly at the base of the pectoral fin.

Inductions were carried out with the treatments in three different periods (blocks), where each experimental unit consisted of one breeding tank (1,000 L) located within the laboratory. All fish in the same breeding tank received the same inducer (Figure 1). Four breeding tanks were used in each period. In these tanks, a continuous flow of water renewal between 5.75 and 7.20 L/s was maintained. The water temperature was maintained between 25.6 and 28.8 ºC, with a mean pH ranging from 6.6 to 7.2, and dissolved oxygen levels ranging from 3.9 to 6.2 mg/L. The photoperiod during the experiment was set at 13:11 (light:dark).

In each experimental unit (breeding tank), 20 females and 20 males were allocated, with semi-natural reproduction occurring after induction according to the pre-established treatments. Of the 40 fish in each breeding tank, five females and five males were euthanized an hour before the predicted spawning time. The euthanized females were used for the evaluation of the gonadosomatic index, while the remaining ones were used to assess reproductive traits.

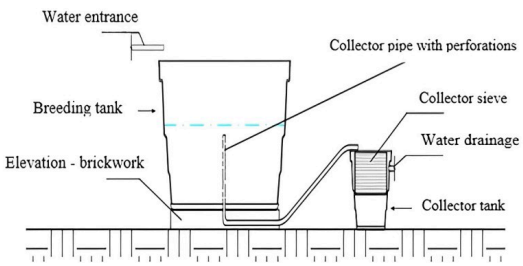

Figure 1. Illustration of the breeding tank and the egg collector tank for the induced reproduction of *Astyanax lacustris* in a semi-natural system.

2.3. Reproductive traits

The eggs retained in the collector tanks were collected and distributed into three sieves for each treatment, with 2 mL of eggs placed in each sieve from each collector tank. All sieves were placed in 60-L incubators with a water flow of 9 L/min. After eight hours of incubation, triplicates of 600 eggs were counted to determine the fertilization rate and subsequently, the hatching rate. To determine the hatching rate, after fixation in alcohol (70%), larvae and bad eggs were counted from each sieve.

The following reproductive traits were evaluated: Degree-hours for spawning (water temperature × hours until spawning – measured from the second application of CPE and from the single application of Ovopel® and buserelin acetate); Spawning rate - [(number of spawned females/number of induced females) x 100]; Fertilization rate [(number of viable eggs/total number of eggs) x 100]; Hatching rate [(number of hatched larvae/total number of eggs) x 100]; Absolute fecundity (total number of eggs collected per tank/number of spawned females); Relative fecundity (total number of eggs collected per tank/total sum body weight of spawned females per tank in g); and Larvae/Average weight of females (g) = Relative Fecundity*(Hatching rate/100). To estimate the number of eggs in each collector tank, the following procedure was adopted: all the eggs were collected from each tank, placed in a 200 mL beaker, gently suspended, and then three 5 mL samples were taken to count the eggs. To determine which females spawned, in general, those with a flaccid abdomen after collecting eggs in semi-natural spawnings are checked; however, this assessment is very difficult in lambari. In this study, all live females were considered to have spawned. Therefore, the spawning rate results in the same values as female survival. The gonadosomatic index [(ovaries weight (g)/average weight of females (g) * 100] and oocytes per gram of ovary (counts were performed using around 0.3 g of oocytes in triplicate) were determined in euthanized fish. The survival of males and females (%) and Fulton's condition factor $K=W/(SL^3)$, [W = weight (g) and SL = standard length (cm)] were also evaluated.

2.4. Statistical analysis

The experiment employed a randomized complete block design with three treatments (inducers: CPE, Ovopel®, and buserelin acetate) and three blocks (different periods). In the control group (induction with saline solution), no spawning occurred. Each breeding tank was considered a replication, and the reproductive traits were related to the average of all animals in the tank. All dependent variables underwent Shapiro-Wilk and Levene tests for normality and homogeneity of variances, respectively. As the assumptions were met in all cases, the variables were analyzed by a model with two independent variables (ANOVA - Two Way, Block Design, Treatment and Block), followed by Student's t-test for multiple comparisons. The "females' survival" and "males' survival" variables underwent angular transformation. All analyses followed the recommendations of Zar (2010) and were conducted using the Statistical Analysis System (SAS, 2002). The significance level used in all tests was 0.05.

3. Results

Spawning occurred in all evaluated treatments, except for the control group (solely injected with saline solution). No significant differences were found between fish groups from different treatments regarding weight, standard length, or condition factor of both females and males (Table 1).

Table 1. Mean values of reproductive traits obtained using different inducers in semi-natural induced reproduction of *Astyanax lacustris* in the same reproductive period.

Reproductive trait[1]	Inducer[2]			CV (%)	*P*-value[3]
	CPE	Ovopel®	Buserelin acetate		
Female weight (g)	13.01[a]	12.40[a]	12.21[a(4)]	5.88	0.451
Male weight (g)	5.60[a]	5.60 [a]	6.28[a]	7.80	0.223
Female standard length (cm)	7.60[a]	7.57[a]	7.80[a]	2.90	0.455
Male standard length (cm)	6.40[a]	6.03[a]	6.00[a]	3.91	0.193
KFE	2.97[a]	2.89[a]	2.58[a]	12.75	0.448
KMA	2.14[a]	2.56[a]	2.93[a]	15.38	0.161
Degree-hours	183.20[b]	204.93[a]	193.18[ab]	3.69	0.049
Ovary weight (g)	1.24[a]	1.36[a]	0.91[b]	8.75	0.0136
Gonadosomatic index	9.55[a]	10.95[a]	7.50[b]	7.66	0.010
Oocytes/g of ovary	6204.8[a]	5688.9[a]	5264.8[a]	10.02	0.247
Oocytes/female	7724.47[a]	7724.93[a]	4802.27[b]	13.93	0.029
Spawning rate (%)[5]	98.46[a]	99.24[a]	97.00[a]	7.14	0.621
Absolute fecundity	5880[a]	6072[a]	4368[b]	9.18	0.026
Relative fecundity	451.67[a]	489.70[a]	357.23[b]	4.00	0.002
Fertilization rate (%)	62.40[c]	72.33[b]	82.30[a]	3.78	0.002
Hatching rate (%)	57.10[b]	65.30[a]	65.50[a]	4.13	0.026
Larvae/female weight	258.0[b]	319.80[a]	234.09[b]	6.48	0.009
Female survival (%)[5]	98.46[a]	99.24[a]	97.00[a]	7.14	0.621
Male survival (%)[5]	99.24[a]	97.00[a]	94.28[a]	10.21	0.484

[1]KFE – female condition factor; KMA – male condition factor. [2]Inducers – Carp Pituitary Extract (CPE), Ovopel® and Buserelin acetate. [3]P-value of analysis of variance. [4]Mean values followed by common letters in the row do not differ statistically by Student t test at a 5% significance level. [5]Values presented in percentage, resulting from the inverse transformation of the angular transformation.

Degree-hours for reproduction were lower (P<0.05) in fish induced with CPE compared to those induced with Ovopel®. However, the degree-hours of females induced with buserelin acetate did not differ (P>0.05) from the values of animals induced with CPE and Ovopel® (Table 1). Regarding the traits of ovary weight and gonadosomatic index of females, values were higher (P<0.05) in fish induced with CPE and Ovopel®, which did not differ from each other (P>0.05).

For the number of oocytes per gram of ovary and spawning rate (%), no significant differences were found between treatments. However, the number of oocytes per female as well as absolute and relative fecundity were lower (P<0.05) in females induced with buserelin acetate compared to those induced with CPE and Ovopel®.

Buserelin acetate was the inducer that provided the highest (P<0.05) fertilization rate, followed by Ovopel®, and finally CPE. For the hatching rate, buserelin acetate and Ovopel® showed better results (P<0.05) when compared to CPE. The percentage of larvae per female weight was higher (P<0.05) in fish induced with Ovopel®. There was no significant difference in female or male survival (P>0.05) (Table 1).

4. Discussion

Current concerns regarding the use of CPE in South American fish species, coupled with the high costs of this inducer, have stimulated research into alternative protocols, particularly those involving GnRH analogue inducers. In this study, it was evident that both inducers proposed as alternatives to CPE resulted in effective spawnings in the *A. lacustris* species, yielding better outcomes in some evaluated traits.

The origin and site of exogenous hormone action produced by synthetic hormonal inducers could explain the observed increase in degree-hours in the spawning of *A. lacustris* females. While the Ovopel® inducer acts on the pituitary gland, stimulating fish to produce their own gonadotropins, CPE acts directly on the gonads, resulting in a faster response. This difference in degree-hours for spawning between the two inducers, based on their action origin, has been previously observed in another South American neotropical species, *Colossoma macropomum*, as reported by Souza et al. (2018). Nonetheless, the value obtained with the buserelin acetate inducer, which also acts on the pituitary gland, resulted in a degree-hour value intermediate to the other two inducers. This may be associated with the amino acid profile of the GnRH analogue hormones in both inducers. Ovopel®'s GnRH is a mammalian (modified) hormone analogue (Das, 2004), which has a different structure from the GnRH analogue of the hormone buserelin acetate (Arabacı et al., 2004). Another factor that may influence degree hours is the presence of metoclopramide in Ovopel®, which inhibits the action of dopamine and may increase the synthesis of GnRH.

The evaluation of the gonadosomatic index (GSI) is important as it enables the analysis of different phases of the reproductive cycle through the development of germ cells, which increase simultaneously with gonadal maturation (Kime, 1995), particularly in females. The lower GSI of females induced with buserelin acetate may indicate that this inducer was less effective in the final maturation of oocytes. This observation is supported by the lower values obtained for ovary weight, number of oocytes per female, absolute fecundity, and relative fecundity of this inducer compared to Ovopel® and CPE. It is important to note that there were no significant differences between treatments for morphometric variables such as weight and standard length of fish, as well as condition factor in both males and females. Thus, these variables are not expected to interfere with the results of reproductive traits. After induction, the Gonadosomatic Index values found in this study closely align with those reported in another study with *A. lacustris* induced with CPE (Felizardo et al., 2012).

One of the main reproductive traits to be analyzed in induced reproduction is the fertilization rate, which accurately estimates oocyte quality (Bobe and Labbé, 2010). In the present study, females induced with buserelin acetate demonstrated superior results in the fertilization rate. This observation can be explained by the lower relative and absolute fecundity of buserelin acetate compared to CPE and Ovopel®, likely resulting in a higher insemination dose for this treatment. Even in semi-natural reproduction, the insemination dose (number of spermatozoa/oocytes) influences reproduction, as evidenced in studies on *Salminus brasiliensis* by Sanches et al. (2009) and *Brycon insignis* by Souza et al. (2022). It is noteworthy that despite the superior fertilization rate in the buserelin acetate treatment, the hatching rate did not differ from the Ovopel®-induced fish, indicating an improvement in the quality of oocytes with this inducer, as reflected in the higher number of larvae per female.

This study provides evidence that Ovopel® is a suitable inducer for induced reproduction of the yellow-tailed lambari. Similar results were obtained for *Colossoma macropomum* [Martins et al. (2017) in males; and Souza et al. (2018) in females], *Brycon henni* (Lenis et al., 2009), and *Leiarius marmoratus* [Araújo et al. (2014) in males]. Although buserelin acetate induced spawning, which was not evidenced in the control group (without hormone induction), adjustments in this protocol may offer an alternative to enhance the reproductive efficiency of this inducer compared to the traditional protocol with carp pituitary extract. This scenario with buserelin acetate was also observed in *Colossoma macropomum* females by Konzen-Freitas et al. (2020) and in females of *Piaractus brachypomus* (Chaves-Moreno et al., 2012), while Andrade et al. (2014) was unsuccessful in the fertilization of *Prochilodus lineatus*.

In the reproductive evaluation of the *Astyanax altiparanae* species, studies by Abreu et al. (2022) with the luteinizing hormone-releasing hormone analog (LHRHa; with and without dopamine antagonist) and Abreu et al. (2021) with the salmon gonadotropin-releasing hormone analog (sGnRHa) found reproductive performance results inferior to the traditional protocol with CPE. Conversely, this study suggests that the reproductive inducer Ovopel® could serve as an alternative to the traditional protocol with CPE for the *A. lacustris* species. It proved to be a

more suitable reproductive inducer by exhibiting a better fertilization rate, hatching rate, and larvae/female weight compared to the CPE protocol. Additionally, it displayed superior values for ovary weight, gonadosomatic index, oocytes/female, absolute fecundity, relative fecundity, and larvae/female weight compared to buserelin acetate. In conclusion, Ovopel® provides the best results for the induced reproduction of yellow-tailed lambari (*A. lacustris*) in a semi-natural system, suggesting its potential as a substitute for the traditional protocol with carp pituitary extract.

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