Influence of Photoperiod over Morphometric and Hematological Parameters of Juvenile Piracanjubas (*Brycon orbygnianus*)

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Abstract: The aim of this study was to evaluate morphometric and hematological parameters of juveniles Piracanjubas treated with two distinct photoperiod. Twenty nine fish with 100 g, in a factorial design were performed in 18 h light:6 h dark (18L:6D) and 6L:18D two different photoperiod, and morphometric and hematological parameters were evaluated at 45 d and 90 d after photoperiod treatment. Benzocaine 1% was used for euthanasia. Fish were weighed and measured, so as visceral fat, liver and gonads. Hematocrit, total count of white cells, lymphocytes and neutrophils were counted. Results showed that there was no significant relationship between bodyweight and standard length. Significant interaction ($P = 0.00018$) had been found between the fat index (FI) and gonado-somatic index (GSI) and the photoperiod and time. At 90 d, the fish kept in 18L:6D had a lower rate of fat than the fish kept in 6L:18D ($P < 0.05$). The GSI was higher in the group 6L:18D compared with the 18L:6D ($P < 0.005$). There was no interaction between photoperiod and period of treatment for total leukocyte count, however with 90 d, the total leukocyte count was decreased ($P < 0.05$). Moreover, decrease of the hematocrit from 58.1% to 46.9% and an increase of lymphocytes from 31.5% to 43.5% had been found in fish kept in 18L:6D after 90 d ($P < 0.05$). Fish kept in 6L:18D presented an increase in segmented neutrophils and lymphocytes ($P < 0.05$). It is concluded that photoperiod maintained at 6L:18D for Piracanjubas permits a greater gonadal development and an amount of immune cells over the photoperiod maintained in 18L: 6D.

Key words: Circadian rhythm, fish maturity, cellular immune response, photoperiod.

1. Introduction

Piracanjuba (*Brycon orbignyanus*) is a migratory fish, native from Uruguay and Parana basin in Southwestern Brazil. This specie is sensitive to environmental degradation and is quite explored in sport fishing [1]. Therefore, its natural population has declined, being necessary efforts to reproduce in captivity, as captive characteristics can be rapid growth, omnivorous and easy adaptation to artificial

feeding [2].

Sexual maturity of this specie is reached at the age of three years [3], however, the production of these fish presents a series of obstacles, being mainly the high rate of cannibalism and competition for space and food. This dispute determines a heterogeneous lot, because of the energy spent by the territorial dispute and high mortality rate [4, 5]. Consequently, it is necessary to develop management systems that determine the increased survival rate and decrease of the age of first maturity, thereby increasing its precocity and prolificacy.

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Among the parameters that can be changed to stimulate reproduction, photoperiod has been cited in several species [6-8]. Circadian rhythms determine the alterations in the hypothalamic-pituitary-gonadal axis, leading to release of gonadotropin-releasing hormone (GnRH) [8]. The increase in photoperiod determines the synthesis of hormones, such as steroids, insulin and catecholamines, while its reduction causes an increase in the levels of growth hormone and melatonin [6].

However, the effect of light on induction of puberty in fish is still unknown. Metabolic signals and environmental cues contribute to the onset of gonadal development [9], and changes induced by puberty determine the decrease in the gene expression of several proteins by dopamine [10]. Dopamine is known as the inhibitory hypothalamic-pituitary-gonadal axis in fish. Furthermore, melatonin have the ability to inhibit the hypothalamic-pituitary-gonadal axis via dopaminergic receptors [7].

Hormonal changes caused by the action of photoperiod can interfere with the physiological processes of teleost fish, and hematological parameters can use as an indicator of these changes. The basic proteins present in granules of leukocytes have defense function against microorganisms, causing their death. When they are released after the rupture of these cells [11], they also present great source of glycogen [12].

In teleost fish, the variation in the number of defense cells may indicate changes in the environment and stress [13-15]. However, several factors may contribute to quantitative variations in fish blood elements. These include length, weight, nutritional state, seasonal cycle and age [16, 17], and the environment in which the animal is maintained [18, 19].

The aim of this study was to evaluate morphometric and hematological parameters in juveniles Piracanjubas held in two distinct light:dark (L:D) photoperiod.

2. Materials and Methods

2.1 Animal Housing and Maintenance

The fish were donated by Eletrobras Furnas, Minas Gerais, Brazil and came from the reproductive period of 2013-2014. Twenty nine Piracanjubas with average weight 100 g and average length 15 cm were selected. Initially, the fish were kept for 45 d in a recirculation system and a photoperiod 12 h light:12 h dark (12L:12D) for adaptation. Later, these juveniles were assigned in five 500 L tanks of polyethylene, with a density of seven fish per box, with constant aeration in closed recirculating system for 45 d. The boxes were kept in vivarium of the Federal University of Lavras (UFLA), and photoperiod was controlled individually by timers with 200 lux lamps used [20]. The photoperiodic treatments were performed with two periods of 18L:6D and 6L:18D, and these were chosen to mimic the summer and winter when the day or night longer present, respectively.

A blackout cloth was placed on the edge of the lid. The management was carried out only when the light from both boxes was lit. The management consisted of feeding commercial feed containing 35% crude protein, collecting food debris and siphoning the bottom twice a day in box.

The food and cleaning operations of the box were performed at the same time in the two photoperiodic treatments, and the interval between the feeds was 6 h, at which time the boxes lights were lit. The water quality parameters were performed one time per week. The room light was kept off when there was no steering. Two collection periods were carried out to evaluate morphometric and hematological measurements at 45 d (12 fish, three of each box) and at 90 d (17 fish, seven from photoperiod 18L:6D and 10 from photoperiod 6L:18D). Fish anesthesia was performed using 1% benzocaine. All the experimental procedures were carried out following the standards of the Ethics Committee on Animal Use (CEUA-UFLA).

2.2 Morphometric Evaluation

For blood collect, fish were sedated with 1% benzocaine, with 5 mL syringes with EDTA by puncture of venal caudal. After blood collection, fish were allocated at benzocaine solution until opercular movement stopped. Fish body weight, standard and total body length were measured [21]. Caelomatic cavity diameter in three different position, anterior (M_1), middle (M_2) and posterior (M_3), were measured using a digital pachimeter. Gonads, liver [21] and visceral fat were weighed for the calculation of gonado-somatic index (GSI), hepatic and visceral fat index.

2.3 Blood Analysis

Hematocrit evaluation was performed with a microhematocrit tube, centrifuged for 5 min at 10,000 rpm. Total erythrocyte, thrombocytes and leukocytes were performed using a dilution of 9.9 mL of Natt-Herrick's solution (Conroy, 1998) added with 0.1 mL of blood (1: 1000) which was homogenized for 3 min and using a Neubauer chamber [17].

For number of erythrocytes/mm³, the number of erythrocytes in five quadrants of newbauer chamber was multiplied 1,000 (dilution factor). For total number of leukocytes, the number of leukocytes counted on newbauer's chamber was multiplied 1,000 (dilution factor). The same was done for number of thrombocytes.

For differential counting of leukocytes an optical microscope (Nikon E200) was used with 400 and 1000-fold increases. The differential count is a percentage count that differentiates the different leukocytes present in each blood sample. Values were expressed as a percentage.

The design was completely randomized in the scheme of subdivided plots, with two replicates. In the plots, the effects of the photoperiod (6 h and 18 h light) were evaluated, and in the subplots, the time effects (45 d and 90 d) were evaluated. The analysis of variance was performed to verify the effect of the

photoperiod, the time and the interaction of two, and the interaction was deployed when necessary. For the analysis of the data, the program R was used.

3. Results

Body weight, total body length, standard length, liver weight and measures of caelomatic (M_1 , M_2 and M_3) did not show significant difference when comparing the two photoperiod (Table 1) nor interaction of these variables over the time. For photoperiod 18L:6D, weight was 121.0 g, total length 20.4 cm, standard length 17.6 cm, hepato-somatic index (HSI) 1.4, and caelomatic measure M_1 , M_2 and M_3 were 20.7, 19.9 and 15.9 cm, respectively. For photoperiod 6L:18D, the same variables were 134.3 g, 20.3, 18.0, 1.3, 20.7, 20.5 and 14.2 cm, respectively. Variables did not show significant interaction, when an analysis was made comparing the influence separately of the photoperiod and the time of treatment.

However if the photoperiod effect is excluded and the averages are compared, it is possible to perceive the increase in the total and standard length of the Piracanjubas. The fish at 90 d were larger (Table 2). The weight of Piracanjubas at 45 d was 117.4 g and at 90 d 136.0 g. Considering the evaluations at 45 d and 90 d, it was observed that Piracanjubas grew, and significantly ($P < 0.05$) increased their total and standard length (18.8 cm at 45 d and 21.3 cm at 90 d), independent of the photoperiod. Standard length showed the same pattern, 15.3 cm at 45 d and 19.4 cm at 90 d ($P < 0.05$). However, HSI (1.8 at 45 d and 1.1 at 90 d) and caelomatic measurements M_1 , M_2 and M_3 showed no statistically significant difference for photoperiodic treatment or time of treatment (Tables 1 and 2).

An interaction between photoperiod and days of treatment was observed for visceral fat index and GSI (Figs. 1 and 2). From Fig. 1, it is possibly observed that at 45 d, GSI was 0.12 for photoperiodic treatment 18L:6D and 0.14 for 6L:18D, being similar. However

Table 1 Evaluation of morphometric parameters of Piracanjubas in different photoperiods.

Variables	Photoperiod (light:dark)	
	18L:6D	6L:18D
Weight (g)	121.0	134.3
Total length (cm)	20.4	20.3
Standard length (cm)	17.6	18.0
Hepato-somatic index (HSI)	1.4	1.3
Caelomatic measure 1 (M ₁ ; cm)	20.7	20.7
Caelomatic measure 2 (M ₂ ; cm)	19.9	20.5
Caelomatic measure 3 (M ₃ ; cm)	15.9	14.2

Table 2 Evaluation of morphometric parameters of Piracanjubas after different treatment days, independent of the photoperiod.

Variables	Time of treatment	
	45 d	90 d
Weight (g)	117.4	136.0
Total length (cm)	18.8 ^a	21.3 ^b
Standard length (cm)	15.3 ^a	19.4 ^b
Hepato-somatic index (HSI)	1.8	1.1
Caelomatic measure 1 (M ₁ ; cm)	20.7	20.7
Caelomatic measure 2 (M ₂ ; cm)	19.3	20.8
Caelomatic measure 3 (M ₃ ; cm)	13.6	15.7

Different letters in columns indicate statistical difference ($P < 0.05$).

at 90 d, the GSI for the photoperiod 18L:6D was 0.25 and for photoperiod 6L:18D was 0.71 ($P < 0.05$). So GSI was higher for juveniles Piracanjubas maintained in photoperiodic treatment 6L:18D during 90 d.

In Fig. 2, it is possible to observe the fat index in fish kept at 18L:6D presented a decrease tendency (2.78 at 45 d and 1.86 at 90 d), although no significant difference ($P > 0.05$) was identified. While juvenile Piracanjubas kept in 6L:18D showed a fat index 2.01 at 45 d and 2.95 at 90 d, showing significant increase in fat index. Among the hematological variables, there was a significant interaction ($P < 0.05$) between photoperiodic treatment and days of treatment for hematocrit, percentage of segmented neutrophils and lymphocytes (Table 3). Hematocrit was higher for Piracanjuba maintained at 18L:6D. Fish kept at 18L:6D photoperiod presented a decreased hematocrit from 58.1% at 45 d to 46.9% at 90 d, however it was not significant. Fish kept at 6L:18D presented 43.8% of hematocrit at 45 d and 42.7% at 90 d, and those results are not significant. However, the hematocrit of fish kept on photoperiod 18L:6D was higher than of

those maintained at 6L:18D ($P < 0.05$) (Table 3).

For total leukocytes, total thrombocytes, basophils, eosinophils, rods neutrophils, monocytes and leukocyte granulocyte-PAS positive (LG-PAS), there was no interaction between photoperiod and day of treatments (Tables 4 and 5).

When comparing segmented neutrophils in different photoperiod, it is possible to observe that for fish kept at 18L:6D during 45 d was 58.1% and for fish kept under 6L:18D was 50.1% at the same period. For fish kept at 6L:18D, hematocrit was of 43.8% at 45 d and 42.7% at 90 d, showing a higher number for the last one ($P < 0.05$). Segmented neutrophils were higher for Piracanjuba kept at 6L:18D when compared with fish kept at 18L:6D at 90 d. For fish kept at 18L:6D, linfocytes were 31.5% at 45 d and 43.5% at 90 d.

Piracanjubas kept at 6L:18D photoperiod, linfocytes were 40.5% at 45 d and 81.5% at 90 d. Fish kept at 6L:18D presented a higher percentage of linfocytes than those kept at 18L:6D at 90 d.

When evaluating the effect of days treatment, a numbers of total leukocyte was observed in fish kept for

90 d (Table 5). However, this effect was not observed when comparing the different photoperiods (Table 4). Thrombocytopenia was observed in fish kept at 18L:6D. It was also observed that fish at 90 d presented a lower amount of thrombocytes/mL (Table

5). Lower levels of basophyls were observed in fish kept at 6L:18D (Table 4), so as in fish kept for 90 d (Table 5). Significant differences were not found for eosinophils, monocytes, rod neutrophils and LG-PAS (Tables 4 and 5).

Fig. 1 Evaluation of gonado-somatic index (GSI) and the interaction between time and photoperiod.

Lowercase letters indicate differences between days of treatment and uppercases indicate difference between the photoperiodic treatment ($P < 0.05$; $P = 0.0018$).

Fig. 2 Evaluation of fat index and the interaction between time and photoperiod.

Lowercase letters indicate difference between treatment days and uppercase letters indicate difference between photoperiods used. ($P < 0.05$; $P = 0.0018$).

Table 3 Variables that showed interaction between days of treatment and the photoperiod.

Variables	Photoperiod (light:dark)	Days	
		45	90
Hematocrit (HT) (%)	18L:6D	58.1 ^{aA}	46.9 ^{aB}
	6L:18D	43.8 ^{aA}	42.7 ^{aA}
Segmented neutrophils (%)	18L:6D	58.1 ^{aA}	48.0 ^{aA}
	6L:18D	50.1 ^{aA}	69.7 ^{bB}
Linfocytes (%)	18L:6D	31.5 ^{aA}	43.5 ^{aA}
	6L:18D	40.5 ^{aA}	81.5 ^{bB}

Lowercase letters indicate differences between photoperiodic treatments at lines (18L:6D and 6L:18D); uppercase letters indicate differences between days of treatment at rows (45 d and 90 d) ($P < 0.05$).

Table 4 Values averages of the variables, when comparing the photoperiods.

Variables	Photoperiod (light:dark)	
	18L:6D	6L:18D
Total leukocytes/mL	7.6×10^4	7.3×10^4
Total trombocytes/mL	9.0×10^{6a}	11.4×10^{6b}
Basophyls/mL	0.8 ^a	0.2 ^b
Eosinophyls/mL	2.3	0.6
Rodsneutrophils/mL	4.8	5.9
Monocytes/mL	2.5	3.7
LG-PAS/mL	0.3	0.5

Lowercase letters indicate differences between treatments ($P < 0.05$).

Table 5 Evaluation of hematological parameters in Piracanjubas kept in a recirculation system for 90 d.

Variables	Time of treatments (days)	
	45	90
Total leukocytes/mL	9.50×10^{4a}	4.50×10^{4b}
Total trombocytes/mL	1.64×10^{7a}	1.67×10^{6b}
Basophyls/mL	0.91 ^a	0.23 ^b
Eosinophyls/mL	0.16	0.11
Rodneutrophils/mL	6.66 ^a	3.83 ^b
Monocytes/mL	4.10	2.40
LG-PAS/mL	0.75	0.20

Different letters indicate statistical difference ($P < 0.05$).

4. Discussion

Handling used in intensive fish farms during the process of production or capture can promote increase or decrease of well-being, directly influencing the productive and reproductive parameters. Management is important, because it allows the maximum expression of genetic potential [22].

Changes in coelomatic measurements M_1 , M_2 and M_3 were not observed. The results indicate that fish were not able for reproduction, since they were juvenile. Bulging of coelomatic cavity would be an indicator of reproduction [22]. Accumulated visceral

fat is related to excess of energy. In Piracanjubas maintained in 6L:18D, there was a greater accumulation of visceral fat, indicating storage of energy reserves, necessary for growth, periods of lack of food or winter anorexia [23]. Fig. 2 shows an increasing of total and standard length, which was influenced by the period of treatment but not by photoperiod, as opposed to fat index. Literatures indicate that Piracanjubas juveniles have the ability to store lipids, but are not adept at using this source to increase growth rates [2].

Consequently, the accumulation of visceral fat may

be related to the gonadal increase, which would indicate a better preparation of the juveniles for the reproductive event in the pubertal age. Although it has been identified that the visceral fat does not have the capacity to synthesize, it is related to gonadal development, since there is a correlation between somatic growth, environmental factors and onset of puberty, which may affect gonadal maturation and fish growth [24].

However, GSI was influenced by the photoperiod. Fish kept in 6L:18D had a higher GSI than those maintained in 18L:6D at 90 d. Melatonin is a hormone synthesized by pineal gland and produced during the night. This hormone is known as inhibitor of some fish reproduction, due to inhibition of GnRH from hypothalamus and diminish of oestradiol concentrations in brain. Both hormones are implicated in the induce of the onset of gonadal maturation [25]. However, the results in present study demonstrate that fish kept at lower photoperiod (6L:18D) presented higher GSI.

It is possible that the increase in fish activity caused by the increase of the photoperiod promoted an inhibition of gonadal growth, due to the energetic deviation necessary for homeostasis correction [26], overlapping the light stimulating mechanism on the gonadal pituitary hypothalamic axis, thus reducing the weight of the gonad in the Piracanjubas maintained in longer photoperiods.

The hematocrit of fish kept at 18L:6D for 45 d was higher than that of fish kept at 6L:18D ($P < 0.05$). This blood parameter is related to activity levels in teleost fish and increased demand for oxygen [27]. During the daytime period, there is an increase in the concentration of hormones, such as corticosteroids and catecholamines [6], and it is probable that fish kept in 18L:6D photoperiod showed higher amounts of those hormones, influencing their movement and consequently the hematocrit.

In the present work, the different photoperiods may have functioned as activators of the immune response, via damage associated molecular patterns (DAMPs).

This type of response act as sensors, inducers or mediators of sterile inflammatory stress responses through receptor recognition of the immune system [28, 29]. A leukopenia was observed in the fish kept during 90 d. In fish, there are still few studies of the influence of activation by DAMPs and the immunological consequences [30-33]. Vera-Jimenez and Nielsen [34] describe different patterns of leukocyte response in fish after stimulation by pathogen-associated molecular pattern molecules (PAMPs) and DAMPs. Lima et al. [35] report that recirculation systems for intensive production determine a decrease in the immune defense capacity of fish, which can be indicated by the decrease of total leukocytes and total thrombocytes.

The fish kept for 90 d presented low number of basophils and a decrease in the number of rods neutrophils, indicating that long periods of maintenance in recirculation systems make the mechanisms of compensatory response dysfunctional [26], and these compensatory responses are related to the altered metabolism and the number of cells in the immune system [36].

In fish, stress often causes leukopenia characterized by lymphopenia and neutrophilia [37]. The influence of photoperiod was observed on lymphocytes and segmented neutrophils separately, but not in relation to total leukocytes. Fish kept in 6L:18D photoperiod for 90 d presented higher amounts of segmented neutrophils and lymphocytes than fish kept at this time in 18L:6D. Falcon et al. [38] comment that stress severity may reduce the total number of circulating cells related to organic defense, corroborating the results found.

The total leukopenia associated with lymphopenia induced in Piracanjubas kept at photoperiodic treatment 18L:6D may be indicative of stress, which promotes a decrease in the number of circulating cells related to organic defense. Leukopenia may be secondary to recruitment of cells, mainly neutrophils representing 60% of white blood cells to tissues due to

activation of the innate immune response by DAMPs. It is possible that the association between the photoperiod and the maintenance of these fish may increase the challenge, promoting a cumulative effect on the response to stress. In addition, thrombocytes showed a decrease in numbers in fish kept in larger photoperiod, which could be caused by stress, since they also have a defense function [39].

5. Conclusions

Thus, it is possible to affirm that the increase of the photoperiod determines a decrease of visceral fat and GSI, but does not influence the somatic growth of these animals. In addition, the increase in photoperiod promoted an increase in hematocrit, but also a reduction of total leukocytes and total thrombocytes, with a reduction of segmented neutrophils and lymphocytes. Therefore, the photoperiod increase is indicated to promote puberty induction, and the excess light promote a stress and energy deviation to maintain the homeostasis. However, it is not indicated for juvenile Piracanjubas in recirculation systems, since the reduction of gonadal tissue and adipose tissue in the future may negatively influence the selection of these fish for possible reproduction. The immunomodulation caused by the photoperiod should be studied in more detail and in future works to the conclusion that although a leucopenia occurs with the excess of light, it can be transitory and with the final protective effect.

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