REFERÊNCIA

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EFFECT OF PHOTOPERIOD STRESS ASSESSMENT AND LOCOMOTOR ACTIVITY OF FEMALE LAMBARI (*Astyanax bimaculatu*)

Efeito do fotoperíodo na avaliação do estresse e na atividade locomotora em fêmeas de lambari (*Astyanax bimaculatus*)

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ABSTRACT

Some studies and scientific investments have been done in aquaculture aiming to minimize the stress of the fish due to different factors, as management, nutritional status, water quality, temperature, photoperiod, and salinity. This study aimed to verify the effect of photoperiod on locomotor activity and plasma levels of cortisol and glucose in female Lambari. One hundred and twenty female Lambari adult were maintained in aquaria of 20 liters each in a completely randomized design with three treatments (T1 = 0Light: 24Dark, T2 = 12L: 12D, T3 = 24L: 0D) and four replications. The daily locomotor activity was registered during 15 days using an infrared photocell. After 40 days of experiment and previous fasting for 24 hours, fish were euthanized by a lethal dose of benzocaine. Female Lambari featured a diurnal rhythm of locomotor activity. Lambari subjected to 12:12 and LD photoperiod presented higher cortisol (12L:12E: 190.00 ± 37.73 ng/mL e 24L:0E: 148.850 ± 32.77 ng/mL) and locomotor activity levels and also lower survival rates (30.0 ± 7.07%) when compared to the LD photoperiod (cortisol: 85.570± 7.99 ng/mL, survival rates: 72.5± 4.330%). The glucose concentration (32.167 ± 22.73 mg/dL) and the growth (7.050±0.59cm) of fish subjected to LD photoperiod was significantly lower than in the other treatments. We conclude that light plays an important role in the growth, behavior and welfare of female Lambari. Besides, our data highlighted that fish submitted to a long light period present a more stressed and more aggressive status when compared to fish submitted to a continuous darkness.

Index terms: Fish, synchronizer, glucose, cortisol, swimming behaviour.

RESUMO

Alguns estudos científicos e investimentos têm sido realizados na aquicultura com o objetivo de minimizar o estresse dos peixes, por diversos fatores, como a gestão, a salinidade do estado nutricional, qualidade da água, temperatura, fotoperíodo e salinidade. Este trabalho teve como objetivo verificar o efeito do fotoperíodo na atividade locomotora e níveis plasmáticos de cortisol e glicose em fêmeas de lambari. Cento e vinte fêmeas de lambaris adultas foram mantidas em aquários de 20 litros cada um, em delineamento inteiramente ao acaso, com três tratamentos (T1 = 0 Luz:24 Escuro, T2 = 12L:12E, T3 = 24L:0E) e quatro repetições. A atividade locomotora diária dos lambaris foi registrada durante 15 dias por meio de uma fotocélula infravermelha. Após 40 dias de experimento os peixes, mantidos a jejum de 24 horas, foram previamente anestesiados com benzocaína. As fêmeas de lambaris apresentaram um ritmo de atividade locomotora diurna. Lambaris submetidos aos fotoperíodos 12L:12E e 24L:0E apresentaram maiores níveis de cortisol (12L:12E: 190.00 ± 37.73 ng/mL e 24L:0E: 148.850 ± 32.77 ng/mL) e atividade locomotora, além de menores taxas de sobrevivência (30,0 ± 7,07%) em relação ao regime de 0L:24E (cortisol: 85.570± 7.99 ng/mL, taxa de sobrevivência: 72.5± 4.330%) A concentração de glicose (32.167 ± 22.73 mg/dL) e o crescimento (7.050±0,59cm) dos peixes submetidos a 24L:0E foi significativamente menor ($p<0,05$) em relação aos outros tratamentos. Conclui-se que a luz desempenha um importante papel no crescimento, comportamento e bem-estar de fêmeas de lambaris. Além disso, os dados evidenciaram que os peixes submetidos a períodos longos de luz ficam mais estressados e agressivos em relação ao período de escuridão contínua.

Termos para indexação: Peixe, sincronizador, glicose, cortisol, comportamento natatório.

INTRODUCTION

Rotation and translation of the Earth submit the living organisms to cyclic changes of environmental factors. All life forms respond to the sun and moon cycles, as well as to seasonal changes. Those responses are called biological clock or circadian rhythm (Carr et al. 2006). Most of the biochemical, physiological, and behavioral events of the living things are rhythmic. The factors external to the rhythmic nature clock that influence the biologic rhythm are called synchronizers or "zeitgeber. The photoperiod and temperature cycles are the major synchronizers of daily and annual rhythms (Fálcon et al. 2010).

The Lambari (*Astyanax bimaculatus*) is a Brazilian native species of the Characidae family. This small species easily accepts artificial feeding and has a good potential for

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the aquiculture, used as bait, tidbit, and as an ornamental fish (Sato; Fenerich-Verani; Verani, 2006).

The effect of environmental variables on growth, survival, and physiological responses of fish has been very well studied. Some studies and scientific investments have been done in aquaculture aiming to minimize the stress of the fish due to different factors, as management, nutritional status, water quality, temperature, photoperiod, and salinity (Bani et al. 2009). Inadequate artificial conditions like limited space, high stock density, lack of food, and photoperiod, can influence the aggressive behavior in fish (Hecht; Piennar 1993; Villamizar; Garcia-Alcazar; Sánchez-Vazquez, 2009; Blanco-Vives et al. 2010; Navarro; Navarro 2012; Navarro et al., 2013).

The photoperiod is an environmental stimulus related to the light length period over a day. The intensity and increase of this light time change according to the seasons and the climate of the region (Bromage; Porter; Randal, 2001). The photoperiod, together to other synchronizers, can shape the circadian and/or annual rhythms, influencing the fish growth, survival, and reproduction (Mendonça et al. 2009). According to Biswas and Takeuchi (2002), the photoperiod, among other factors, is the one with the highest influence on the biological rhythm of the animals, by affecting the weight gain, food intake, energy use, locomotion, as well as other physiological parameters.

Regarding the behaviors' rhythm, fish species are classified as diurnal, nocturnal, or twilights, according to their activity peak (Oliveira et al. 2009). The photoperiod is a key factor synchronizing the fish locomotor activity. Bayarri et al. (2002), Observed in *Solea senegalensis* a higher locomotor activity through the night, with 84.3% of this activity during this period. Yokota and Oishi (1992) verified in a higher diurnal activity for *Oryzias ladipes* during the summer, independent of the water layer.

Cortisol, the main glucocorticoid synthetized by the interrenal tissue of fish, plays an important role in the response to stress, behavior, osmoregulation, metabolism, growth, reproduction, and immune function (Wendelarr et al. 1997). In teleost fish, the elevation of plasma cortisol and glucose is the main response to stress, largely used as indicators of this response. Cortisol is used to characterize the primary response, and glucose, the secondary response to stress (Martínez-Porchas; Martinez-Córdova; Ramos-Enriquez, 2009).

Besides, the photoperiod manipulation with focus on better growth of fish has become an interesting subject for the commercial production of several species (Taylor; Migaud 2009). The literature contains little information on the photoperiod influence on growth, behavioral and physiological patterns of Brazilian fish native species. Thus, the goal of the present study was to analyze the photoperiod effect on the locomotor activity and on the cortisol and glucose plasma levels of female Lambari.

MATERIALS AND METHODS

This experiment was conducted at the Department of Veterinary Medicine, Federal University of Lavras - UFLA, Physiology and Pharmacology Laboratory, for 40 days, from January 25th to March 07th, 2010.

Animals and facilities

For this experiment, it was used 120 female Lambari adult, *Astyanax bimaculatus,* from the Federal University of Viçosa – UFV, weighing 5.18 ± 1.80g and length of 6.50 ± 0.80 cm. Sexual differentiation was conducted according to (Navarro et al., 2006). After 15 days of acclimatization, fish were distributed in 12 aquaria of 20 L each, in a randomized experimental design, with three treatments and four replications. The treatments differed in the photoperiod simulation (Light:Dark cycle - LD) as follows: (T1= 0:24 light:dark (0L:24D; DD); T2= 12L:12D; LD; T3= 24L:0D LL).

The experimental aquaria were kept in a closed recirculating system, in which water from the aquaria was captured and passed through a mechanical and biological filter of 250 L for later use; this system maintains water quality. The water temperature controlled by a thermostat at 27° C.

Dissolved oxygen, pH, and temperature were measured daily. The oxygen was supplied by aerators, with 640mL min^{-1} water flow, measured by a digital oximeter (YSI Bernauer, Blumenau, Brazil).The pH and temperature were measured by digital a multi-parameter measurer pH com um pHmetro portátil da marca Bernauer F-1002, Blumenau, Brazil.

Groups of four aquaria were kept isolated under a lighting system controlled by individual timers and one 20 W fluorescent lamp with a constant intensity of 1173 lx on the water surface, measured by a digital light meter Model LDR-208 (Instrutherm, São Paulo, São Paulo, Brazil). The lamp for the 12L: 12D lighting program was always turned on at 07:00 h and turned off at 19:00, through the automated system timer brand Brasfort. Moreover, the lighting programs in the 0L:24D and 24L:0D lamps remained switched off and on, respectively, throughout the experimental period.

During the feed management, fish were fed with a commercial diet containing 28% CP and 3,100 kcal DE kg^{-1} (Tropical NUTRON Hi-Fi). The amount of diet given daily was 3% body weight, divided into 2 daily meals, (9:00 a.m. and 4:00 p.m.). After 15 minutes each meal, the leftovers were removed.

The survival rate was calculated by the formula TS = Sf / Si x 100, Sf = final survival, Si = Initial Survival.

Locomotor activity

The fish were fed twice a day at fixed times, with a commercially extruded feed containing 28% crude protein pellets, 2 mm in diameter, at a rate of 3% body weight.

In order to assess the locomotor activity of the fish, the aquaria were equipped with photocells (Omron, model E3S-AD62, Kyoto, Japan) that were positioned in the center and to the front of the aquaria. The photocells were connected to a channel board (USB-1024HLS, Measurement Computing, Norton, Massachusetts, USA) and to a computer. The photocells worked continuously, emitting a beam of infrared light, and each interruption caused by a fish was counted and recorded on a data sheet of a specialized program (DIO98USB, University of Murcia, Spain) every 10 min (Blanco-Vives; Sanchez-Vázquéz, 2009). During the 15 days of experiments were conducted, behavioral observation sessions, lasting 20 minutes for each treatment to complement the behavioral analyzes.

Collection procedures and biochemical analysis

By the end of the experiment, blood samples were taken with 1mL syringes by caudal puncture to measure blood glucose, and check indicators of stress and differences caused by the different photoperiods. Of each treatment, 12 fish (three of each repetition) were fasted for 24 hours and anesthetized with benzocaine 400 mg L^{-1} to start the collecting procedures. Data of weight and total length of each fish were taken with a balance accurate to 0.001g and a digital caliper, respectively.

An aliquot of the blood sample was used to quantify the blood glucose through a glucose digital monitor (ACCU – CHEC SOFTCLICK – United States). The most part of the collected blood was placed into eppendorf tubes with heparin and kept on ice until completing all blood samplings. The collected blood was then centrifuged for 10 minutes at 5000 rpm in an eppendorf centrifuge (model 5415C). Later, the heparinized plasma (supernatant) was removed using a digital pipette. The collected plasma was stored in labeled eppendorf tubes for further hormone analysis. All plasmatic material was frozen at -20°C until analysis.

For determining cortisol, the plasma was analyzed using the ELISA technique (Cortisol 96t. Elisa Kit – Human).

The statistical analyses were done by the software SAS, being the mean values compared by the SNK test at 5% significance.

RESULTS AND DISCUSSION

During the experimental period, the average values of temperature 28.67± 0.73° C, pH 6.65± 0.68, and dissolved oxygen 5.23± 0.85 mg L^{-1} were within the acceptable range for rearing fish (Sato; Fenerich-Verani; Verani, 2006).

During the 15 monitoring days, the Lambari under a 24L:0D cycle presented an intense swimming activity throughout the period (Figure 1). In the 12L:12D treatment, the Lambari had a higher activity during the light phase and an activity peak around 8:00 clock AM (Figure 2). It means that during the light phase the Lambari locomotor activity was 4.254 min^{-1}, while during the dark phase it was 1.581 min^{-1}. In the treatment 0L:24D, fish featured a low swimming activity for almost the entire period, except the activity peak observed around 8:00 clock AM (Figure 3).

The activity peak was detected both in fish submitted to 12L:12D and 0L:24D treatments, before the fist feed period, probably this is due to the anticipatory behavior of fish in relation to anticipated perception of the feeding period. According to Chandroo et al. (2004), these cognitive characteristics are called declarative representations, which imply in selective attention to stimuli, capability to anticipate and have expectancies, as well as a flexible and integrated focus of the behavioral responses. The formation of declarative mental representations is a prerequisite to the sentience (awareness of sensations and feelings) and this has been described for fish regarding contexts of social interaction, spatial memory, and learning (Galhardo; Oliveira, 2006). Matioli et al. (1997) suggested that the substance P (neuropeptide implied in some neuronal plasticity) stimulates the memory and learning of goldfish (*Carassius auratus*) in the context of duties with specific motivation (hunger).

In this study, the Lambari, a native species of the tropics where the natural photoperiod remains close to 12L:12D (Fálcon et al. 2010), presented a diurnal activity rhythm during the artificial manipulation of the light cycle. Other species like zebrafish, *Danio rerio*, has a striking diurnal activity, being almost inactive during the night (Blanco-Vives; Sanchez-Vásquez 2009). This preference to be active in certain periods of the day may be species specific and can be associated with genetic inheritance or with adaptation to the habitat (food availability, predation, etc.) or it can depend on sensory factors, such as need the vision to capture the food (Oliveira et al. 2009).

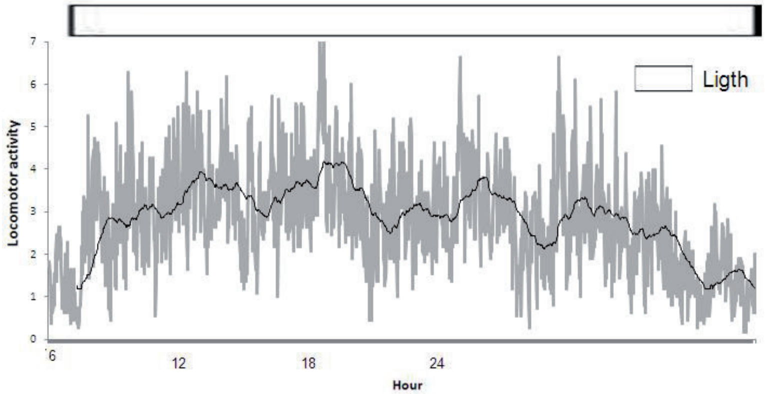

Figure 1 – Daily locomotor activity of female Lambari (*Astyanax bimaculatus*) under a 24L:0D photoperiod. The white bar at the top indicates the light period.

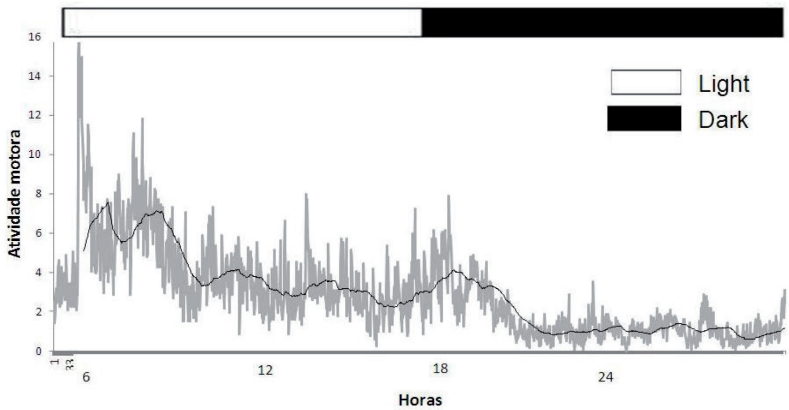

Figure 2 - Daily locomotor activity of female Lambari (*Astyanax bimaculatus*) under a 12L:12D photoperiod. The White and black bars at the top indicate the light and dark periods, respectively.

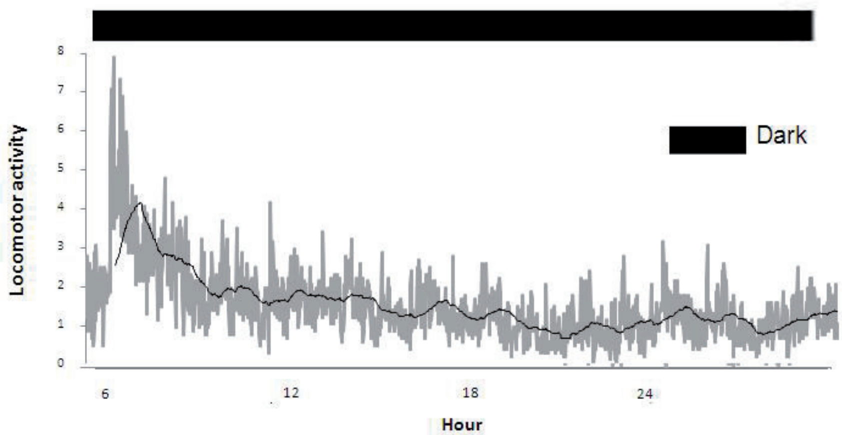

Figure 3 – Daily locomotor activity of female Lambari (*Astyanax bimaculatus*) under a 0L:24D photoperiod. The black bar at the top indicates the dark period.

The present study showed higher levels of cortisol ($p<0.05$) in the treatments 24L:0D (148.850 ng mL^{-1}) and 12L:12D (190.000 ng mL^{-1}) when compared to the treatment 0D:24D (85.570 ng mL^{-1}) (Table 1). Furthermore, we observed increased aggressiveness and sores on the body in fish under long photoperiods. However, Lambari under the 24L:0D cycle presented significantly ($p<0.05$) lower glucose levels (Table 1). Lambari submitted to treatment 24L:0D and 12L:12D presented low survival (30%) when compared to the treatment 0L:24D (72.5%) (Table 1).

Thus, the photoperiod is a key factor on synchronizing the locomotor activity in fish (Bayarri et al. 2004). This increased locomotor activity during long light periods can be justified by the higher levels of cortisol in the treatments 24L:0D and 12L:12D and aggressive behavior when compared to the treatment 0L:24D. These results agree with Leonardi and Klempau (2003) who demonstrated that an artificial 24L:0D photoperiod induced a response to stress in rainbow trout (*Oncorhynchus mykiss*) with a significant increase in cortisol plasma level after 2 months of experiment. The extended light period followed by a more aggressive behavior was also observed in silver catfish (*Rhamdia quelen*) (Piaia; Towsend; Baldisserotto, 1999).

Some studies verified that high levels of cortisol decrease the production of melatonin, probably due to the inhibition of arylalkylamine N-acetyltransferase (AANAT), an important enzyme for the melatonin biosynthesis in fish pineal gland (Benyassi et al. 2001; Yanthan; Gupta 2007; Nikaido et al. 2010, Larson et al. 2004). In this way, high levels of cortisol in the treatments 24L:0D and 12L:12D may have reduced the synthesis of melatonin and consequently increased the fish aggressiveness. The pineal gland hormone, released in a higher amount under low or absent light (Ekström; Meissl 1997; Bayarri; Madrid; Sánchez-Vazquez; 2002). Munro (1986) observed the role of melatonin in reducing aggressive responses in the blue acara *Aequidens pulcher*.

The greater luminosity in the treatments 24L:0D and 12L:12D followed by the increased swimming activity and aggressiveness (wounds on the body of death fish) justify the low survival (30%) of the Lambari submitted to these light cycles when compared to the treatment 0L:24D (72.5%) (Table 1). According to Almazán-Rueda et al. (2004), an increased swimming activity may lead to a higher probability of encounters between fish, increasing the susceptibility of attacks.

Several authors addressed this negative relationship between long light periods and low fish survival. Almazán-Rueda, Schrama and Verreth (2005) observed a reduction of the survival rate in *Clarias gariepinus* submitted to 24 hours of light. This fact was also registered for *Scophthalmus maximus* juveniles by Imsland, Folkvorde and Steffansson (1995). Similarly, Sigholt et al. (1995) observed a low survival of atlantic salmon (*Salmo salar*), during its early life under a continuous light. Other researchers verified high survival rates in *Hoplias lacerdae* larvae kept under low light intensity and total darkness (Luz; Portella, 2002). However, Reynalte-Tataje et al. (2002) analyzed piracanjuba *Brycon orbignyanus* larvae and observed a higher survival in the treatment with 24 hours of light, and the lowest survival, in the treatment without light.

Moreover, the lower glucose levels by Lambari submitted to 24L:0D (Table 1), probably, the intense swimming activity throughout the period of continuous light led to a greater exhaustion, followed by a higher demand for energy, and glucose breakdown into lactate by the anaerobic glycolysis process in the white muscle to meet motor activities rather than growth processes. Thus, although no significant difference was found in the final weight, the total length was significantly smaller in fish subjected to a continuous light cycle (24L:0D) (Table 1). Otherwise, several studies on fish showed a

Table 1 – Plasma levels of glucose and cortisol, final weight (g), total length (cm) and survival rate of female Lambari (*Astyanax bimaculatus*) according to the photoperiod.

Tratament	24L:0D	12L:12D	0L:24D
Glucose (mg/dL)	32.17 ± 22.73^{b}	65.18 ± 28.00^{a}	51.85 ± 20.48^{a}
Cortisol (ng/mL)	148.85 ± 32.77^{a}	190.00 ± 37.73^{a}	85.57 ± 7.99^{b}
Final weight (g)	6.476 ± 1.67^{a}	7.017 ± 1.78^{a}	6.745 ± 1.28^{a}
Total length (cm)	7.050 ± 0.59^{b}	8.100 ± 0.63^{a}	7.886 ± 0.52^{a}
Surviving rates %	30.00 ± 7.07^{b}	30.00 ± 7.07^{b}	72.50 ± 4.33^{a}

Averages in the same line with different superscript are significantly different according SNK test ($p<0.05$). Average ± EPM.

positive relationship between continuous exposure to light, body weight, somatic growth, and feed intake. This was observed for the atlantic salmon (Oppedal et al. 1997); atlantic cod, *Gadus morhua* (Taranger et al. 2006; Hansen et al. 2001), *Melanogrammus aeglefinus* (Davie et al. 2007), *Pagrus major* (Biswas et al. 2010), *Oplegnathus fasciatus* (Biswas et al. 2008), and *Sparus aurata* (Ginés et al. 2004). Nevertheless, the photoperiod manipulation did not influence the total length and body weight of *Verasper moseri* (Amano et al. 2004) and *Oreochromis niloticus* (Campos-Mendoza; McAndrew; Coward, 2004).

CONCLUSIONS

Light plays an important role in the behavior and welfare of female Lambari (*Astyanax bimaculatus*). Besides, data suggest that this species has a diurnal activity rhythm, and that fish submitted to a long light period have a greater aggressiveness than those fish under a continuous dark period.

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