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MONITORING WHOLE BLOOD, PLASMA AND SERUM VARIABLES OF NILE TILAPIA DURING 24 HOURS, AFTER CAPTURE STRESS*

Diana Meira ALMEIDA[1]
Maria Letizia PETESSE[2]
Leonardo TACHIBANA[3]
Danielle de Carla DIAS[4]
Renata Guimarães MOREIRA[5]
Maria José Tavares RANZANI-PAIVA[3]

[1] Instituto de Pesca, Agência Paulista de Tecnologia dos Agronegócios/Secretaria de Agricultura e Abastecimento – APTA/SSA, Av. Francisco Matarazzo, 455, CEP 05001-900, São Paulo, SP, Brasil.

[2] Instituto de Pesca, Av. Francisco Matarazzo, 455 CEP 05001-900, São Paulo, SP, Brasil.

[3] Instituto de Pesca, Agência Paulista de Tecnologia dos Agronegócios/Secretaria de Agricultura e Abastecimento – APTA/SSA, Instituto de Pesca, Av. Francisco Matarazzo, 455 CEP 05001-900, São Paulo, SP, Brasil. E-mail: mase@pesca.sp.gov.br (corresponding author).

[4] Instituto de Pesca, Av. Francisco Matarazzo, 455 CEP 05001-900, São Paulo, SP.

[5] Universidade de São Paulo – USP, Instituto de Biosciências, Departamento de Fisiologia, Rua do Matão, trav. 14, 101, salas 220/219B, CEP 05508-090, São Paulo, SP, Brasil.

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ABSTRACT

Stress is know as responsible for the decrease in immunity and reduced survival, growth, and reproductive capacity of animals. Besides, organisms respond to certain biological rhythms, such as circadian, to which animals naturally adjust. This study aimed to monitor the plasma (lactate and total protein), serum (cortisol and cholesterol) and whole blood (glucose) variables in tilapia in a 24 h cycle. A set of 49 aquariums with 5 individuals each were used. All of them, at the beginning of the experiment, were subjected to the same stress of capture and handling. The animals from the first aquaria were sampled after 15 min of stress. Blood samples from animals of the remaining aquarium were sequentially drawn at 30-min intervals. Radar charts were used to visualize the hourly change in the measured variables. Significant differences of each analysis between the measured variables by aquarium and the phases of the circadian cycle (light-dark) and morning-afternoon-night-dawn intervals, were evaluated using the Mann-Whitney and Kruskal-Wallis tests, respectively. A positive relationship between cortisol and glucose levels was found. Most analysis, except cholesterol and lactate did not show any significant difference between light and dark periods, and the intensity and frequency of peaks observed were not deleterious to individuals.

Key words: fish; stress; circadian cycle; management.

MONITORAMENTO DAS VARIÁVEIS DO SANGUE TOTAL, PLASMA E SORO DE TILÁPIA-DO-NILO, DURANTE 24 HORAS, APÓS ESTRESSE DE CAPTURA

RESUMO

O estresse é conhecidamente responsável pela diminuição da imunidade e redução da sobrevivência, crescimento e capacidade reprodutiva dos animais. Além disso, os organismos respondem a certos ritmos biológicos, como o circadiano, aos quais se ajustam naturalmente. Este estudo teve como objetivo monitorar as variáveis plasmática (lactato e proteína total), soro (cortisol e colesterol) e sangue total (glicose) na tilápia em um período de 24 h. Quarenta e nove aquários com 5 indivíduos cada foram utilizados. Todos eles, no início do experimento, foram submetidos ao mesmo estresse de captura e manuseio. Os animais do primeiro aquários foram amostrados após 15 min do estresse. Os demais As amostras de sangue de indivíduos de cada aquário foram sequencialmente em intervalos de 30 min. Somente os indivíduos do primeiro aquário foram analisados após 15 minutos de estresse. Os gráficos de radar foram usadas para visualizar a variação horária nas variáveis medidas. Diferenças estatisticamente significativas entre a média dos indivíduos de cada aquário, para as fases do ciclo circadiano (luz-escuro) e os intervalos manhã-tarde-noite-madrugada foram respectivamente avaliadas utilizando os testes Mann-Whitney e Kruskal-Wallis. Observou-se relação positiva entre os níveis de cortisol e glicose. As análises, com exceção de colesterol e lactato, não mostraram diferença significativa entre períodos de luz e sombras, e a intensidade e a freqüência dos picos observados não foram prejudiciais aos indivíduos.

Palavras-chave: peixes; estresse; ciclo circadiano; manejo.

INTRODUCTION

Tilapia are among the fastest-growing commercial fish groups in the world, especially due to increased production in China and other developing countries such as Brazil. Among the various species, those of the genus *Oreochromis* are the most used in aquaculture (HEMPEL, 2002).

Proper management, in particular, is essential to the success of aquaculture and consists of monitoring aspects such as water quality, food, stocking density and animal health. The adequate control of these aspects aims to provide fish well-being in the culture so that they can express their best growth potential (BARTON and IWAMA, 1991). However, the variability of conditions in the culture environment, such as exposure to natural environmental factors (daily variations in light/dark period and temperature and presence/absence of rainfall, and changes in water quality) or routine interventions in the cultivation system (capture techniques, handling of fish for biometrics and transport) may alter the homeostasis of individuals (BARCELLOS *et al*., 2001; SAMPAIO and FREIRE, 2016), triggering stress responses.

The causes of fish stress are practically unavoidable when it comes to routine management, and for operational reasons intrinsic to aquaculture, individuals are subjected to acute or chronic stress conditions. The triggered responses, classically described by SELYE (1950) as general adaptation syndrome, correspond to a series of physiological changes that may be of the primary type (hormonal changes - for example, cortisol), secondary type (changes in glucose, lactic acid, and hepatic and muscle glycogen) or tertiary type (changes related to growth impairment, changes in behavior and increased susceptibility to diseases). In general, when certain limits are exceeded, recovery capacity and fish performance during the successive reestablishment phase can be compromised (MOMMSEN *et al*., 1999). These observations justify the need to monitor the behavior of some blood variables to better understand the consequences of handling on the physiology of individuals so that stress conditions can be quickly detected.

According to VOLPATO and BARRETO (2001) and LÓPEZ-OLMEDA *et al*. (2013) important effects on homeostasis may also derive from changes in the endogenous rhythms of a living being in response to certain environmental stimuli. In fact, many physiological and behavioral processes in organisms are rhythmic, occurring within a 24-h period and are referred to as circadian or nictemeral cycles (ROENNEBERG and MERROW, 2002).

Biological rhythms have been classified according to their periodicity: ultradian, cycles that are repeated at intervals of up to 20 h; circadian, for intervals of 20 to 28 h; and infradian for intervals longer than 28 h (HERRERO *et al*., 2003). The circadian cycle is considered the most important synchronizing environmental factor of the biological rhythms and is present in the most varied groups of vertebrates and invertebrates, where it is therefore one of the most studied (LÓPEZ-OLMEDA *et al*., 2013). All circadian systems consist of at least three elements: (1) an afferent pathway that transmits information from the environment; (2) one or more oscillators capable of generating oscillation; and (3) efferent pathways, through which the oscillator regulates the expression of various rhythms. It can be said that photoreceptors are the first way of capturing and identifying the environmental cycle (VERA *et al*., 2007; SCHRECK and TORT, 2016).

Circadian clocks increase the innate ability of organisms to survive constant environmental changes by enabling them to efficiently anticipate periodic events such as availability of food, light and reproduction, among others. An individual who is simply driven by external changes is at a disadvantage relative to one that is regulated by a flexible and anticipatory endogenous clock. Organisms endowed with this ability to anticipate periodic events were evolutionarily viable and survived, preserving it to this day in the various forms that biological clocks have assumed in different species (PANDA *et al*., 2002; ROENNEBERG and MERROW, 2002; BRANDSTÄTTER, 2003; PARANJPE and SHARMA, 2005). The ability to respond to light is a universal aspect of clocks in all organisms, on which the rest/activity cycle is adjusted (LÓPEZ-OLMEDA *et al*., 2013; SCHRECK and TORT, 2016).

There has been constant research on the manifestations of the circadian rhythm, seeking to confirm and better understand locomotor, reproductive and alimentary rhythms (OLIVEIRA *et al*., 2009; KULCZYKOWSKA and SÁNCHEZ-VAZQUEZ, 2010). These studies have demonstrated the importance of the light-dark cycle as a biological rhythm synchronizer. Among these, knowledge of the pattern of locomotor activity of species is essential for captive breeding purposes, contributing to the selection of the most suitable species for a given growing region, as well as assisting in management practices (such as establishment of feeding schedules), and these aspects are factors that support the success of the enterprise. Accordingly, there are reports of daily variations in the levels of glucose, cortisol, digestive enzymes, lactate, cholesterol and proteins associated with synchronization by the feeding cycle (VERA *et al*., 2007; LÓPEZ-OLMEDA *et al*., 2009; MONTOYA *et al*., 2010; DEL POZO *et al*., 2012).

In fish, amphibians and reptiles are identified as ocular circadian oscillators, with properties described for invertebrates, such as mollusk and crustaceans. Circadian cycles in fish, amphibians, etc, are survival strategies that seek the best possible fit between an animal's physiology and some predictable events, such as sunrise (MADRID *et al*., 2001).

The objective of this study was to monitor the plasma, serum and whole blood variables (cortisol, glucose, cholesterol, lactate and monitorplasma protein) in Nile tilapia (*Oreochromis niloticus*) subjected to management stress in the 24-h cycle. In particular, we sought to answer the following questions: How do these variables vary over 24 h? Which variables are most affected by stress? Can the observed variations be related to the influence of the circadian cycle? Which part of the cycle has the most influence on the concentrations of blood variables?

METHODS

The fish used in this study had a mean length of 15.02 ± 1.20 cm and a mean weight of 66.60 ± 16.20 g. The fish were initially placed in two 1000L aquaria, with a 12 / 12h photoperiod, with constant aeration, temperature between 24 and 25 °C and the water parameters checked daily. The fish were fed twice a day (morning and afternoon) and 12 hours before the experiment, the animals were no longer fed.

The experiment started at 7:00 h and ended at 7:00 h on the following day, totaling 24 h. During the experiment period, the animals received no feed. Were used a set of 49 aquariums of 60 L, with water flow and constant aeration, temperature controlled at 25ºC and containing 5 individuals per aquarium, totaling 245 fish. All fish were subjected to the same capture stress (performed with a dip net) and handled for biometrics (total length and weight), remaining out of water for one minute. This protocol

was considered representative of a management stress condition normally present within an aquaculture enterprise.

Each fish was removed from the aquarium and immediately anesthetized with clove oil (25 mg L^{-1} in a 40 L bucket of water), and blood was collected by caudal vessel puncture. The first blood sampling occurred 15 min after application of the stressor and later at 30 min, 1:00 h, 1:30 h and so on until 24:00 h. Two syringes were used to collect blood, one heparinized and one non-heparinized. Plasma was obtained by centrifuging the collected blood with heparinized syringes and placed in in snap cap microcentrifuge tubes of 2 mL and the serum, obtained with syringes without heparin was placed also in snap cap microcentrifuge tubes of 2 mL, stored in a refrigerator at 4 °C and centrifuged after the end of the experiment. Centrifugation was at 2000 x g, for 10 min. The photoperiod was 12h light and 12h dark.

The serum concentrations of cortisol (µg dL^{-1}) and cholesterol (mg dL^{-1}) were respectively determined with the Elisa-DBC® kit and Trinder® colorimetric enzyme system.

A drop of blood from the non-heparinized syringe was used to measure the glucose concentrations (mg dL^{-1}) with the Accu-Check Activ Performa® glucometer.

Plasma concentrations of lactate (mmol L^{-1}) and total protein (g L^{-1}) were respectively determined with the Trinder® lactate oxidase system and the biuret reagent.

The values of the whole blood, plasma and serological variables measured in the individuals belonging to each time interval were initially submitted to the descriptive analysis, determining the mean ± standard error, median and observed minimum and maximum values.

Radar charts were prepared for analysis of the results using the mean of the individuals of each aquarium. To better visualize the temporal distribution of plasma (lactate and total protein), serum (cortisol, cholesterol) and whole blood (glucose) variables over 24 h, the observation period was divided into 12/12 h light and dark cycles and 6-h intervals coinciding with the morning, afternoon, night and dawn. In the charts, the values were placed followed the clockwise direction with the initial value of 00:00 h corresponding to midnight.

The Mann-Whitney test was used to evaluate significant differences in the variables measured in the 12/12 h (light-dark) cycle. The Kruskal-Wallis test was applied to the 6 h intervals to show which part of the day the peaks of the measured variables occurred. The use of the non-parametric alternative was justified by the lack of the parametric requirements for normality and homogeneity of the variances (ZAR, 2010). When the Kruskal-Wallis test was significant ($p<0.05$), the *a posteriori* multiple comparison test was used to identify which intervals differed one from the other. To avoid pseudoreplication, the statistical tests were applied on the variables mean values by aquarium.

The Spearman correlation was performed to verify the relationship between the variables. This was preferred over the Pearson correlation because of the lack of normality of the measured variables. All statistical tests were performed using Statistica software (StatSoft, version 7).

RESULTS

The grouping of the data by phases of the circadian cycle allowed us to show in a clearer and more synthetic way the dynamics of the whole blood, plasma and serum variables over the observed period.

Cortisol

The mean and standard error of the mean of cortisol for all in fish in the 49 aquarium were 5.75 ± 0.44 µg dL^{-1} and values varied between 1.38 and 14.93 µg dL^{-1}, with a median of 4.93 µg dL^{-1}.

In Figure 1A, corresponding to the 12/12h cycle, it can be observed that four cortisol peaks occurred at 11:00 h (13.396 µg dL^{-1}),

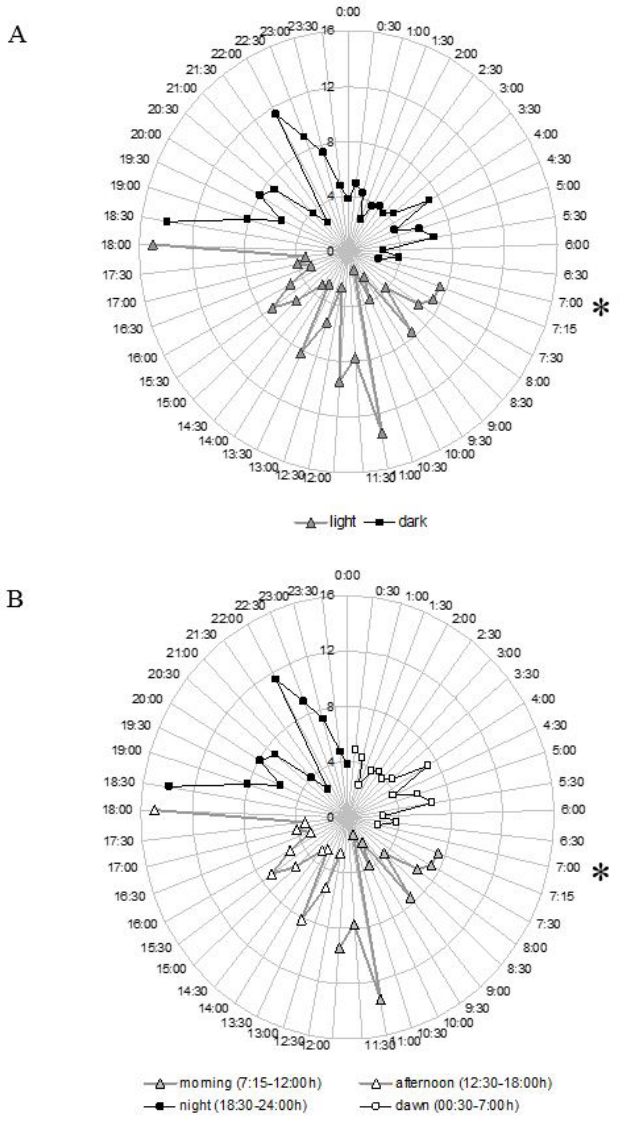

Figure 1. Medium variation on the plasmatic concentration of cortisol (µg dL^{-1}) of Nile tilapia *(O. niloticus)* during the 24 h. (A) light-dark; (B) morning, afternoon, night, dawn; *start of experiment.

at 18:00 h (14.934 µg dL^{-1}), at 18: 30 h (13.934 µg dL^{-1}) and 22:00 h (11.377 µg dL^{-1}). In particular, the peaks from 18:00 h and 18:30 h occurred corresponding to the light-dark passage. When considering the 6 h intervals, there was a clear decrease in the values at dawn (Figure 1B).

The test for the 12/12h cycle did not show significant differences between light and dark (n=49; U=296.0; p=0.95) and the same was for the 6h intervals (n=49, H=6.12, p=0.10). Despite the observed peaks and stabilization around minimum values in the dawn, the observed cortisol fluctuations were not substantial throughout the experiment.

Glucose

The mean and standard error of the mean of glucose for all fish in the 49 aquarium were 86.66 ± 6.65 mg dL^{-1} and levels ranged from 39.40 to 207.80 mg dL^{-1}, with a median of 66.80 mg dL^{-1}.

In Figure 2A, corresponding to the 12/12 h cycle, it can be observed that there were four glucose peaks at 13:30 h (201.8 mg dL^{-1}), 20:00 h (207.8 mg dL^{-1}), 22:30 (197 mg dL^{-1}) and 01:00 (206.4 mg dL^{-1}).

By analyzing the 6 h intervals (Figure 2 B), it was possible to observe a lower fluctuation of glucose values only in the morning showing that glucose response to handling stress was not immediate. Tendency for lower glucose levels in the latter part of dawn (from 05:00 to 07:00h).

The statistical tests applied to the two cycles considered did not show significant differences. However, the Spearman correlation between cortisol and glucose was positive with correlation coefficient r=0.44 and significant (p<0.05), confirming the relationship between the variables (Figure 3).

Lactate

The mean and standard error of the mean of fish's lactate for all 49 aquarium were 0.04 ± 0.00 mmol L^{-1} and values varied between 0.02 and 0.08 mmol L^{-1}, with a median of 0.03 mmol dL^{-1}.

In Figure 4A, it can be seen that the most intense peaks occurred at the beginning of the light period at 07:30 h. (0.075 mmol L^{-1}) and at the end of the dark period at 6:30 h (0.064 mmol L^{-1}) and 7:00 h (0.0836 mmol L^{-1}). Analyzing the values in the 6 h intervals, it was observed that fluctuations in lactate response occurred especially at night and dawn (Figure 4B).

The Mann-Whitney test applied to the 12/12 h cycle data, however, showed no significant difference between light and dark (n=49, U=246.50, p=0.29). The Kruskal-Wallis test, on the other hand, showed a significant difference between the 6 h intervals (n=49; H=8.79; p=0.03). The *a posteriori* multiple comparison test revealed that the morning period was different from night (Figure 5),

Fluctuations over 24 h rarely exceeded 0.05 mmol L^{-1} and the lowest values were observed in the late afternoon between 16:00 h

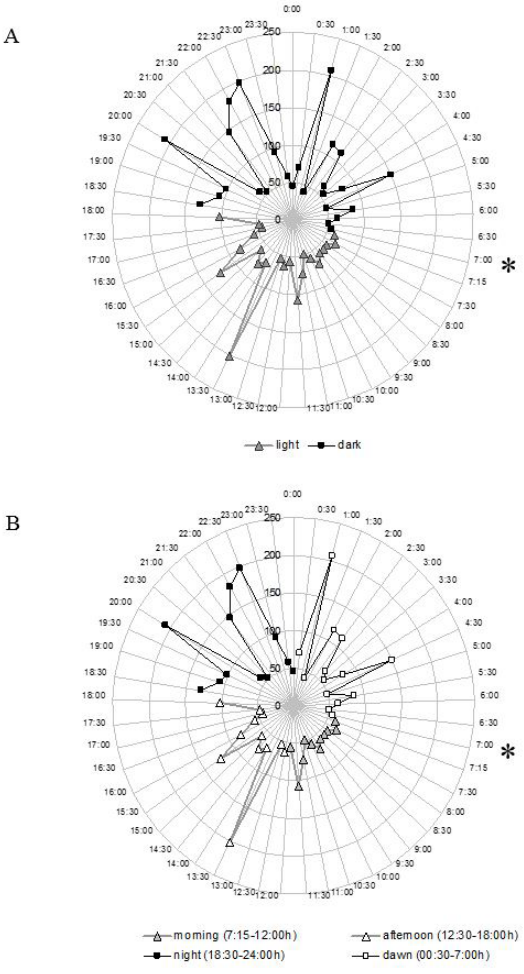

Figure 2. Medium variation on the plasmatic concentration of glucose (mg dL^{-1}) of Nile tilapia *(O. niloticus)* during the 24 h. (A) light-dark; (B) morning, afternoon, night, dawn; *start of experiment.

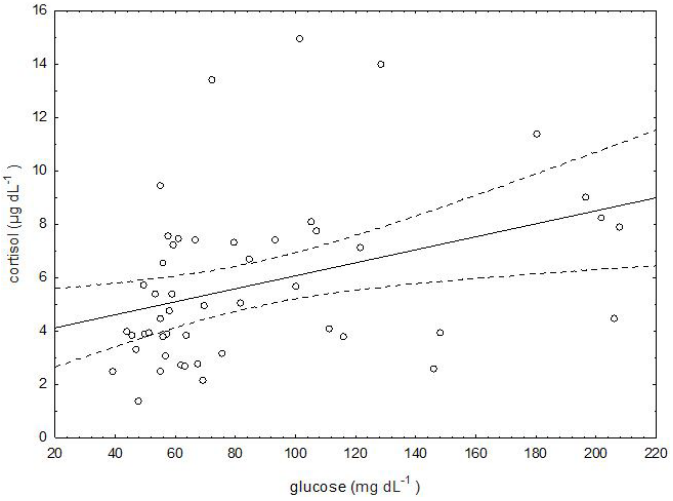

Figure 3. Spearman correlation between plasmatic cortisol and whole blood glucose of Nile tilapia (*O. niloticus*) during the 24 h.

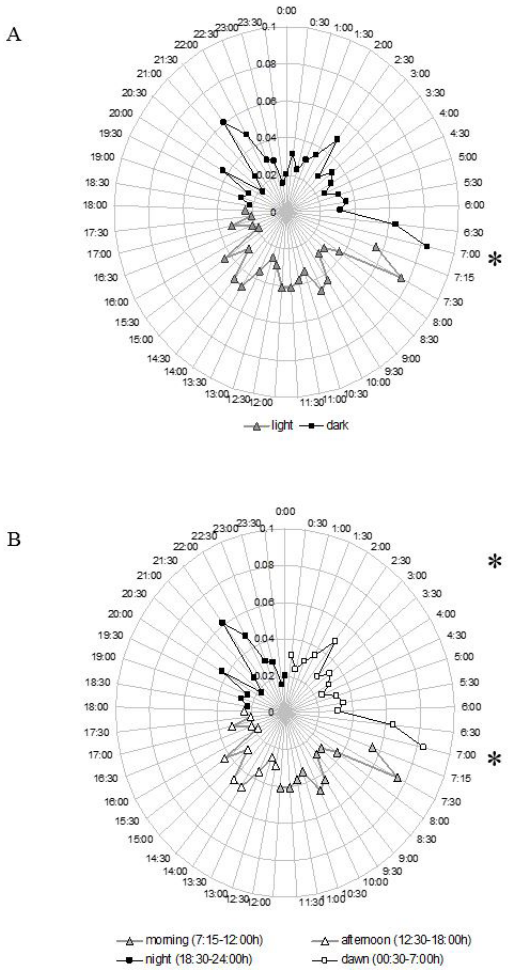

Figure 4. Medium variation on the plasmatic concentration of lactate (mmol L^{-1}) of Nile tilapia *(O. niloticus)* during the 24 h. (A) light-dark; (B) morning, afternoon, night, dawn; *start of experiment.

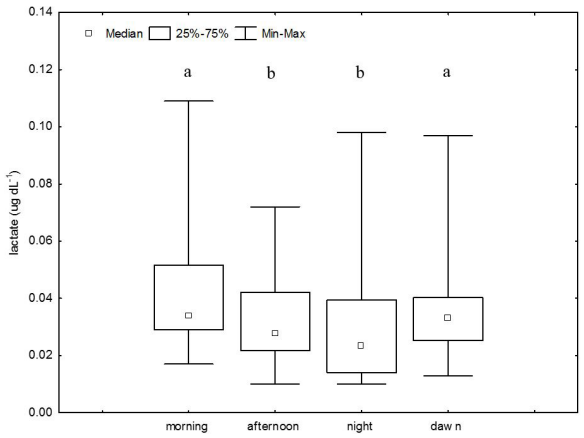

Figure 5. Box-Whisher plot distribution values of lactate (mmol L-1) of Nile tilapia (O. *niloticus*) during the 24 h. The letters show the multiple comparison test result (different letters = statistically significant difference among mean in the four periods) (n=49).

and 18:00 h. In general, it was observed that lactate responded quickly to the stimuli and fluctuated throughout the 24-h period,

Cholesterol

The mean and standard error of the mean for cholesterol for all fish in the 49 aquarium were 0.04 ± 0.00 mg dL^{-1} and levels ranged from 0.02 to 0.08 mg dL^{-1}, with a median 0.04 mg dL^{-1}. Overall, the cholesterol response fluctuated over the observation period and began to normalize around 17:30 h.

Analyzing the values in relation to the 12/12h cycle (Figure 6A), there was a clear decrease in values in the dark period. When considering the 6 h intervals (Figure 6B), this occurred in part of the night and dawn, indicating that in these time intervals, the values tended to stabilize; that is, the frequency and the intensity of the peaks decreased.

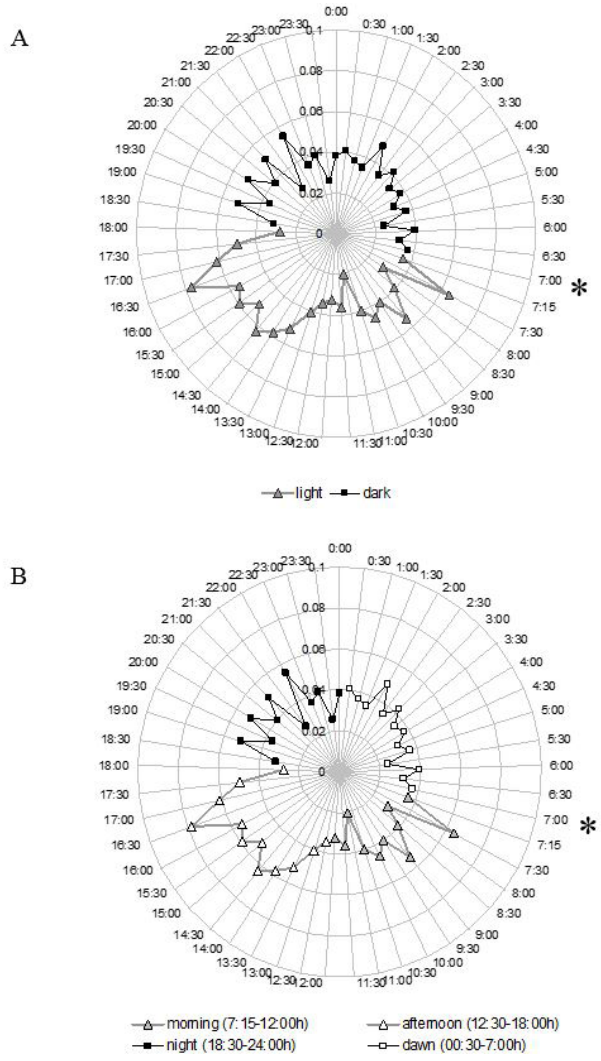

Figure 6. Medium variation on the plasmatic concentration of cholesterol (mg dL^{-1}) of Nile tilapia *(O. niloticus)* during the 24 h. (A) light-dark; (B) morning, afternoon, night, dawn; *start of experiment.

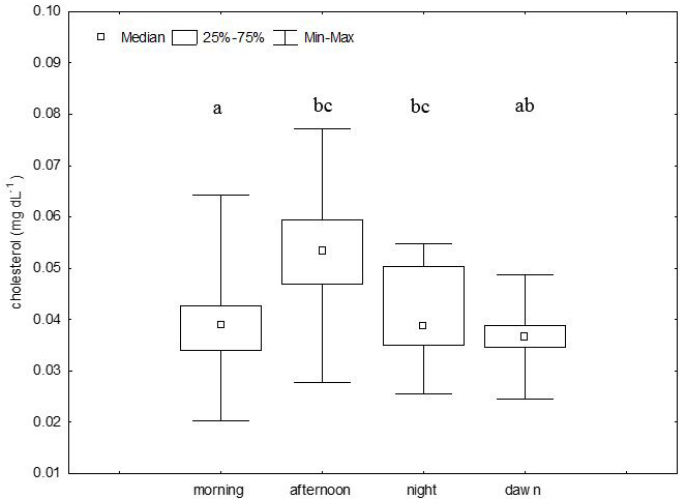

Figure 7. Box-Whisher plot distribution values of cholesterol (mg dL-1) of Nile tilapia (O. *niloticus*) during the 24 h. The letters show the multiple comparison test result (different letters = statistically significant difference among mean in the four periods) (n=49).

The Mann-Whitney test applied to the 12/12 h cycle data showed a significant difference between light and dark (n=49; U=189.0; p=0.027), as well as the Kruskal-Wallis test applied at intervals of the 6 h (n=49, H=10.54, p=0.014). In this case, the *a posteriori* multiple comparison test (Figure 7) revealed that the afternoon period was statistically different from the dawn. In fact, the highest values of cholesterol occurred in this period.

Total protein

The mean and standard error of mean of total protein for fish in all 49 aquarium were 0.03 ± 0.00 g dL^{-1} and levels ranged from a minimum of 0.02 g dL^{-1} to a maximum of 0.05 g dL^{-1}, with a median of 0.03 g dL^{-1}.

In Figure 8A, it can be seen that two peaks occurred in the light period at 8:00 h (0.048 g dL^{-1}) and 14:00 h. (0.043 dL^{-1}), and a peak occurred during the dark period at 18:30 h (0.040 g dL^{-1}), but in general, there were similar levels in the two parts of the cycle. This was also the case for the 6 h intervals (Figure 8B). The peaks at 8:00 h and 18:30 h could be related to the passage from dark to light, while that at 16:30 p.m. could be related in addition to the effect of handling. Somehow, protein variability was limited, indicating that it was not affected by the circadian cycle or handling. The limited variability of total protein was confirmed by both the Mann-Whitney and Kruskal-Wallis test, which showed no significant difference between dark and light (n=49, U=280.0, p=0.70) and morning, afternoon, night and dawn (n=49, H=0.331, p=0.95).

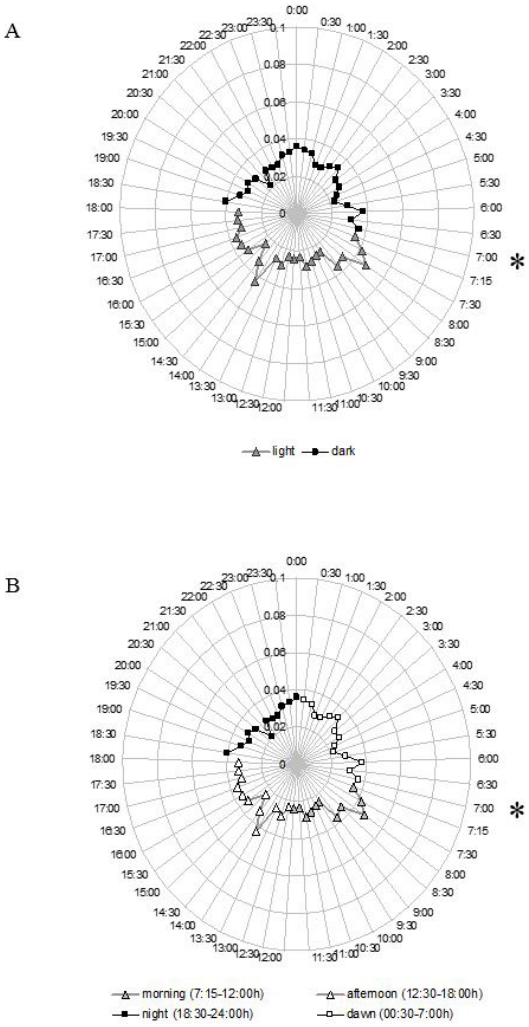

Figure 8. Medium variation on the plasmatic concentration of total proteins (g dL^{-1}) of Nile tilapia *(O. niloticus*) during the 24 h. (A) light-dark; (B) morning, afternoon, night, dawn; *start of experiment.

DISCUSSION

The objective of this study was to determine the changes that could occur during day and night in plasma, serum and whole blood variables of Nile tilapia, to help future studies in which stress would be tested at different times. These changes were closely related to light/dark periods and should be taken into account in new studies. There was found that all variables displayed a daily rhythm.

Cortisol, the main corticosteroid in fish, is considered a good indicator for the evaluation of primary stress (BARTON, 2002). The baseline level of cortisol varies between different species of fish, and for Nile tilapia (*O. niloticus*), as a daytime fish, it is 2.08 ± 1.9 µg dL^{-1} (VOLPATO and BARRETO, 2001). In situations of acute stress, such as in animal handling, a rapid increase in

cortisol can occur, reaching values between 4 and 20 µg dL^{-1}, which may return to baseline within 24 h (ROCHA *et al*., 2004). In the present study, cortisol showed variation over 24 h, but in the dawn interval cortisol values tended to stabilize; that is, there was a decreases in the frequency and intensity of the peaks, indicating the recovery of the physiological response. The results of BARCELLOS *et al*. (2001) show that 24 h was not enough time for *Rhamdia quelen* males and females to recover to pre-stress cortisol level.

The stress condition might have been related to both the circadian cycle and handling stress, but the absence of significant differences between the various parts of the cycle indicated that cortisol oscillations did not affect the well-being of individuals. At this purpose, BARCELLOS *et al*. (1999) considered normal values lower than 8 µg dL^{-1}. We also observed that the response of cortisol was not immediate, occurring after 4 h of exposure to stress and the passage from dark to light. On the contrary, the reaction was more rapid in the case of the passage of light to dark, showing marked oscillations during the night and returning to stable levels only in the dawn.

In relation to response time, there are reports in the literature demonstrating differences in the time of cortisol release between different species of fish (VIJAYAN and MOON, 1994; EINARSDÓTTIR and NILSSEN, 1996; WENDEELAR-BONGA, 1997; BARCELLOS *et al*., 2001; LÓPEZ-OLMEDA *et al*., 2013). BARTON *et al*. (1980); BIRON and BENFEY (1994) observed in tilapia a decrease in cortisol concentration after stress, which also occurred in the present study.

The pattern we observed here showed that capture management, represented by the handling and post-transfer maintenance can generate stress conditions, but the observed cortisol fluctuations were not substantial throughout the experiment.

Glucose is recognized a characterizing the secondary response to stress in fish. Physiological (secondary) reactions occur naturally in the aquatic environment and are generally positive for organisms allowing rapid reactions in the case of stimuli such as escape from predators or helping to adapt to new environmental conditions (BARTON and IWAMA, 1991; NOLAN *et al*., 1999; DAVIS and PETERSON, 2006; BOSISIO *et al*., 2017).

The increase in blood glucose levels (hyperglycemia), recognize as a secondary effect of stress, may be induced by cortisol and this occurs by two mechanisms: 1) stimulation of liver glycogen breakdown (glycogenolysis) and 2) the body being induced to synthesize glucose from non-carbohydrate precursors, besides the stimulation of the replacement of liver glycogen (glyconeogenesis) (VIJAYAN *et al*., 1991; WENDEELAR-BONGA, 1997). The relationship between cortisol and glucose increase was also observed by JENTOFT *et al*. (2005) in rainbow trout, where levels were three times higher than baseline after 3 h of stress application.

Glucose peaks in the present study, were positively related to those of cortisol, occurring with a delay of about 2 h from that. According to the literature, after the increase in the concentration of emergency hormones in the blood caused by a stressor agent, there is a significant increase in glucose concentration, preparing the animal to face an emergency situation (JENTOFT *et al*., 2005).

In general, increased lactate concentrations are observed as a response to stress as a consequence of cortisol elevation (MOMMSEN *et al*., 1999). Animals under stress have increased concentrations of cortisol and catecholamines. Cortisol acts on the liver and increases glycogenolysis and glucose release, whereas catecholamines increase the release of lactate by the peripheral tissues (CARNEIRO and URBINATI, 2002).

Under conditions of prolonged stress, there will be intense degradation of muscle glycogen, forming large amounts of lactic acid (BARTON *et al*., 2011). The conversion of pyruvate to lactate occurs more or less continuously and, in situations of tissue hypoxia, the amount of lactate that will be released into the bloodstream increases further (BOLTON, 2007). If anaerobic conditions persist, generalized overload occurs and tissues are no longer able to recycle this product, and lactate will accumulate. As a consequence, situations of intense stress can lead to muscle exhaustion (BARTON, 2002). Under normal conditions, the reuse of lactate produced in muscle during exercise is induced by low cortisol levels, and in oxidative processes, increased lactate utilization (MILLIGAN and FARRELL, 1991) is stimulated to provide aerobic energy in fish for their locomotor functions (swimming).

In this regard, IVERSEN *et al*. (1998), in a study with Atlantic salmon, *Salmo salar*, observed high initial lactate levels when compared to other fish. The authors interpreted this result as a consequence of the intense swimming of the school in the breeding tank. Thus, the value found by the authors at the time after the stimulus (0.075 mmol L^{-1}) showed that the fish were under stress as a direct response to the limited space available and the inability to swim freely. However, these same authors found a different response for Atlantic salmon (*Salmo salar*); lactate values increased after the stimulus and remained high for up to 48 h after handling (IVERSEN *et al*., 1998).

The values found in the present study showed that lactate varied throughout the period probably as a response to the maintenance conditions due to the little space available and the inability to swim freely in the aquarium used for the experiment. They also showed that the response was rapid after stress and was in correspondence with the passage from dark to light. During the observed period, lactate showed higher values in the morning than in the night, confirming the effect of stressor application and 12/12 h circadian cycle. Similar results were found by LÓPEZ-OLMEDA *et al*. (2013), who reported that lactate values began to increase at the end of the dark period.

Cholesterol concentrations were found to change over the observed period. The analysis of the data in relation to the circadian cycle showed higher values in correspondence with the light period. It was also observed that the highest values occurred in the afternoon indicating that the cholesterol response to stressor application was not rapid. The aspect that can be emphasized to explain this result is that during the experiment the fish were not fed, and therefore, they could have used lipid reserves to supplies the energy demand. We believe that this conditions did not affect the observed results because the duration of the experiment was limited in time (24 h), and it has been demonstrated that biological rhythms continue to be expressed for days, months or years after

suppression of the synchronizing agent (BARCELLOS *et al*., 1999), depending on the species and experimental conditions. These rhythms, obtained after the suppression of the synchronizing agent, are known as free-course rhythms and are expressions of endogenous biological clocks.

There were no changes in plasma total protein concentrations. Increased proteins may be related to increased cortisol, which will cause increased gluconeogenesis and protein catabolism (MOMMSEN *et al*., 1999). PETERS *et al*. (1980) also found no differences in total protein levels in fish subjected to non-intense stress, stating that the mobilization of proteins as an energy source is dependent on the intensity of the stress to which the animal is subjected. CARNEIRO and URBINATI (2002) also did not find statistical differences in mean total protein between samples, suggesting that young animals show greater resistance to handling stress. Our results are in agreement with these authors, since almost all the values found were lesser than 0.04 g dL^{-1} and did not shows marked fluctuations. This also agrees with the fact that all the individuals used were young and that the applied stress was not enough to trigger a response in this variable.

CONCLUSION

We found that certain blood variables varied during 24 h and that those most affected were lactate and cholesterol. Only cholesterol showed a relationship with the circadian cycle with higher values in the light part. With relation to the most physiologically affected times (6h intervals), cholesterol showed the higher picks in the afternoon and lactate in the morning. . The information obtained contributes to the individuation of reference values of whole blood, plasma and serum variables aimed at the optimization of management practices in tilapia culture.

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