PORTABLE POINT-OF-CARE DEVICE AS ALTERNATIVE TOOL FOR MONITORING BLOOD GLUCOSE IN LAMBARI *Astyanax altiparanae*: STRESS AND SEX-SPECIFIC EFFECTS*

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ABSTRACT

The efficacy of a point-of-care (POC) blood glucometer (Accu-Chek Active®) was evaluated in adult lambaris *(Astyanax altiparanae)*, comparing the values obtained by this equipment to those obtained by the laboratory enzymatic method. The glycemia of adult males and females and the variations resulting from transporting in juveniles were also evaluated 0, 1, 24, 48 and 72 hours after procedure. Data were submitted to ANOVA, comparing the means by the Tukey´s Test (P> 0.05) and a regression analysis was performed to establish the correlations between values obtained by the two methods. The blood glucose values obtained by POC meter were 11.2% lower, in absolute terms, but did not differ significantly from those recorded by the laboratory enzymatic method, with linear correlation (R = 67.7%) between the methods. Higher glucose levels were recorded for males; glycemia increased 334.3% immediately after transportation, remained elevated (113.3%) up to 48 hours and returned to basal levels (fasting) after 72 hours. It was concluded that POC Accu-Chek Active® is an effective tool for glucose determination in lambari; there are sex-dependent differences in relation to blood glucose levels; the species is sensitive to stress transporting and glycemia is an immediate and appropriate physiological indicator for this evaluation.

Key words: glucometer; handheld; portable clinical analyser; transporting.

GLICOSÍMETRO PORTÁTIL COMO FERRAMENTA ALTERNATIVA PARA MONITORAMENTO DA GLICOSE SANGUÍNEA EM LAMBARIS *Astyanax altiparanae*: EFEITOS DO ESTRESSE E SEXO

RESUMO

A eficácia do medidor portátil de glicemia (Accu-Chek Active®) foi avaliada em lambaris adultos *(Astyanax altiparanae)*, comparando-se os valores registrados com os obtidos por meio do método enzimático laboratorial. A glicemia de machos e fêmeas adultos e as variações decorrentes do transporte em juvenis também foram avaliadas 0, 1, 24, 48 e 72 horas após o procedimento. Os dados foram submetidos à ANOVA, comparando-se as médias pelo Teste de Tukey (P> 0,05), realizando-se posterior análise de regressão para estabelecimento das correlações entre os valores obtidos pelos dois métodos. Os valores de glicemia obtidos pelo glicosímetro foram 11,2% menores, em termos absolutos, mas não diferiram daqueles registrados pelo método laboratorial, observando-se correlação linear (R = 67,7%) entre ambos. Maiores concentrações de glicose foram registradas para os machos; a glicemia aumentou 334,3% imediatamente após o transporte, permaneceu elevada (113,3%) até 48 horas e retornou aos níveis basais (jejum) após 72 horas. Concluiu-se que o glicosímetro portátil Accu-Chek Active® é uma ferramenta eficaz para a determinação da glicose em lambari; existem diferenças dependentes do sexo em relação aos níveis de glicose no sangue; a espécie é sensível ao estresse de transporte, sendo a glicose um indicador fisiológico imediato e apropriado para esta avaliação.

Palavras-chave: glicosímetro; medidor clínico portátil; transporte.

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INTRODUCTION

Hematological and biochemical characteristics reflect the internal state of the organism and can be used to evaluate the nutritional, reproductive and health status of the fish. Therefore, monitoring of blood parameters is important for study of metabolic imbalances, handling trauma, cellular damage caused by exposure to chemical agents, infections and physiological changes due to stress, which can be detected even before clinical signs are evident (BAHMANI *et al*., 2001; TAVARES-DIAS and MORAES, 2004; DE PEDRO *et al*., 2005; TAVARES-DIAS *et al*., 2007; SVOBODOVÁ *et al*., 2009; SATHEESHKUMAR *et al*., 2011; HARTER *et al*., 2014; STOOT *et a*l., 2014; BOSISIO *et al*., 2017). Blood glucose is a commonly parameter used to assess the physiological status and sanity of the fish and its concentration expresses the level of mobilization of hepatic and muscular glycogen reserves in stress situations (SVOBODA *et al*., 2001; ENDO *et al*., 2009; YONEMORI *et al*., 2009).

Glucose concentration varies according to the species and individuals, biotic factors such as sex, stage of development and feeding or abiotics, such as water temperature and seasonal variations, which may affect hepatic glycogen stores (BARTON *et al*., 1988; WEDEMEYER *et al*., 1990; HEMRE *et al*., 2002; SOENGAS and ALDEGUNDE, 2002; IWAMA *et al*., 2004; COZ-RAKOVAC *et al*., 2005; PRASAD and CHARLES, 2010; BARBIERI and BONDIOLI, 2015).

Procedures as the capture, biometry, vaccination and transportation induce increase of blood glucose levels, which characterizes the "secondary response to acute stress" that provides energy support for the reestablishment of fish homeostasis (BARTON and IWAMA, 1991; MORGAN and IWAMA, 1997; WENDELLAR BONGA, 1997; BARTON, 2002). This increase arises initially from the activity of catecholamines (VIJAYAN and MOON, 1992), is maintained by the later release of cortisol and depends on the intensity and exposure time to stressor agent, species, size and nutritional status of the fish (BARTON, 2000).

Therefore, the determination of blood glucose levels is a simple and low cost tool that when analyzed in association with the cortisol provides the evaluation of physiological stress responses of fish, either in intensive production or in laboratory research conditions (BARTON, 1997; CARNEIRO *et al*., 2009; SVOBODOVÁ *et al*., 2009).

Regardless the variable investigated, blood collection is a necessary and ordinary procedure in studies related to fish physiology (SHI *et al*., 2010, STOOT *et al*., 2014). However, blood sampling is difficult when research is performed small specimens or individuals, considering the small size of their vessels and the small blood volume (EAMES *et al*., 2010). The significant distance between the collection site (field situations) and the laboratory can affect the results, once physical and chemical changes occur during the storage and transportation of the samples (BEECHAM *et al*., 2006).

The storage of whole blood can also affect the concentrations of glucose and lactate, because erythrocytes are nucleated and show intense metabolic activity while refrigeration of blood samples may be inappropriate considering fish ectothermia (WELLS and PANKHURST, 1999). Therefore, the use of portable equipment for *in situ* determination of glucose is an important diagnostic alternative which provides immediate evaluations in laboratory or field conditions (HARRENSTIEN *et al*., 2005; BEECHAM *et al*., 2006; COOKE *et al*., 2008; HARTER *et al*., 2014; STOOT *et al*., 2014).

The use of point-of-care (POC) device to measure physiological blood parameters related to veterinary diagnostics and laboratory research has become popular due to its speed of measurement (seconds) and *in loco* portability over traditional laboratory methods but it should be considered that these equipments were developed based on human blood samples and that there are differences between species and taxonomic classes; therefore, species-specific validation studies to confirm the accuracy and reliability of these tools are still necessary (STOOT *et al*., 2014).

Validation studies of portable glucometers have already been performed in coho salmon *Oncorhynchus kisutch* (IWAMA *et al*., 1995), rainbown trout *Oncorhynchus mykiss* (WELLS and PANKHURST, 1999; HARTER *et al*., 2014), Nile tilapia *Oreochromis niloticus* (EVANS *et al*., 2003), black e blue rockfish *Sebastes melanops* e *S. mystinus* (HARRESTIEN *et al*., 2005), channel catfish *Ictalurus punctatus* (BEECHAM *et al*., 2006), bonefish *Albula vulpes* (COOKE *et al*., 2008), zebrafish (EAMES *et al*., 2010) and ballan wrasse *Labrus bergylta* (LECLERCQ and MIGAUD, 2014).

The yellow-tailed lambari, *Astyanax altiparanae,* is a small fish of the family Characidae, native of

Brazil and previously classified as *A. bimaculatus*, of omnivorous habit with insectivorous tendency (ANDRIAN *et al.*, 2001). Although initially considered an invader of fish ponds, represents today an interesting species for fish farming due to its high prolificacy, adaptation to environmental conditions and early growth (GARUTTI, 2003; ABIMORADI and CASTELLANI, 2011).

The species is considered a significant part of the diet of larger carnivorous fish (GARUTTI and BRITSKI, 2000), is appreciated in the human diet, used as live bait for sport fishing and can be industrialized in canned form, which characterizes it as promising for the market (PORTO-FORESTI *et al*., 2005). The objectives of this work were to evaluate the potential and validate the efficacy of a portable commercial glucometer (Accu-Chek Active - Roche®), originally developed for monitoring human blood glucose, to investigate sex-dependent and stress effects on glycemic profile in lambaris, *Astyanax altiparanae.*

METHODS

Fish

Lambaris of the species *Astyanax altiparanae* were obtained from induced spawning performed at São Paulo Agribusiness Technology Agency (APTA) Pirassununga, São Paulo State, Brazil, and the experiment was conducted at APTA and Laboratory of Fish Behavior, Department of Basic Sciences, College of Animal Science and Food Engineering, University of São Paulo, Brazil.

The water quality indicators (temperature, pH and dissolved ammonia, nitrite and oxygen concentrations) were continuously monitored using, respectively, digital thermometer (Checktemp - Hanna Instruments Brazil), portable pH meter (ExStik II - Extech Instruments), portable ammonia and nitrite meters (HI 93700 and HI 93707 - Hanna Instruments Brasil) and dissolved oxygen meter (DO-5519 - Lutron Electronic Enterprise).

Fish were fed *ad libitum* twice daily (8:00 and 17:00 hours) with a commercial extruded ration (Ambar Amaral Group®, Santa Fé do Sul, São Paulo, Brazil) with 2 mm grain diameter and 32% crude protein, until 18 h prior to assays. Procedures were approved by the Animal Research Ethics Committee at Faculty of Animal Science and Food Engineering, University of São Paulo Protocolo - 20062011).

Anesthesia and blood sampling

The assays were performed after induction to the stage of deep anesthesia with clove oil in the proportion of 50 mg.L^{-1} of facility water, according to the methodology described by PEREIRA-DA-SILVA *et al*. (2009) and whole blood samples were collected in up to 1 min by puncturing the caudal vein (100 µL) using syringes containing sodium heparin (Heptar® - Lot 184728A).

Blood Glucose in captive conditions

Adult fish (n = 30; 15.0 ± 4.6 g) from a tank (30 m^3) were caught using dip net and anesthetized to proceed the blood samplings. An aliquot of each sample (10 µL) was immediately transferred to a test strip used for determination of whole blood glucose (mg.dL^{-1}) in loco on a point-of-care (POC) blood analyzer (Accu-Chek Active - Roche®) and the remainder of the samples was immediately stored in Eppendorf tube and centrifuged 3000 rpm to separate the plasma, which was maintained in freezer (-20°C) until determination of glucose by enzymatic method - glucose oxidase (Laborlab® kit, Guarulhos, SP, Brazil - Lot 0610210), with absorbance evaluated in a spectrophotometer at 505nm.

Glicemic profile of males and females

Adult fish (males n = 40; 10.5 ± 1.5 g; females n = 40; 11.4 ± 1.5 g) were captured and anesthetized for sexing and subsequent blood sampling to determine the glucose levels by the POC device, as described in the previous item.

Glicemic profile after transporting

To evaluate the stressor effect of transporting 95 juveniles (6.0 ± 0.4 g) were packed in a polyethylene bag (50L) containing 25L of facility water, inflated with oxygen and tied with rubber strips for transportation (density 5 fish.L^{-1} of water), through a paved road for 45 minutes. Then, blood sampling were performed at 0 (arrival to laboratory), 1, 24, 48 and 72 hours after the procedure (n = 19 fish per treatment) and during this period, the fish were fasted and grouped in a tank (250 L).

Glucose levels were measured using the POC device and the values were compared to those

obtained for fish from a control group (n = 19), not submitted to transportation and that were acclimatized to laboratory conditions.

Statistical Analysis

The data showed as the mean ± standard deviation presented a normal distribution (Shapiro-Wilk normality test) and were submitted to ANOVA one-way, comparing the means by Tukey's Test ($p < 0.05$). Subsequently a regression study was performed to verify the correlation between the values of blood glucose recorded using the conventional laboratory enzymatic method or the POC device.

RESULTS

The temperature remained at 26.2 ± 0.6, pH 7.4 ± 0.1, the concentration of dissolved oxygen 5.6 ± 0.5 $mg.L^{-1}$ and the concentrations of ammonia and nitrite respectively 0,32 ± 0.06 $mg.L^{-1}$ and 0.06 ± 0.01 $mg.L^{-1}$.

The mean glucose levels of fish from captive conditions and recorded by means of the POC device or the enzymatic laboratorial method are presented in Figure 1.

The values of blood glucose recorded using the POC were 11.2% lower, but there was no significant difference between the methods. After the regression study, a significant correlation (R = 67.7%) was found between the values obtained by means of the POC or the laboratory method (Figure 2). The blood glucose levels recorded for males were higher than females (Figure 3). The blood glucose levels increased 334.3% immediately after transporting and although a significant reduction of glucose was observed one hour after the procedure, the values remained at high levels up to 48 hours (113.3%), returning to baseline levels after 72 hours (Figure 4).

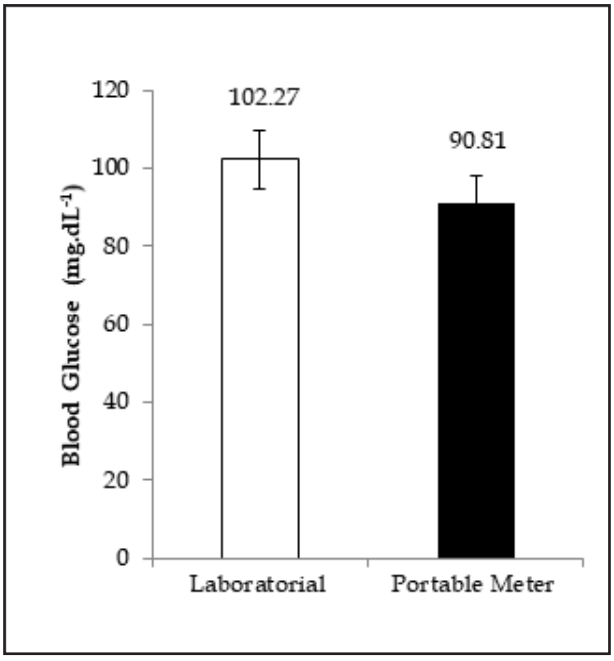

Figure 1 . Mean ± SE comparison of glucose levels for lambaris *(Astyanax altiparanae)* (n=30), as determined by POC device (Accu Chek Active - Roche®) and laboratorial enzymatic method (Laborlab®). ANOVA and Tukey's test were used to determined statistical differences ($p<0.05$) and means followed by (*) indicates statistical differences between the treatments.

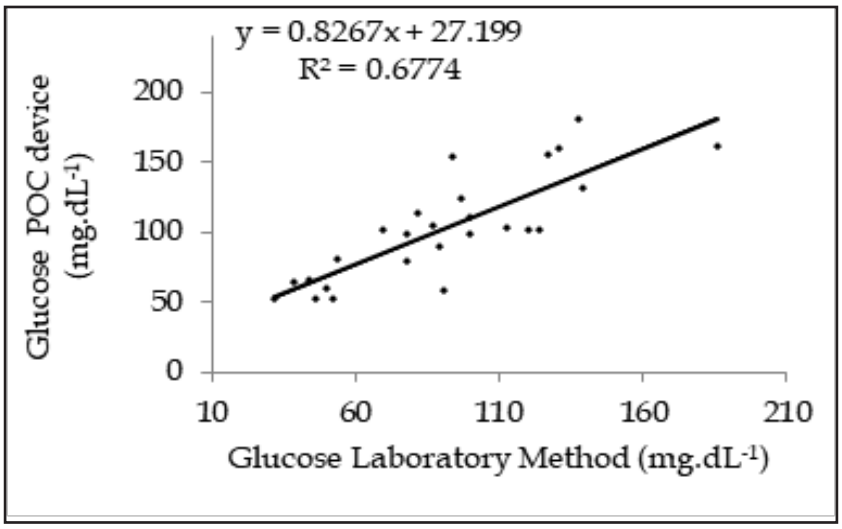

Figure 2 . Regression analysis of blood glucose levels for samples obtained from lambaris, *Astyanax altiparanae* (n=30) using POC device (Accu Chek Active - Roche®) and laboratorial enzymatic assay (Laborlab®).

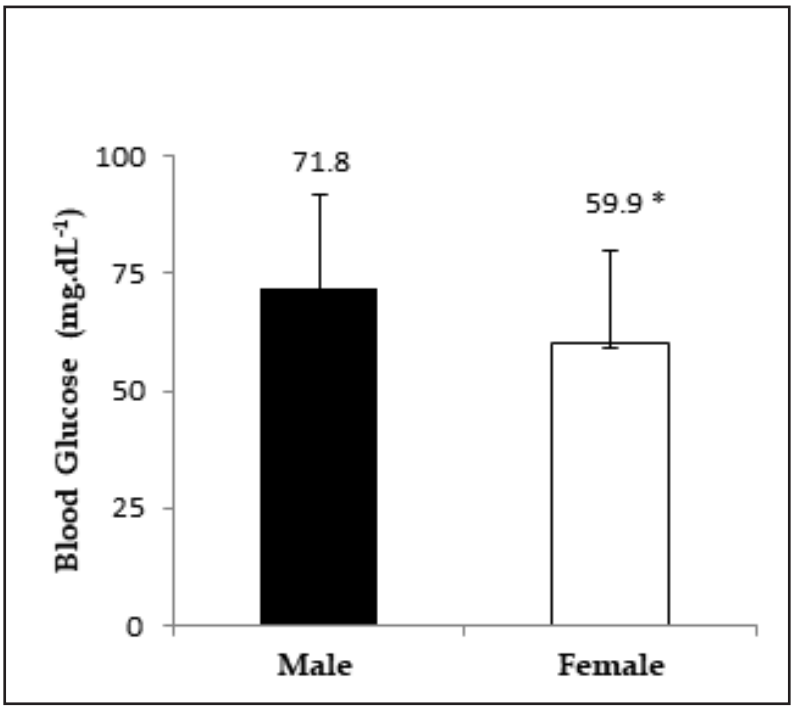

Figure 3 . Mean ± SD comparison of glucose levels between males (n=40) and females (n=40) lambaris *(Astyanax altiparanae)* after capture, as determined by POC device (Accu Chek Active - Roche®). ANOVA and Tukey´s test were used to determined statistical differences ($p<0.05$) and means followed by (*) indicates statistical differences.

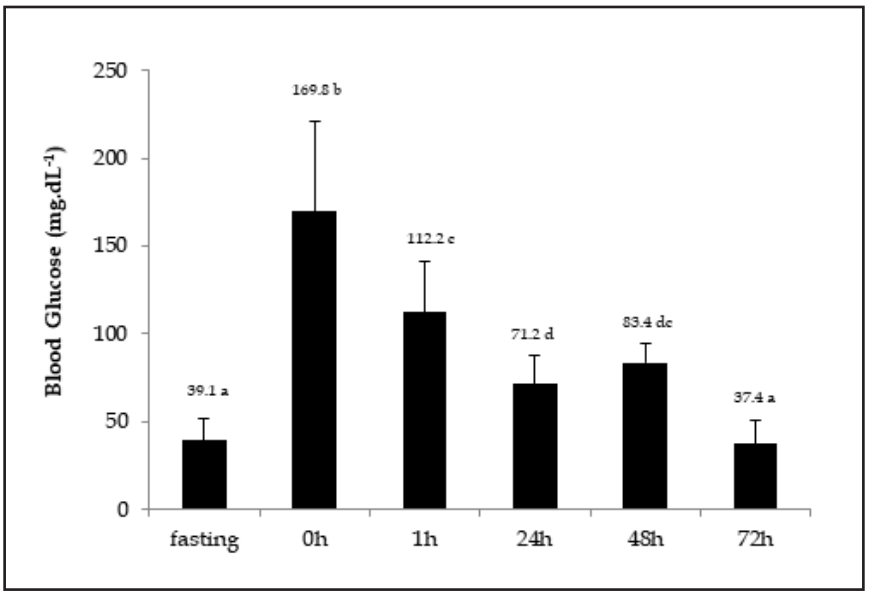

Figure 4 . Mean ± SD comparison of glucose levels for lambari *(Astyanax altiparanae)* after transporting, as determined by POC device (Accu Chek Active - Roche®). ANOVA and Tukey's test were used to determined statistical differences ($p<0.05$) and means followed by different lower case letters indicates statistical differences between the treatments.

DISCUSSION

The recorded values for the water quality indicators remained in the range considered adequate for the maintenance of tropical fish species (VINATEA, 2004).

The glucose oxidase laboratory method, used for clinical evaluations in human blood, is also routinely used for the determination of plasma glucose in fish (EAMES *et al*., 2010). It is based on the glucose reaction of the sample with the enzyme glucose oxidase which, in the presence of oxygen, forms gluconic acid. This, in turn, combines with hydrogen peroxide (H_2O_2) and peroxidase and a chromogenic substance (usually toluidine and dianisidine) forming a colored product whose absorbance is measured in a spectrophotometer (TRINDER, 1969).

In general, portable glucose meters are based on the glucose reaction with the enzymes glucose oxidase or PQQ glucose dehydrogenase (pyrroloquinoline quinone-dependent glucose-mediated dehydrogenase reaction), the principles of which are detailed in the review by HONES *et al*. (2008). COOKE *et al*. (2008) recorded higher blood glucose values (approximately 9.3%) for fish using POCs but in general the values obtained using these devices are lower (WELLS and PANKHURST, 1999), which can be attributed to total blood utilized, unlike the conventional laboratory method that employs the plasma for such evaluations (COOKE *et al*., 2008). Furthermore, the measurements and calculations of many variables are based on constants and algorithms of human blood and the differences between species and taxonomic classes must be considered (HARTER *et al*., 2014).

BEECHAM *et al*. (2006) observed in *I. punctatus* blood glucose levels 30% lower when the POC device was utilized and attributed the differences to storage period of heparinized blood until centrifugation, a commonly problem in field samplings. WELLS and PANKHURST (1999) and STOOT *et al*. (2014) suggest the use of portable glucometers for recording relative rather than absolute values, considering that these devices underestimate glucose values. In fact the values recorded in the present study were lower than those recorded for the same samples using the enzymatic laboratory method but this difference was only 11.2%.

EAMES *et al*. (2010) evaluated the efficacy of four portable devices for glucose determination in zebrafish *Danio rerio*, a biological model of extremely small size and widely used in research related to disorders of carbohydrate metabolism in humans. The authors considered as advantages the speed of reading (seconds) and *in loco* portability of POCs over traditional laboratory methods, once these

equipments are adapted to the precision standards determined by the International Organization of Standardization (ISO 15197, 2013), which establish 25% as the maximum variability for the mean values recorded, compared to those obtained by conventional laboratory methods, in 95% of the tested samples. According to the label of Accu-Check®, utilized in this study and originally developed for determination of the human blood glucose, 99% of the tested samples meet the minimum performance criteria required by European Standard (ISO 15197, 2013).

A linear relationship between the blood glucose values recorded by means of a portable glucometer and by the laboratory enzymatic method was observed in this study, as verified in rainbow trout *O. mykiss* (WELLS and PANKHURST, 1999), Nile tilapia *O. niloticus* (EVANS *et al*., 2003), black rock fish S. melanops and adult blue rockfish *S. mystinus* (HARRENSTIEN *et al*., 2005), channel catfish *I. punctatus* (BEECHAM *et al*., 2006), and bonefish *A. vulpes* (COOKE *et al*., 2008). Considering this linear relationship and that application of correction factors was already proposed for zebrafish *D. rerio* (EAMES *et al*., 2010), black rock fish *S. melanops* and adult blue rockfish *S. mystinus* (HARRENSTIEN *et al*., 2005) and ballan wrase *L. bergylta* (LECLERCQ and MIGAUD, 2014), it is suggested posterior investigations in order to determine specific correction factor for lambari, in order to make feasible the use of portable device Accu-Chek® for determination of absolute values in this species.

Blood glucose levels may vary depending on the water temperature, diet and sex of the fish. In colder periods lower blood glucose concentration is observed due to reduced food intake and increased use of energy reserves (SHERIDAN and MOMMSEN, 1991; BANI and VAYGHAN, 2011). Significant variations resulting from energy displacement required for ovarian maturation or even fasting periods were observed in species that migrate to spawn (SVOBODA *et al*., 2001; SVOBODOVÁ *et al*., 2009; PRASAD and CHARLES, 2010). SVOBODA *et al*. (2001) recorded high concentrations of plasma glucose in females of the *Tinca tinca* species evaluated during the reproductive period and suggested an effect of gonadal development on this parameter. Otherwise lower levels of glucose were observed in common carp females *Cyprinus carpio*, response that was attributed to the accumulation of glycogen in the ovaries during maturation (SVOBODOVÁ *et al*., 2009) and in females *Rutilus frisii kutum*, an anadromous species of the Cyprinidae family that exhibits energy displacement for egg production and faces periods of fasting during migration to spawning (PRASAD and CHARLES, 2010). However no sex effect on mean values of blood glucose in *Clarias batrachus* (KUMARI and AHSAN, 2011) and zebrafish *D. rerio* (EAMES *et al*., 2010) were observed. In this study the fish weight were similar and the temperature and fed were controlled, therefore the lower blood glucose levels recorded for females suggest a sex-dependent effect on glycemia.

The increasing blood glucose is a classical secondary response of fish to stress (BARTON, 1997; MORGAN and IWAMA, 1997; BARTON, 2002) and results mainly from the release of catecholamines, mainly epinephrine (MAZEAUD *et al*., 1977; RANDALL and PERRY, 1992; REID *et al*., 1998) which promotes the phosphorylation of the enzyme glycogen phosphorylase, resulting in an increase in glycogenolysis and, consequently, in blood glucose levels, in order to meet the increase in the energy demand (VIJAYAN and MOON, 1992; WEDEMEYER, 1996; WENDELAAR BONGA, 1997). Transporting is a stressful procedure for intensive fish farming, since it includes handling, capture and confinement at high densities, with consequent changes in water quality, mainly due to the increase in temperature and reduction of oxygen, which causes a series of physiological changes in order to reestablish homeostasis (CARNEIRO and URBINATTI, 2001; URBINATI and CARNEIRO, 2004; URBINATI *et al*., 2004; ACERETE *et al*., 2004; BRANDÃO *et al*., 2006; TAKAHASHI *et al*., 2006; OLIVEIRA *et al*., 2009). Hyperglycemia is an immediate responses of fish after transportation and was observed in native species in Brazil as matrinxã *Brycon cephalus* (CARNEIRO and URBINATTI, 2001, 2002), tambaqui *Colossoma macropomum* (GOMES *et al*., 2003; CHAGAS *et al*., 2012), jundiá *Rhamdia quelen* (CARNEIRO *et al*., 2009 a) and pirarucu *Arapaima gigas* (GOMES *et al*., 2006; BRANDÃO *et al*., 2008).

In this study, the blood glucose levels increased significantly after transporting and this response should not be attributed to the activity of catecholamines that have a short half-life (clearance up to 10 minutes), due to tissue accumulation/ binding and metabolic degradation (ROTHWELL *et al*., 2005) but rather to cortisol. Considering the aim of this research was to evaluate the efficacy of the portable glucometer, cortisol variations were

not recorded, and it can only be inferred that the maintenance of hyperglycemia must have occurred due to the activity of this hormone, whose release occurs approximately after one hour with return to basal levels after six hour (BARTON *et al*., 2002; IWAMA *et al*., 2006).

The physiological stress responses due transporting are intense and dependent on the species and intensity, duration and type of the management and the return to the basal blood glucose levels is an indicative of fish recovery. Blood glucose returned to basal levels 24 hours after transporting in tambaqui *C. macropomum* (GOMES *et al*., 2003; CHAGAS *et al*., 2012), pirarucu *A. gigas* (GOMES *et al*., 2006), jundiá *Rhamdia quelen* (CARNEIRO *et al*., 2009) and matrinxã *Brycon amazonicus* (CARNEIRO and URBINATTI, 2002; URBINATTI *et al*., 2004). BRANDÃO *et al.* (2008) observed the time 48 hours in pirarucu *A. gigas* and CARNEIRO and URBINATTI (2001) 96 hours in matrinxã *B. amazonicus*. TAKAHASHI *et al*. (2006) registered progressive reduction in blood glucose in pacu *P. mesopotamicus* mantained in ground tank (after 72 hours) and laboratory (after 120 hours). In this study the reestablished of glucose basal levels occurred only 72 hour after a short period transporting, indicating the stressor potential of transporting for the species and also interspecific variability of the responses.

CONCLUSIONS

The Accu-Chek Active® portable glucometer represents an efficient tool and can be used in laboratory or field to determine the glycemia of lambari, a species whose small size makes it difficult to collect significant volumes of blood samples. There are sex-dependent differences with respect to blood glucose levels which are lower in females; the species is sensitive to stress transporting and glycemia is an immediate and appropriate physiological indicator for this evaluation.

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REFERENCES

ABIMORAD, E.G.; CASTELLANI, D. 2011 Exigências nutricionais de aminoácidos para o lambari-do-rabo-amarelo baseadas na composição da carcaça e do músculo. *Boletim do Instituto de Pesca, 37*(1): 31-38.

ACERETE, L.; BALASCH, J.C.; ESPINOSA, E.; JOSA, A.; TORT, L. 2004 Physiological responses in Eurasian perch *(Perca fluviatilis, L.)* subjected to stress by transport and handling. *Aquaculture*, *237*(1-4): 167-178

ANDRIAN, I.F.; SILVA, H.B.R.; PERETTI, D. 2001 Dieta de *Astyanax bimaculatus* (Linnaeus, 1758) (Characiformes, Characidae), da área de influência do reservatório de Corumbá, Estado de Goiás, Brasil. *Acta Scientiarum Biological Sciences*, *23*(2): 435-440.

BAHMANI, M.; KASEMI, R.; DONSKAYA, P.A. 2001 Comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). *Fish Physiology and Biochemistry, 24*(2): 135-140.

BANI, A.; VAYGHAN, A.H. 2011 Temporal variation in haematological and biochemical indices of the Caspian kutum, *Rutilus frisii kutum. Ichthyology Research*. *58*(2): 126-133.

BARBIERI, E.; BONDIOLI, A.C.V. 2015 Acute toxicity of ammonia in Pacu fish *(Piaractus mesopotamicus* , Holmberg, 1887) at different temperatures levels. *Aquaculture Research*, *46*(3): 565-571.

BARTON, B.A. 1997 Stress in finfish: past, present and future a historical perspective. In: IWAMA, G.K.; PICKERING, A.D.; SUMPTER, J.P.; SCHRECK, C.B. *Fish stress and health in aquaculture.* Society for Experimental Biology Seminar Series 62. Cambridge University Press, New York. p. 1-33.

BARTON, B.A. 2000 Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. *North American Journal of Aquaculture*, *62*(1): 12-18.

BARTON, B. A. 2002 Stress in fishes: A diversity of responses with particular reference to changes

in circulating corticosteroids. *Integrative and Comparative Biology*, *42*(3): 517-525.

BARTON, B.A.; IWAMA, G.K. 1991 Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Reviews of Fish Diseases, 1*(1): 3-26.

BARTON, B.A.; SCHRECK, C.; FOWLER, L. 1988 Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile Chinook salmon. *The Progressive Fish Culturist*, *50*(1): 16-22.

BARTON, B.A.; MORGAN, J.D; VIJAYAN, M.M. 2002 Physiological and condition-related indicators of environmental stress in fish. In: ADAMS, S.M. *Biological indicators of aquatic ecosystem stress*. American Fisheries Society, Bethesda, Maryland. p. 111-148.

BEECHAM, R.V.; SMALL, B.C.; MINCHEW, C.D. 2006 Using portable lactate and glucose meters for catfish research: Acceptable alternatives to established laboratory methods? *North American Journal of Aquaculture*, *68*(4): 291-295.

BOSISIO, F.; REZENDE, K. F. O.; BARBIERI, E. 2017 Alterations in the hematological parameters of Juvenile Nile Tilapia (*Oreochromis niloticus*) submitted to different salinities. *Pan-American Journal of Aquatic Sciences, 12*(2):146-154.

BRANDÃO, F.R.; GOMES, L.C.; CHAGAS, E.C. 2006 Respostas de estresse em pirarucu *(Arapaima gigas)* durante práticas de rotina em piscicultura. *Acta Amazonica*, *36*(3): 349-356.

BRANDÃO, F.; GOMES, L.C.; CRESCENCIO, R.; CARVALHO, E.S. 2008 Uso de sal durante o transporte de juvenis (1kg) de pirarucu *(Arapaima gigas). Acta Amazonica, 38*(4): 767-771.

CARNEIRO, P.C.F.; URBINATTI, E.C. 2001 Salt as a stress response mitigator of matrinxa, *Brycon cephalus* (Günther), during transport. *Aquaculture Research*, *32*(4): 297-304.

CARNEIRO, P.C.F.; URBINATI, E.C. 2002 Transport stress in matrinxã, *Brycon cephalus* (Teleostei; Characidae) at different densities. *Aquaculture International*, *10*(3): 221-229.

CARNEIRO, P.C.F.; KAISELER, P.H.S.; SWAROFSKY, E.A.C. BALDISSEROTTO, B. 2009 Transport of jundiá *Rhamdia quelen* juveniles at different loading densities: water quality and blood parameters. *Neotropical Ichthyology*, *7*(2): 283-288.

CHAGAS, E.C.; ARAÚJO, L.D.; BOIJINK, C.L.; INOUE, L.A.K.A.; GOMES, L.C.; MORAES, F.R. CHAGAS, E.C. 2012 Respostas de tambaquis ao estresse por transporte após alimentação com dietas suplementadas com b-glucano. *Biotemas*, *25*(4): 221-227.

COOKE, S.J.; SUSKI, C.D.; DANYLCHUK, S.E.; DANYLCHUK, A.J.; DONALDSON, M.R.; PULLEN, C.; BULTE´, G.; O'TOOLE, A.; MURCHIE, K.J.; KOPPELMAN, J.B.; SHULTZ, A.D.; BROOKS, E.; GOLDBERG, T.L. 2008 Effects of capture techniques on the physiological condition of bonefish *(Albula vulpes)* evaluated using field physiology diagnostic tools. *Journal of Fish Biology*, *73*(6): 1351-1375.

COZ-RAKOVAC, R.; STRUNJAK-PEROVIC, I.; HACMANJEK, M.; POPOVIC, N.T.; LIPEJ, Z.; SOSTARIC, B. 2005 Blood chemistry and histological properties of wild and cultured sea bass *(Dicentrarchus labrax)* in the North Adriatic Sea. *Veterinary Research Communications*, *29*(8): 677-687.

DE PEDRO, N.; GUIJARRO, A.I.; LÓPEZ-PATIÑO, M.A.; MARTÍNEZ-ÁLVAREZ, R.; DELGADO, M.J. 2005 Daily and seasonal variation in haematological and blood biochemical parameters in tench *Tinca tinca*. *Aquaculture Research*, *36*(12): 1185-1196.

EAMES, S.C.; PHILIPSON, L.H.; PRINCE, V.E.; KINKEL, M.D. 2010 Blood sugar measurement in zebrafish reveals dynamics of glucose homeostastis. *Zebrafish*, *7*(2): 205-213.

ENDO, H.; YONEMORI, Y.; HIBI, K.; REN, H.; HAYASHI, T.; TSUGAWA, W.; SODE, K. 2009 Wireless enzyme sensor system for real-time monitoring of blood glucose levels in fish.

Biosensors and Bioelectronics, *24*(5): 1417-1423.

EVANS, J.J.; SHOEMAKER, C.A.; KLESIUS, P.H. 2003 Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae* in Nile Tilapia. *Journal of Aquatic Animal Health, 15*(3): 202-208.

GARUTTI, V. 2003 *Piscicultura ecológica*. São Paulo: Universidade de São Paulo. 336p.

GARUTTI, V.; BRITSKI, H.A. 2000 Descrição de uma espécie nova de *Astyanax* (Teleostei: Characidae) da bacia do alto rio Paraná e considerações sobre as demais espécies do gênero na bacia. *Comunicações do Museu de Ciências e Tecnologia, Série Zoologia*, *13*(1): 65-88.

GOMES, L.C.; ROUBACH, R.; ARAUJO-LIMA, C.A.R.M.; CHIPPARI-GOMES, A.R.; LOPES, N.P.; URBINATI, E.C. 2003 Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. *Journal of the World Aquaculture Society*, *34*(1): 76-84.

GOMES, L.C.; ROUBACH, R.; CAVERO, B.A.S.; PEREIRA-FILHO, M.; URBINATI, E. C. 2006 Use of salt during transportation of air breathing pirarucu juveniles *(Arapaima gigas)* in plastic bags. Aquaculture, *256*(4): 521-528.

HARTER, T.S.; SHARTAU, R.B.; BRAUNER, C.J.; FARRELL, A.P. 2014 Validation of the i-STAT system for the analysis of blood parameters in fish. *Conservation Physiology, 2*(1): 1-12.

HARRESTIEN, L.A.; TORNQUIST, S.J.; MILLER-MORGAN, T.J.; FODNESS, B.G.; CLIFFORD, K.E. 2005 Evaluation of a point-of-care blood analyzer and determination of reference ranges for blood parameters in rockfish. *Journal of the American Veterinary Medical Association*, *226*(2): 255-265.

HEMRE, G.-I.; MOMMSEN, T.P.; KROGDAHL, A. 2002 Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition*, *8*(3): 175-194.

HONES, J.; MULLER, P.; SURRIDGE, N. 2008 The technology behind glucose meters: test strips. *Diabetes Technology and Therapeutics*, *10*(1): 1-10.

ISO - International Organization for Standardization. 2013 In vitro diagnostic test systems - requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus. *ISO 15197: 2013*. Geneva: International Organization for Standardization, 46p.

IWAMA, G.K.; MORGAN, J.D.; BARTON, B.A. 1995 Simple field methods for monitoring stress and general condition of fish. *Aquaculture Research, 26*(4): 273-282.

IWAMA, G.K.; AFONSO, L.; MATHILAKAT, H. 2004 Stress in fish. In: *Aquanet Workshop on Fish Welfare*, Campbell River. p. 1-9.

IWAMA, G.K.; AFONSO, L.O.B.; VIJAYAN, M.M. 2006 Stress in fish. 2006 In: EVANS, D. H. CLAIBORNE, J. B. *The Physiology of fishes*, Taylor and Francis, USA. p. 319-342.

KUMARI, B.; AHSAN, J. 2011 Acute exposure of arsenic tri-oxide produces hyperglycemia in both sexes of an Indian Teleost, *Clarias batrachus* (Linn.). *Archives of Environmental Contamination and Toxicology*, *61*(3): 435-442.

LECLERCQ, E.; DAVIE, E.; MIGAUD, H. 2014 The physiological response of farmed balla wrasse *(Labrus bergylta)* exposed to an acute stressor. *Aquaculture,* 434: 1-4.

MAZEAUD, M.M.; MAZEAUD, F.; DONALDSON, E.M. 1977 Primary and secondary effects of stress in fish. *Transactions of the American Fisheries Society, 106*(3): 201-212.

MORGAN.; J.D.; IWAMA.; G.K. 1997 Measurements of stressed states in the field. In: IWAMA, G.W.; PICKERING, A.D.; SUMPTER, J.P.; SCHRECK, C.B. *Fish stress and health in aquaculture*. University Press, Cambridge, p. 247-270.

OLIVEIRA, J.R.; CARMO, J.L.; OLIVEIRA, K.K.C.; SOARES, M.C.F. 2009 Cloreto de sódio, benzocaína e óleo de cravo-da-índia na água de transporte de tilápia-do-Nilo. *Revista Brasileira de Zootecnia, 38*(7): 1163-1169.

PEREIRA-DA-SILVA, E.M.; OLIVEIRA, R.H.F.; RIBEIRO, M.A.R.; COPPOLA, M.P. 2009 Efeito anestésico do óleo de cravo em alevinos de lambari. *Ciência Rural*, *39*(6): 1851-1856.

PORTO-FORESTI, F.; CASTILHO ALMEIDA, R.; FORESTI, F. 2005 Biologia e criação do lambari-do-rabo-amarelo *(Astyanax altiparanae)*. In: BALDISSEROTO, B.; GOMES, L.C. *Espécies Nativas para piscicultura no Brasil*. Universidade Federal de Santa Maria, Santa Maria. p. 105-120.

PRASAD, G.; CHARLES, S. 2010 Haematology and leucocyte enzyme cytochemistry of a threatened yellow catfish *Horabagrus brachysoma* (Gunther 1864). *Fish Physiology and Biochemistry*, *361*(3): 435-443.

RANDALL, D.J.; PERRY, S.F. 1992 Catecholamines. In: HOAR, W.S.; RANDALL, D.J. *Fish physiology,* Academic Press, New York, p. 255-300.

REID, S.G.; BERNIER, N.J.; PERRY, S.F. 1998 The adrenergic stress response in fish: control of catecholamine storage and release. *Comparative Biochemistry and Physiology, 120*(1): 1-27.

ROTHWELL, S.E.; BLACK, S.E.; JERRETT, A.R.; FORSTER, M.E. 2005 Cardiovascular changes and catecholamine release following anaesthesia in Chinook slamon *(Oncorhynchus tshawytscha)* and snapper *(Pagrus auratus)*. *Comparative Biochemistry and Physiology, 140*(3): 289-298.

SATHEESHKUMAR, P.; ANANTHAN, G.; SENTHIL KUMAR, D.; JAGADEESAN, L. 2011 Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. *Comparative Clinical Pathology, 21*(6): 1187-1191.

SHERIDAN, M.A.; MOMMSEN, T.P. 1991 Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch. General Comparative Endocrinology*, *81*(3): 473-483.

SHI, X.; ZHUANG, P.; ZHANG, L.; CHEN, L.; XU, B.; FENG, G.; HUANG, X. 2010 Optimal starvation time before blood sampling to get baseline data on several blood biochemical parameters in Amur sturgeon, *Acipenser schrenckii. Aquaculture Nutrition, 16*(5): 544-548.

SOENGAS, J.L.; ALDEGUNDE, M. 2002 Energy metabolism of fish brain. *Comparative Biochemistry and Physiology B*, *131*(3): 271-296.

STOOT, L.J.; CAIRNS, N.A.; CULL, F.; TAYLOR, J.J.; JEFFREY, J.D.; MORIN, F.; MANDELMAN, J.W.; CLARK, T.D.; COOKE, S.J. 2014 Use of blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conservation Physiology, 2*(1): 1-12.

SVOBODA, M. KALAB, P.; SVOBODOVA, Z.; KOURIL, J.; HAMACKOVA, J.; SAVINA, L.; VYKUSOVA, B. 2001 Biochemical profile of blood plasma of tench *(Tinca tinca L.)* during pre and post spawning period. *Acta Veterinaria Brno, 70*: 259-268.

SVOBODOVÁ, Z.; SMUTNÁ, M.; FLAJŠHANS, M.; GELA, D.; KOCOUR, M.; MÁCHOVÁ, J.; KROUPOVÁ, H. 2009 Differences in biochemical profiles among spawners of eight common carp breeds. *Jounal of Applied Ichthyology, 25*(6): 734-739.

TAKAHASHI, L.S.; ABREU, J.S.; BILLER, J.D.; URBINATI, E.C. 2006 Efeito do ambiente pós-transporte na recuperação dos indicadores de estresse de pacus juvenis, *Piaractus mesopotamicus*. *Acta Scientiarum Animal Science*, *28*(4): 469-475.

TAVARES-DIAS, M.; MORAES, F.R. 2004 *Hematology of teleosts fish*. Villimpress, Ribeirão Preto, 144 p.

TAVARES-DIAS, M.; MORAES, F.R. 2007 Haematological and biochemical reference intervals for farmed channel catfish. *Journal of Fish Biology, 71*(2): 383-388.

TAVARES-DIAS, M.; BARCELLOS, J. F. M.; MARCON, J.L; MENEZES, G.C.; ONO, E.A.; AFFONSO, E.G. 2007 Hematological and biochemical parameters for the pirarucu *Arapaima gigas* Schinz, 1822 (Osteoglossiformes, Arapaimidae) in net cage culture. *Electronic Journal of Ichthyology, 2*(2): 61-68.

TRINDER, P. 1969 Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Journal of Clinical Pathology, 22*(2): 158-161.

URBINATI, E.C.; CARNEIRO, P.C.F. 2004 *Práticas de Manejo e Estresse dos Peixes em Piscicultura Intensiva*. In: CYRINO, J.E.P.; URBINATI, E.C.; CASTAGNOLLI, N. *Tópicos Especiais em Piscicultura Tropical*. TecArt, São Paulo, p. 171-193.

URBINATI, E.C.; ABREU, J.S.; CAMARGO, A.C.S.; PARRA, M.A.L. 2004. Loading and transport stress in juvenile matrinxã *(Brycon cephalus)* at various densities. Aquaculture, *229*: 389-400.

VIJAYAN, M.M.; MOON, T.W. 1992 Acute handling stress alters hepatyc glycogen metabolism in food deprived rainbow-trout *(Oncorhynchus mykiss)*. *Canadian Journal of Aquatic Science*, *49*(11): 2260-2266.

VINATEA, L. 2004 *Princípios químicos de qualidade da água em aqüicultura*. UFSC, Florianópolis, 345p.

WEDEMEYER, G.A. 1996 *Physiology of Fish in Intensive Culture Systems*. Chapman and Hall, New York, 232p.

WEDEMEYER, G.A.; BARTON, B.; MC LEAY, D. 1990 Stress and acclimation. In: SCHRECK, C.B.; MOYLE, P.B. *Methods for Fish Biology*. American Fisheries Society, Bethesda, p.451-489.

WENDELAAR BONGA, S.E. 1997 The stress response in fish. *Physiological Reviews*, *77*(3): 591-625

WELLS, R.M.G.; PANKHURST, N.W. 1999 Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein a stress indicator in fish. *Journal of the World Aquaculture Society, 30*(2): 276-284.

YONEMORI, Y.; TAKAHASHI, E.; REN, H.; HAYASHI, T.; ENDO, H. 2009 Biosensor system for continuous glucose monitoring in fish. *Analytica Chimica Acta, 633*(1): 90-96.