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Intermittent sustained swimming in 'matrinxã' *Brycon amazonicus* (Bryconidae: Bryconinae): hematological and metabolic responses

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In fish, studies on a wide variety of physiological effects of exercise have been reported since a long time. It has been attributed special attention to some types of exercise, however, its application as a healthful practice in the rearing and welfare of farming fish is rising in last few years. In this particular, long-term intermittent sustained swimming (ISS) has been not yet explored. In this work, the freshwater fish *Brycon amazonicus* was submitted to (ISS) for 30 days at velocity of 1.0 body-length sec^{-1} for 12h interspaced by 12h under still water. Hematology and metabolism were evaluated. Exercised fish decreased 30% the erythrocyte number and hemoglobin was unvaried. The stores of liver glycogen and muscular triacylglycerol (TAG) were increased and the metabolic profile was typically aerobic. The slight decrease of liver (TAG) plus the full metabolic and hematic trait allow investing in this kind of exercise a beneficial practice in the rearing of fish species.

Há muito tempo, tem sido relatada uma ampla variedade de efeitos fisiológicos em peixes sob exercício. Tem sido dada especial atenção a alguns tipos de exercício, mas, sua aplicação como prática salutar na criação e para o bem estar dos peixes vem crescendo nos últimos anos. Neste caso, a natação sustentada intermitente (ISS) por longos períodos ainda não foi explorada. Neste trabalho, o peixe de água doce *Brycon amazonicus* foi submetido a (ISS) por 30 dias à velocidade de 1,0 comprimento corporal s^{-1} por 12h intervalado de 12h sob regime de água sem movimento. A hematologia e o metabolismo foram avaliados. Os peixes exercitados diminuíram 30% a contagem de eritrócitos e o teor de hemoglobina permaneceu constante. Os estoques de glicogênio hepático, o teor de triacil glycerol muscular (TAG) aumentou e o perfil metabólico foi tipicamente aeróbico. O discreto aumento de TAG hepático, além das características hematológicas e do metabolismo como um todo, instiga-nos investigar este tipo de exercício como uma prática benéfica na criação de peixes.

Key words: Adaptation, Exercise, Fish, Glycogen, Metabolism, Triacylglycerol.

Introduction

For several reasons the fish welfare is a current concern either for ethical account or for commercial basis. Since several decades ago the physiological responses of fish to exercise have been studied to understand many biological aspects such as ion balance, metabolism and energetic interchanges; however, the studies, to the greatest extent, were focused in strenuous exercise. Initial interest for such subject was in attempting to clear some effects observed in the wild when fish are presumably exposed to fatigue. In fact, some situations such as catch and release sport fishing (Cooke & Sneddon, 2007), commercial fishing 'throwback' (Neilsen *et al.*, 1989), netting and electro-fishing for biological surveys (Bracewell *et al.*, 2004), and transportation, confinement and handling in aquaculture and fish-stocking practices (Schwalme & Mackay, 1985; Fraser & Beamish, 1969; Barton *et al.*, 1986; Woodward & Strange, 1987) can lead fish to fatigue. The resultant stress from fatigue can be injurious and several times lethal (Black, 1958; Wood *et al.*, 1983). Other kinds of exercise have been yet assayed and from the metabolic point of view it can be generally classified as anaerobic or aerobic. Sustained swimming is an aerobic kind of exercise (Davie *et al.*, 1986) and has been reported as advantageous to the rearing of the tropical freshwater fish *Brycon amazonicus* (Arbeláez *et al.*, 2011). In this sort of exercise the fish is able to keep an upright position against the water stream all over the swimming period without reach exhaustion or muscle fatigue or muscle lactate accumulation. In this particular, the velocity is low and the fish are able to swim for more than 200min or even indefinitely. In the wild, several species are observed under sustained swimming, *e.g.*, during the feeding and

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migration (Jobling, 1994; Davison, 1997; Holk & Lykkeboe, 1998; Azuma *et al*., 2002).

Over the exercise a metabolic up-regulation occurs, increasing the flux of metabolites and integrating the energetic supply or demand with the body compartments. It is well established that white and red muscle are involved in the over range of sustained swimming speeds (Pritchard *et al*., 1971; Johnston & Goldspink, 1973a; Johnston & Moon, 1980). However, both tissues show distinct behaviors. White muscle is widely reported as lactate producer and glycogen consumer during sustained swimming (Johnston & Goldspink, 1973b). This feature is remarkable in fish submitted to strenuous exercise but can be changed after adaptation to sustained swimming (Milligan *et al*., 2000). Consume of glycogen followed by lactate production can be attributed to cortisol levels in exhaustive exercise (Pagnotta *et al*., 1994; Eros & Milligan, 1996). The adaptive feature resultant from sustained swimming encourages thinking upon the possibility of reducing occasional and undesirable effects of stress through the use of this practice and/ or derivatives. There are evidences that sustained swimming enhances the growth rate (Young & Cech Jr., 1994; Azuma *et al*., 2002; Hackbarth & Moraes, 2006; Moraes *et al*., 2009), the feeding conversion and the efficiency of nutrient utilization (Davison, 1997; Ogata & Oku, 2000; Yogata & Oku, 2000; Hackbarth & Moraes, 2006; Moraes *et al*., 2009), the survival rate (Totland *et al*., 1987; Takle *et al*., 2010), and reduces the aggressiveness in some species (Arbeláez-Rojas & Moraes, 2009; Kieffer *et al*., 2009).

Different results can be expected from sustained swimming when the exercise endurance varies in both, intensity and/ or frequency. This type of exercise for long term has been proposed to improve the farming of several species. One has been observed a correlation between sustained swimming intensity and the fish performance in accordance with species, *eg*: rainbow trout *Salmo gairdneri* (= *Oncorhynchus mykiss*) (Houlihan & Laurent, 1987), salmon *Salmo salar* (Davison, 1997), brown-trout *Salmo trutta* (Bugeon *et al*., 2003), striped bass *Morone saxatilis* (Young & Cech Jr., 1994), red sea bream *Pagrus major* (Forster & Ogata, 1996), masu salmon *Oncorhynchus masou masou* (= *Oncorhynchus masou*) (Azuma *et al*., 2002), Japanese flounder *Paralichthys olivaceus* (Ogata & Oku, 2000) and yellowtail *Seriola quinqueradiata* (Yogata & Oku, 2000). Concerning the hematological parameters, it has been reported distinct changes as well (Lowe & Wells, 1997; Moraes *et al*., 2009). As fish is submitted to sustained swimming under moderate intensities some useful metabolic changes have been observed such as a shift of protein to lipid and carbohydrate catabolism to fill usual energetic demands (Moyes & West, 1995; Davison, 1997; Wood, 2001; Richards *et al*., 2002; Hackbarth & Moraes, 2006; Arbeláez-Rojas & Moraes, 2009; Moraes *et al*., 2009). In addition, sustained swimming may result in changes of hemodynamic parameters such as blood flow, blood capillar diameter and beating frequency (Sandblom *et al*., 2005; Dunmall & Schreer, 2003; Altimiras & Larsen, 2000). However, the authors do not know upon any experimental assay with intermittent sustained swimming (ISS) in fish. Indeed, this case is more close to actual circumstances since in the wild the fish, as any other animals, do not exercise all over the time. Even in larger systems of sustained swimming the fish usually move into schools but eventually look for different spots in the water column to take some moments under resting.

The authors assumed that this natural characteristic should be experimentally simulated, studied and occasionally explored for some fish species. The elected species was 'matrinxã', a fresh water migratory teleost that lives mainly in streams and rapids of well aerated water. In addition, it is fully adapted to farming conditions for displaying excellent meat and convenient body growth traits. The farming conditions usually do not explore the trait of being 'matrinxã' a natural high-performance swimmer. We predicted that fish under ISS would have enhanced the metabolic fitness as compared with those living in still water or even under continuous sustained swimming. Three body compartments play a core role in the metabolism of fish over the exercise span; liver, muscle and blood. In such concern, hematological responses of the blood and some interrelations between liver and muscle were evaluated.

Material and Methods

Juvenile 'matrinxã' *B. amazonicus*, from the fish farm Águas Claras, Mococa, SP, were transported to the lab and held outdoors to acclimate for 30 days in 2000L tanks under filtered and aerated water. The new water conditions were: Temperature 24.5 ± 1.3°C; pH 7.3 ± 0.2; dissolved oxygen 5.9 ± 0.4mg L^{-1}; and ammonia 0.45 ± 0.07 mg ml^{-1}. After the acclimation period, nearly fifty fish were sampled and transferred to anesthetic bath with 40mgL^{-1} eugenol (Inoue *et al*., 2003). Anesthetized fish were submitted to biometry and 20 animals, classified into a range of 28.4 ± 0.5g of weight and 13.3 ± 0.5cm of length, were randomly and equally distributed into two circular fiber-tanks of 200L (10 fish per tank) with 82cm diameter and 55cm high and the same water conditions reported above, wherein they remained for 7 days to recover.

Experimental design

After recovery, the tanks were assigned as ISS (intermittent sustained swimming) and NE (non exercise). The fish in the ISS tanks were submitted to intermittent sustained swimming regimen of 12/12 in which the water was 12h motionless and 12h circulating at 1.0 BL (body-length) sec^{-1}. In the ISS system the water movement was started at morning (6:30 AM) and remained over the day light period (photoperiod nearly 12:12h). This velocity and the experimental swimming system were previously established and reported (Arbeláez-Rojas & Moraes, 2010).

The fish were kept in the experimental conditions for 30 days and the feeding regimen of the acclimation period was maintained the same; fish were fed to satiety twice a day with commercial pellets containing 30% of crude protein (CP), 31% of carbohydrate (CH) and 6.5% of lipid (L). After the experimental span, the ten fish from both conditions were sampled, anesthetized as described above and blood was withdrawn from the caudal vein. A blood aliquot from every fish was transferred to heparinized syringe and centrifuged at 5000 x g to obtainment of plasma. The remained blood in the syringe was used to hematologic determinations. Following the blood sampling, the fish were killed by pinching the spinal cord and samples of white muscle and liver were taken, immediately frozen into liquid nitrogen and preserved at -20^{o} C for posterior analyses.

Hematology

The blood samples were evaluated regarding the microhematocrit (Ht%), the content of hemoglobin (Hb gL^{-1}) according to Drabkin (1948), and the number of red blood cells (RBC 10^{6}mm^{-3}). From those data, the mean corpuscular volume (MCV μ^3), mean corpuscular hemoglobin (MCH pg cell^{-1}) and mean corpuscular hemoglobin concentration (MCHC %) were inferred (Lima *et al*., 1969).

Cell extracts

Neutral, acid or alkaline tissue extracts were performed to determine metabolic intermediates. Acid extracts were done in 20% trichloroacetic acid (TCA) for muscle and liver, and kept the ratio 1:10. The tissue homogenates were performed in a rotative homogenizer with three strokes of 1000rpm for 20sec under ice bath. The homogenates were centrifuged at 12000 x g for 3min and the pellets were discarded. In the neutral extracts, TCA was replaced by distilled water and the homogenates were centrifuged at 13400 x g for 3 min. In the alkaline extracts, tissues were disrupted in 6.0N KOH under boiling water bath for 5min and the ratio tissue: KOH was kept at 1:20 for liver and 1:5 for muscle.

Primary metabolites

Metabolites were colorimetrically determined. In acid extracts the next metabolites were determined: Total sugars (Dubois *et al*., 1956), pyruvate (Lu, 1939) and lactate (Harrower & Brown, 1972). Free amino acids (Copley, 1941) free fatty acids (Norvák, 1965), triglycerides (Lab-Test enzymatic Kit) and protein (Kruger, 1994) were determined in neutral extracts. Glycogen was quantified in alkaline extract (Bidinotto *et al*., 1998). Plasma triglycerides are expressed in mg dL^{-1}, glycogen in μmol of glycosil-glucose (mg of wet tissue)$^{-1}$ and the other metabolites in μmol (mg of protein)$^{-1}$.

Statistics

All values are presented as means ± (SD). In this study, a random assignment design with two independent sample size of 10 fish each were used to compare exercised with non-exercised fish (N = 20) through an unpaired t-test and since no outlier data points were observed the significant differences were considered for a confidence level of 5%. Normality of each metabolic and hematologic variable was checked and the Mann-Whitney test was used as necessary. The data were analyzed by Statistical Analyses System SAS® v.8 software package (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Hematology

The ISS condition over 30 days was effective on the hematological profile of 'matrinxã' (Table 1). The number of red blood cells decreased about 30%; however but equivalent reduction of hematocrit was not observed. This frame means that the erythrocyte volume was enlarged and such enlargement must be attributed to cell swelling. This response could be due to alterations in intracellular or extracellular fluids which are usually accompanied by a redistribution of body fluids (Milligan & Wood, 1982). Considering that the total hemoglobin remained constant but the erythrocyte number decreased over the ISS followed by increase of the RBC volume, the enhancement observed in the content of hemoglobin per cell was expected. From the foregoing, the constant total blood content of hemoglobin was reflected in the unaltered mean cell hemoglobin concentration.

From the hematological parameters it is possible to infer the health condition of the fish since the exercise, though moderate exercise, involves a number of changes in the blood flow, in the vein diameter, and the functions of respiration and oxygenation (Satchell, 1991; Sänger & Pötscher, 2000). Aerobic exercise enhances the muscle capillarization bringing immediate consequences to the whole organism, such as increase of oxygen and capacity of metabolites transport, as well as promoting faster waste of metabolism residues (Sänger & Pötscher, 2000). In addition, the hematological responses of fish submitted to sustained swimming are different between species and change according to the swimming velocity (Hackbarth & Moraes, 2006; Moraes *et al*., 2009; Arbeláez-Rojas & Moraes, 2010).

There is no information on the hematic profile of fish submitted to ISS. This fact hampers any comparison among species but does not hinder an incipient discussion. The ISS

Table 1. Blood parameters of *Brycon amazonicus* submitted to ISS. Juvenile 'matrinxã' were submitted to intermittent sustained swimming (ISS) at 1.0 Body-Length sec^{-1} for 30 days. NE-non exercise; ISS-12/12h (swimming/still); Ht - hematocrit (%); Hb - hemoglobin (g%); RBC - erythrocyte count (10^{6} mm^{-3}); MCV - mean corpuscular volume (m^{3}); MCH - mean corpuscular hemoglobin (μg); CHCM - mean corpuscular hemoglobin concentration (%). Superscript letters mean significant difference between conditions for the same parameter at $p<0.05$.

Blood Parameter	Condition	
	NE	ISS
Ht	33.81±1.5	31.08±3.6
Hb	7.16±0.2	8.44±0.4
RBC	2.83±0.1 ^{A}	1.96±0.2 ^{B}
MCV	134.20±33 ^{B}	193.8±25 ^{A}
MCH	26.74±1,3 ^{B}	56.33±12,2 ^{A}
MCHC	22.22±0.7	24.87±1.4

seemed to result in adaptive effects of the exercise on the blood tissue. The increase of the erythrocyte size should be understood as macrocytosis but no evidence was observed in blood smears, which would be apparent as the number of immature erythrocytes increases. The periodic exposure of fish to ISS should result in daily release of catecholamines and cortisol, which would lead to increase of glomerular filtration rates and urine production, as reported in European eels (Chan *et al*., 1969). This effect could account for a reduced blood fluid volume and a resultant, higher hematocrit (Barton *et al*., 1987). Although the 15% of increase of hemoglobin concentration has been not significantly different, a clear tendency of enhancement was observed. This might be assumed as a frame of anabolism of the red blood cells with biosynthesis *de novo* of hemoglobin and the assembly of correlated proteins. The lower number of erythrocytes in fish held in ISS may carry on changes in osmotic parameters such as blood viscosity. Fish under exercise are supposed to present adjustments in the hemodynamic traits, such as heart beat, blood flow, stroke volume and blood pressure. The hematological responses may be typical of the species and also depend on external factors such as swimming speed (Hackbarth & Moraes, 2006; Moraes *et al*., 2009; Arbeláez-Rojas & Moraes, 2010). Changes in the blood viscosity in consequence of decrease in erythrocyte number and/ or size can be a hematological adaptation to prevent undesirable effects from hemodynamic changes. In addition, the tendency of increase in hemoglobin concentrations was probably to supply an increased metabolic demand from intermittent exercise, holding steady the level of oxygen uptake.

Metabolism

Fish submitted to ISS brought out significant enhancement in the liver stores of glycogen (Table 2). The glycogen biosynthesis is dependent on glucose readiness which comes from feeding or other cell metabolites. The liver glucose was increased in fish under ISS; however, the feeding regimen was the same in both conditions avoiding any interference from the food. Then, the main chance is that glucose comes out from metabolites through the gluconeogenic paths. The level of pyruvate was also increased, and two relevant ways can justify such result; the synthesis from lactate and/ or the deamination of free amino acids. Both paths are distinctly interpreted; prevalence of anaerobic metabolism or utilization of amino acids backbone. The increase of lactate in the liver was likely due to rise of anaerobic metabolism in peripheral tissues to fulfill the energetic demand from the exercise. White muscle is supposed to be the core of lactate production and the main source of it to the liver re-composition of glucose through the Cory cycle. This metabolic pathway is well reported in fish under anaerobic metabolism (Pagnotta & Milligan, 1991; Girard & Milligan, 1992; Turner & Wood, 1983). However, the observed decrease in the concentration of liver amino acids suggests consume of these molecules. In addition, they were likely come from proteins of peripheral tissues, since hepatic protein was invariable, and were also used to produce the backbone of glucose. Hepatic gluconeogenesis has been observed in fish species under sustained swimming (Moose, 1980; Suarez & Mommsen, 1987). The enhancement of the liver glycogen stores should be intriguing at a first glance; however the exercise usually increases the levels of cortisol, and this steroid enhances the gluconeogenic preference. Sustained swimming increases carbohydrate and lipid oxidation driving amino acids to proteins synthesis, which favors the fish growth (Davison, 1997). Increase in muscle protein retention with utilization of lipids and carbohydrates as energetic is observed in *B. amazonicus* submitted to sustained swimming at 1.0 BL sec^{-1} (Hackbarth & Moraes, 2006).

Table 2. Metabolic profile of liver of *Brycon amazonicus* submitted to ISS. Juvenile 'matrinxã' were submitted to intermittent sustained swimming (ISS) at 1.0 Body-Length sec^{-1} for 30 days. NE - non exercise; ISS - 12/12h (swimming/ still). Glycogen (µmol of glycosyl-glucose (g protein) $^{-1}$); glucose (nmol g^{-1}); pyruvate (µmol g^{-1}); lactate (µmol g^{-1}); protein (mg g^{-1}); FAA - free amino acids (µmol g^{-1}); TAG - triacylglycerol (mg g^{-1}), FFA - free fatty acids (µmol g^{-1}); TAG/ FFA ratio. Superscript letters mean significant difference between conditions for the same parameter at $p<0.05$.

Metabolite	Condition	
	NE	ISS
Glycogen	73.22 ± 9^{B}	163.32 ± 10.6^{A}
Glucose	41.37 ± 0.7^{B}	133.82 ± 2.8^{A}
Pyruvate	0.70 ± 0.02^{B}	0.86 ± 0.04^{A}
Lactate	15.09 ± 0.5^{B}	20.82 ± 1.6^{A}
Protein	31.2±0.2	31.8±0.4
FAA	14.53 ± 0.2^{A}	8.57 ± 0.4^{B}
TAG	2.33 ± 0.07^{A}	2.18 ± 0.07^{B}
FFA	1.16±0.31	0.80±0.04

'Matrinxã' under ISS presented slightly lower levels of TAG than those fish kept under motionless waters; however, the FFA remained unaltered. This metabolic picture means that low levels of fat are being mobilized from liver to other peripheral tissues. This biochemical profile seems to be healthful since it is antagonistic to a clinical picture of fat liver. Steatosis, a tissue degeneration of liver, characterized by deposition of lipid droplets, has been observed in fish under several conditions, as artificial diets, and is followed by higher mortality rates. Increase of free mono-unsaturated fatty acids should be the primary cause of steatosis in fish (Spisni *et al*., 1998). Protein regulatory elements plus some FFA-biosynthesis-enzymes are reduced in animals submitted to a daily exercise program (Cintra *et al*., 2012). There are no reports on beneficial effects of intermittent sustained swimming, or even continuous exercise, on steatosis in fish; however, is plausible that this practice should be healthful, and the observed decrease of TAG in exercised fish is suggestive of such benefic consequences.

In white muscle of 'matrinxã', glucose and pyruvate were increased in fish submitted to ISS (Table 3). The invariable content of the glycogen stores suggests that glucose was

Table 3. Metabolic profile of white muscle of *Brycon amazonicus* submitted to ISS. Juvenile 'matrinxã' were submitted to intermittent sustained swimming (ISS) at 1.0 Body-Length sec^{-1} for 30 days. NE - non exercise; ISS - 12/12h (swimming/still). Glycogen (μmol of glycosyl-glucose (g protein)$^{-1}$); glucose (nmol g^{-1}); pyruvate (μmol g^{-1}); lactate (μmol g^{-1}); L/P - lactate/pyruvate ratio; protein (mg g^{-1}); FAA - free amino acids (μmol g^{-1}); TAG - triacylglycerol (mg g^{-1}), FFA - free fatty acids (μmol g^{-1}); TAG/FFA ratio. Superscript letters mean significant difference between conditions for the same parameter at $p<0.05$.

Metabolite	Condition	
	NE	ISS
Glycogen	2.20±0.2	2.86±1.1
Glucose	17.15±2.0^{B}	48.47±7.4^{A}
Pyruvate	0.040±0.1^{B}	0.12±0.1^{A}
Lactate	46.37±3.5	55.00±4.5
L/P	1159	458
Protein	32.20±3.0	26.42±1.3
FAA	28.53±5.0	57.35±9.9
TAG	0.36±0.1^{B}	0.46±0.1^{A}
FFA	0.08±0.01^{A}	0.04±0.1^{B}

taken up from the blood stream. The increase of pyruvate, observed in fish submitted to ISS, comes down on the side of two possibilities; the amino acids breakdown or the enhancement of glycolytic activity. Since the white muscle content of protein and FAA remained constant in the exercised fish, there is a remote chance that pyruvate comes from muscle proteins. The enhancement of anaerobic glycolysis is usually observed in individuals submitted to exhaustive exercise, including fish (Wood & Perry, 1985; Milligan & Wood, 1986; Dobson, & Hochachka, 1987). However, no increase of lactate was observed in 'matrinxã', as usually occurs in fish submitted to strenuous swimming. Actually, the lactate/ pyruvate ratio decreased 2.5 times. This frame is suggestive that muscle pyruvate came from glucose breakdown and the aerobic path was followed. In consequence, the increase of the hepatic lactate observed in fish under ISS was from increased metabolic activity but unlikely from the Cory cycle as discussed above. Similar results were observed in *Piaractus mesopotamicus* and *B. amazonicus* submitted to sustained swimming (Moraes *et al.*, 2009). The glycolytic preference of erythrocytes is toward fermentation and this was likely the source of lactate and the responsible for such an increase in the liver. This metabolic frame of white muscle plus erythrocytes corroborate the assumption of increased metabolic rates of RBC and muscle but prevented any undesirable effect from fermentation, typical from harmful, excessive physical activities.

The increased TAG observed in the white muscle of 'matrinxã' under ISS was due to anabolism of the fatty acids. This fact is observed also in striped bass *Morone saxatilis* submitted, however, to long term sustained swimming (Young & Cech Jr., 1994). The biosynthesis of TAG explains the decrease, or consume, of muscle FFA, which were likely come from exogenous sources such as liver. The enhancement of TAG in white muscle was 21%, and such a metabolic condition associated to liver gluconeogenesis is evidence that ISS leaded the fish to anabolism over the experimental period with clear aerobic preference of the peripheral tissues. This is indicative that ISS was a healthful practice to 'matrinxã' fed with 30% of CP, 31% of CH and 6.5% of L. This particular feeding condition must be observed since the carbon backbone source was likely dietary protein and carbohydrate. Changing these nutrient contents certainly will change the metabolic responses of fish under ISS.

The gluconeogenesis observed in the liver of fish submitted to ISS should be attributed to increased levels of cortisol. In fish, this glucocorticoid is produced by interrenal cells of the head kidney and it is reported to be increased in animals under a wide variety of stimuli, including exercise (Butler *et al.*, 1986). The cortisol profile in fish as response to stressors is not the same among the species, and a considerable diversity can be expected which seems to be consistent within an individual (Pottinger *et al.*, 1992; Romero & Reed, 2008). The physiological response to acute stress is an adaptive mechanism that, for example, facilitates escape from challenging situations (Wingfield *et al.*, 1998). It would be expected that greater level of stress response would have positive fitness effects. However, a stress response is energetically demanding and it results in reallocation of energy away from costly activities as courtship, immune defense, self-maintenance or survival (Ricklefs & Wikelski, 2002; Wingfield *et al.*, 1998). Intermittent sustained swimming cannot be considered an acute stress source; however, the biochemical responses observed in 'matrinxã' are consistent with some physiological effects observed under cortisol increase. Studies correlating glucocorticoids and fitness have yielded inconsistent results (Bonier *et al.*, 2009a; Breuner *et al.*, 2008). It seems that such a relationship is context dependant and influenced by several factors including life history (Bonier *et al.*, 2009b), energetic constraints (Angelier *et al.*, 2010; Cote *et al.*, 2010) and environmental stability (Angelier *et al.*, 2009). Anyhow, increased levels of cortisol and/ or even some consequent effects cannot be considered primary harmful. Correlation between ISS and stress, measured through cortisol and other stress level parameters, must be performed in 'matrinxã' to clear many mechanistic points and accept or not advantageous effects from ISS to introduce it as a practice in rearing systems. Although, in the present work the adaptive changes observed in the blood profile and the liver-muscle metabolism allows to assume that 'matrinxã', reared for 30 days under ISS at 12h of exercise and 12h of resting, is fitted into: 1) preferential aerobic catabolism avoiding some undesirable consequences of acidosis and lactate accumulation, 2) a metabolic frame of biosyntheses, which is a convenient characteristics to juvenile fish over the growth period.

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