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Hematological, morphological, biochemical and hydromineral responses in *Rhamdia quelen* sedated with propofol

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Abstract *Rhamdia quelen* morphophysiological responses to propofol sedation were examined. The purpose was to investigate whether propofol would be a suitable drug to be used in fish transport procedures. Fish were exposed to 0, 0.4 or 0.8 mg L^{-1} propofol for 1, 6 or 12 h in 40 L tanks, simulating open transport systems. Propofol was able to prevent the peak of cortisol levels experienced by the group exposed to 0 mg L^{-1} propofol at 1 h. At 0.4 mg L^{-1}, propofol also preserved the stability of hematological (hematocrit, red blood cell count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration), morphological (red blood cell area), biochemical (cortisol, glucose, lactate, total protein, ammonia, urea, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase) and hydromineral (Na^+, Cl^- and K^+ plasma levels) indicators of stress. Such results suggest that sedation with propofol at 0.4 mg L^{-1} is suitable for *R. quelen* transport.

Keywords Transport · Stress · Hematology · Morphology · Biochemistry

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Introduction

The transportation segment of the fish farming system undoubtedly requires operational expertise and knowledge on the physiology of the subjects. Buin et al. (2013) stated that mortality occurred not only during transport but also and most importantly after the fish had been delivered to the recipient location. The addition of sedative/anesthetic substances to the water of transport has been employed in an attempt to reduce fish death arisen from transport-mediated stress (Ross et al. 2007; Becker et al. 2012; Benovit et al. 2012). The purpose is to induce a calming or sedative state during the procedure so that the perception of the stressful event is minimized and its side effects reduced (Iwama et al. 1989).

The stress response in fish is an adaptive mechanism characterized by a cascade of physiological alterations that constitute a three-phase pattern (Wendelaar Bonga 1997). Firstly, upon stress, the neuroendocrine system releases catecholamines and

corticosteroids into the blood stream. The presence of these stress hormones in the circulatory system precipitates subsequent responses related to energy requirements, such as increases in blood glucose and lactate levels, and variation in plasma electrolytes concentrations, among others. If the second responses are extreme or sustained, the process culminates with whole-animal changes which compromise metabolism, reproductive output and disease resistance and may ultimately lead to mortality (Barton and Iwama 1991; Wendelaar Bonga 1997; Maricchiolo and Genovese 2011).

Despite the purpose of the use of an anesthetic being to mitigate stress, a common report is that the substance itself may pose as a stressor, thus activating the stress response mechanism (Thomas and Robertson 1991; Sladky et al. 2001; Bolasina 2006). According to Zahl et al. (2012), the unwanted side effects of an anesthetic such as respiratory acidosis and osmotic stress reduce the welfare of the fish and, therefore, caution should be taken when such agents are used. Another point to consider is that the efficacy of a given anesthetic depends on variables such as the intensity of the stressor, the fish species, its developmental stage and the environmental conditions (Rotllant et al. 2001; King et al. 2005).

In view of such considerations, the viability of using propofol as a sedative for juvenile silver catfish *Rhamdia quelen* transport was investigated. This anesthetic has recently been proven effective for immersion anesthesia of the same fish species (Gressler et al. 2012a). The experiment was performed in a laboratory-controlled setting in order to verify the sole effect of the anesthetic upon the physiology of the species through the analyses of hematological, morphological, biochemical and hydromineral indicators of stress.

Methods

Animals

Juvenile gray silver catfish ($n = 90$; mean ± SE body mass = 91.44 ± 1.98 g; mean ± SE total length = 20.66 ± 0.15 cm) were acquired from a fish farm in Santa Maria city, southern Brazil, and housed at the Laboratório de Fisiologia de Peixes (LAFIPE) at Universidade Federal de Santa Maria (UFSM). Acclimation lasted 7 days and was performed in 250-L tanks (15 fish/tank) in a semi-static system with constantly aerated dechlorinated well water (200 L/tank) at 21.5 ± 0.08 °C, pH 7.45 ± 0.13 and dissolved oxygen 8.04 ± 0.26 mg L^{-1} (mean ± SE). The water was renewed every second day and the fish were fed commercial pellets for omnivorous fish (42 % extruded crude protein; 4 % fibrous matter; 14 % mineral matter; 2.5 % calcium) once a day. All procedures were conducted with the approval of the Ethics Committee on Animal Experimentation of the UFSM (registration no. 67/2012).

Drug

Propofol (Propotil 1 %; BioChimico; www.biochimico.com.br) was commercially acquired. A pilot study based on the literature-derived values (Gressler et al. 2012a) was performed and two low concentration levels of propofol were established to be used in sedative baths: 0.4 and 0.8 mg L^{-1}. These concentrations were able to induce up to stages 2 and 3a, respectively, as described by Schoettger and Julin (1967).

Experimental design

Twenty-four hours after the last feeding the fish were subjected to one of the following concentrations of propofol: 0 (control), 0.4 or 0.8 mg L^{-1}. Each treatment was further divided into an exposure time of 1, 6 and 12 h, reproducing short, medium and long transport procedures, respectively. For every concentration/time combination, 10 fish were tested (two replicates of five fish each).

The trials were performed in 40-L tanks filled to 50 % of their capacity with the same water used in the acclimation tanks. The proper anesthetic concentration was dispersed in the water if that was the case. The experimental setting simulated transportation in tanks. Nonetheless, in order to guarantee that any observed effect would arise from the anesthetic only, care was taken to prevent common transport interferences such as decline in dissolved oxygen, build-up of ammonia, accumulation of carbon dioxide and reduction in pH from happening. Therefore, loading density was low, constant aeration was provided, and the experiment was carried out under controlled environmental conditions at LAFIPE. Water parameters in the experimental tanks were as follows: 21.8 ± 0.12 °C,

dissolved oxygen 6.5 ± 0.24 mg L^{-1} (Yellow Springs Instruments, Yellow Springs, OH, USA; model Y55), pH 7.4 ± 0.06 (Hanna Instruments, Woonsocket, RI, USA; model HI 8424), total ammonia 0.14 ± 0.02 mg L^{-1} (Verdouw et al. 1978) and un-ionized ammonia 0.002 ± 0.001 mg L^{-1} (Colt 2002).

Fish were hand-transferred to the trial tanks and kept under the confined experimental conditions for the assigned period. Once exposure time had elapsed, fish were individually removed from the tanks and a 2 mL blood sample was immediately taken from the caudal peduncle with heparinized sterile syringes. Biometrics was also performed and fish were euthanized by sectioning the spinal cord.

Whole blood analyses

Hematocrit was measured in microcapillary tubes centrifuged at 10,000×*g* for 10 min and reading was performed with the aid of a hematocrit card reader. Total red blood cells (RBC) count was determined with a Neubauer hemocytometer (Tavares-Dias et al. 2002). The concentration of hemoglobin was assayed by the cyanmethemoglobin method using a spectrophotometer (Brow 1976). The indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe (1934). Blood smears were prepared and then air-dried, fixed in methanol and stained with May-Grünwald (Rosenfeld 1947). Subsequently, with the aid of an image analyzer microscope, ten high-power fields were randomly selected on each blood smear and morphometry of six RBC was observed in each of these fields (Benfey et al. 1984; Dorafshan et al. 2008). All of the morphometric analyzes were performed using the Zeiss Axio Vision System with Remote Capture 4.7 Rel DC—Cannon Power shot G9.

Plasma analyses

The remaining whole blood was placed into microcentrifuge tubes and spun at 3,000×*g* for 10 min. The obtained plasma was collected in microtubes and stored at −25 °C for further analyses.

EIA kits (EIAgenTM Cortisol, Adaltis Italy S.p.A) were used to measure cortisol in unextracted plasma samples. Test specificity was assessed through comparison of the parallelism between the standard curve and serial dilutions of the samples in PBS (pH 7.4). The standard curve ran parallel to the one achieved with serial dilutions of *R. quelen* plasma. A high positive correlation ($r^2 = 0.9818$) was observed between the curves in the linear regression test. Inter- and intra-assay variation coefficients ranged from 9 to 12 % and 6 to 9 %, respectively. Glucose, total protein, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed using kits by Analisa (www.goldanalisa.com.br). Analyses of lactate and alkaline phosphatase (AP) were determined in Labtest kits (www.labtest.com.br). Ammonia was quantified according to Verdouw et al. (1978). Concentrations of Na^+ and K^+ were measured in appropriate diluted samples against known standards using flame photometry (Micronal B262). Chloride levels were assessed via the colorimetric method (Zall et al. 1956).

Statistical analyses

The experimental variables were three propofol concentrations (0, 0.4 and 0.8 mg L^{-1}) and three exposure durations (1, 6 and 12 h). Data are presented as the mean ± standard error (SE). The Bartlett test was used to evaluate normality, and the Levene test was applied to verify homogeneity of variances. Cortisol analyses were made through the Scheirer–Ray–Hare extension of the Kruskal–Wallis test and the Nemenyi test. The remaining parameters were analyzed through a two-way ANOVA and Tukey's test. The Statistica software 7.0 (Stat Soft. Inc., wwwstatsoft.com) was used to make the analyses, and differences were considered significant at $P < 0.05$.

Results

Whole blood analyses

At 0.4 mg L^{-1} propofol, the hematocrit was lower at 6 compared to 12 h, while at 0.8 mg L^{-1} propofol, the hematocrit was lower at 1 h than at the remaining times. The level of this blood index was lower at 0.4 than at 0 and 0.8 mg L^{-1} propofol in fish exposed for 6 h. The concentration of hemoglobin was significantly greater within 6 h of exposure to the highest concentration of the anesthetic. Statistical evidence did not identify any effect on RBC, MCV, MCH or

Table 1 Hematological parameters in *Rhamdia quelen* subjected to 0, 0.4 or 0.8 mg L^{-1} propofol for 1, 6 or 12 h

Groups/ parameters	Hematocrit (%)	RBC (10^6 μL^{-1})	Hemoglobin (g dL^{-1})	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
0 mg L^{-1} propofol						
1 h	28.8 ± 1.08^{Aa}	2.23 ± 0.17	7.19 ± 0.60^{Aa}	125.68 ± 9.82	32.27 ± 1.53	25.49 ± 2.54
6 h	32.0 ± 0.85^{Aa}	2.22 ± 0.16	8.15 ± 0.56^{Aab}	137.81 ± 8.19	37.60 ± 2.09	25.85 ± 2.17
12 h	29.3 ± 0.47^{Aa}	2.46 ± 0.13	7.30 ± 0.60^{Aa}	121.43 ± 5.67	30.51 ± 2.82	24.95 ± 1.89
0.4 mg L^{-1} propofol						
1 h	29.8 ± 1.14^{ABa}	2.20 ± 0.16	7.26 ± 0.60^{Aa}	141.23 ± 9.66	33.55 ± 2.31	24.52 ± 1.97
6 h	27.8 ± 1.07^{Ab}	1.70 ± 0.16	6.19 ± 0.63^{Aa}	145.72 ± 9.03	33.35 ± 1.62	22.49 ± 2.26
12 h	32.9 ± 0.86^{Ba}	2.17 ± 0.12	8.19 ± 0.41^{Aa}	156.97 ± 10.60	38.69 ± 2.57	25.15 ± 1.54
0.8 mg L^{-1} propofol						
1 h	26.1 ± 0.39^{Aa}	2.24 ± 0.19	6.94 ± 0.31^{Aa}	114.84 ± 9.72	33.53 ± 3.70	26.56 ± 0.99
6 h	32.5 ± 1.25^{Ba}	2.25 ± 0.13	9.93 ± 0.59^{Bb}	150.50 ± 13.95	42.08 ± 1.98	31.01 ± 2.22
12 h	31.6 ± 1.16^{Ba}	2.27 ± 0.16	7.30 ± 0.70^{Aa}	146.28 ± 12.46	32.79 ± 2.24	23.38 ± 1.78

Values are mean ± SE. Different upper case letters indicate significant difference between times within the same concentration and different lower case letters indicate significant difference between concentrations at a given sampling by two-way ANOVA and Tukey's test. No significant differences were observed in RBC, MCV, MCH or MCHC

RBC red blood cells, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration

MCHC (Table 1). Propofol exposure reduced the nucleus of the cells (Table 2).

Plasma analyses

The level of cortisol in control group progressively declined from 1 to 12 h. The hormone concentration increased at 6 and 12 h in comparison with 1 h at 0.4 mg L^{-1}. The level of cortisol was greater at 6 than at 1 h exposure to 0.8 mg L^{-1} propofol; the group sampled at 12 h showed higher and lower concentrations of the hormone compared to 1 and 6 h, respectively. Cortisol gradually reduced from the highest to the lowest concentration of the anesthetic at 1 h. At 6 h, cortisol was higher at 0.8 than at 0 and 0.4 mg L^{-1} propofol. The level of the hormone was significantly higher at 0.4 than at 0 and 0.8 mg L^{-1} propofol in fish exposed for 12 h. Glucose concentration did not vary between groups. The content of lactate was significantly lower at 0.8 mg L^{-1} propofol after 1 and 6 h of exposure. Protein, ammonia and urea were not significantly affected by the treatments. The activity of AP showed an increase in fish sampled at 12 h after exposure to 0.8 mg L^{-1} propofol. No statistically significant alterations were observed in ALT or AST (Table 3). At 0 mg L^{-1} propofol, significantly higher Cl^- levels were observed in the group sampled at 12 h compare to 6 h. The level of Na^+ increased after exposure to 0.4 mg L^{-1} propofol for 12 h and decreased at the same sampling time at the highest concentration. There was a significant rise in the level of K^+ in fish exposed to 0.8 mg L^{-1} propofol for 12 h (Fig. 1). Survival during the experiment was 100 %.

Discussion

Whole blood analyses

Hematocrit, RBC, hemoglobin, MCV, MCH and MCHC

Propofol has been described as capable of decreasing ventilatory drive as well as cardiac output and contractility in mammals (Grouds et al. 1985; Pagel and Warltier 1993). Nevertheless, the results obtained for the hematological indices show that the anesthetic produced only mild hemodynamic changes in *R. quelen*. As in the present investigation, Tort et al. (2002), Bressler and Ron (2004) and Filiciotto et al.

Table 2 Red blood cell morphometry in *Rhamdia quelen* subjected to 0, 0.4 or 0.8 mg L^{-1} propofol for 1, 6 or 12 h

Groups/ parameters	Area (μm^2)	Minor axis (μm)	Major axis (μm)	Nucleus area (μm^2)	Nucleus minor axis (μm)	Nucleus major axis (μm)
0 mg L^{-1} propofol						
1 h	123.31 ± 2.90	11.11 ± 0.13	14.19 ± 0.13	19.18 ± 0.64^{Aa}	4.63 ± 0.07^{Aa}	5.38 ± 0.09^{Aa}
6 h	115.88 ± 2.68	10.68 ± 0.10	14.33 ± 0.16	17.37 ± 0.55^{Aa}	4.24 ± 0.07^{Ba}	5.33 ± 0.08^{Aa}
12 h	116.24 ± 2.56	10.70 ± 0.17	14.08 ± 0.14	18.37 ± 0.83^{Aa}	4.42 ± 0.11^{ABa}	5.39 ± 0.11^{Aa}
0.4 mg L^{-1} propofol						
1 h	119.60 ± 1.78	10.98 ± 0.08	14.10 ± 0.14	13.98 ± 0.58^{Ab}	3.86 ± 0.10^{Ab}	4.71 ± 0.08^{Ab}
6 h	114.04 ± 3.13	10.58 ± 0.11	13.94 ± 0.24	13.55 ± 0.38^{Ab}	3.76 ± 0.05^{Ab}	4.71 ± 0.08^{Ab}
12 h	121.40 ± 2.50	11.13 ± 0.10	14.06 ± 0.20	13.32 ± 0.39^{Ab}	3.78 ± 0.05^{Ab}	4.61 ± 0.07^{Ab}
0.8 mg L^{-1} propofol						
1 h	113.95 ± 2.29	10.66 ± 0.15	13.80 ± 0.17	15.00 ± 0.54^{Ab}	4.05 ± 0.08^{Ab}	4.83 ± 0.08^{Ab}
6 h	119.06 ± 1.75	10.92 ± 0.10	14.80 ± 0.12	14.11 ± 0.42^{Ab}	4.77 ± 0.10^{Bc}	4.77 ± 0.10^{Ab}
12 h	119.18 ± 1.55	10.99 ± 0.08	14.01 ± 0.12	12.98 ± 0.27^{Ab}	3.70 ± 0.04^{Cb}	4.60 ± 0.06^{Ab}

Values are mean ± SE. Different upper case letters indicate significant difference between times within the same concentration and different lower case letters indicate significant difference between concentrations at a given sampling by two-way ANOVA and Tukey's test. No significant differences were observed in cell area, minor axis or major axis

(2012) noted a decrease in hematocrit percentage as a result of anesthesia. Nevertheless, increased level of this blood index following anesthetic administration is most commonly reported (Thomas and Robertson 1991; Olsen et al. 1995; Gomulka et al. 2008; Sudagara et al. 2009; Maricchiolo and Genovese 2011; Gressler et al. 2012b; Pádua et al. 2012). Elevated hematocrit percentage may occur due to plasma volume reduction, hypoxia and a combination of RBC swelling and/or release by the spleen as a response to acute stress mediated by catecholamines (Davidson et al. 2000; Tort et al. 2002). In this study, in turn, the reduction in hematocrit may have been an adaptive response without major physiological significance especially because RBC number remained unchanged (Franklin et al. 1993).

The values found for hemoglobin concentration at 0.8 mg L^{-1} propofol after 6 h exposure may indicate that in this group there was a transient requirement for increased blood oxygen-carrying capacity. It was probably achieved by the movement of water from primary to secondary circulation systems, resulting in increased content of hemoglobin (Wells and Weber 1990; Franklin et al. 1993). The capacitance response is a rapid means of preserving oxygen delivery to tissues under hypoxic challenge, which in this case was mostly likely a result of the reduced gill ventilation during sedation or anesthesia, as previously related (Molinero and Gonzalez 1995; Sudagara et al. 2009). Moreover, besides the above-mentioned changes in RBC size or number, which may also be accountable for elevation in hemoglobin, as well as in hematocrit, Speckner et al. (1989) proposed that fish erythrocytes still synthesize hemoglobin while circulating in the peripheral blood. Thus, the increased levels of hemoglobin may reflect enhanced synthesis during low oxygen availability. In line with most of the results observed for hemoglobin in this study, previous works also described no effect of anesthesia on its concentration (Velisek et al. 2005a, b; Pádua et al. 2012).

RBC morphometry

The RBC are the most abundant cells in fish blood and their number and size represent the capacity of oxygen transportation (Fukushima et al. 2012). The RBC area obtained by means of morphometric analyses remained the same throughout the groups, what confirms that the use of propofol did not trigger major changes in the blood oxygen-carrying capacity besides the sole effect seen in the hemoglobin content for one specific experimental group. Morphometry is a valuable and accurate tool for obtaining fish RBC measurements without the interference of other variables, as in the case of MCV. But the literature on it is

Table 3 Biochemical parameters in *Rhamdia quelen* subjected to 0, 0.4 or 0.8 mg L^{-1} propofol for 1, 6 or 12 h

Groups/ parameters	cortisol (ng mL^{-1})	glucose (mg dL^{-1})	lactate (mg dL^{-1})	protein (g dL^{-1})	ammonia (mg L^{-1})	urea (mg dL^{-1})	AP (U L^{-1})	ALT (U mL^{-1})	AST (U mL^{-1})
0 mg L^{-1} propofol									
1 h	403.49 ± 48.51^{Aa}	34.48 ± 3.88	17.07 ± 1.81^{Aa}	5.28 ± 0.40	4.16 ± 0.11	23.47 ± 2.48	23.19 ± 1.45^{Aa}	22.70 ± 1.96	23.51 ± 1.72
6 h	248.07 ± 7.47^{Ba}	34.54 ± 1.82	16.73 ± 1.55^{Aa}	4.73 ± 0.27	4.32 ± 0.23	22.33 ± 3.13	21.59 ± 2.42^{Aa}	22.59 ± 2.33	20.72 ± 2.16
12 h	191.24 ± 19.68^{Ca}	36.27 ± 3.67	13.22 ± 1.52^{Aa}	5.33 ± 0.29	4.03 ± 0.13	27.17 ± 3.54	33.72 ± 2.60^{Aa}	17.11 ± 2.03	23.14 ± 2.43
0.4 mg L^{-1} propofol									
1 h	211.63 ± 38.50^{Ab}	46.67 ± 4.67	12.17 ± 1.05^{Aab}	4.98 ± 0.40	4.08 ± 0.18	34.90 ± 4.55	36.70 ± 3.95^{Aa}	26.05 ± 1.53	25.22 ± 1.29
6 h	241.68 ± 27.78^{Ba}	44.33 ± 4.87	10.83 ± 1.38^{Aab}	5.28 ± 0.26	3.80 ± 0.15	27.83 ± 3.36	28.47 ± 3.36^{Aa}	23.20 ± 1.29	27.89 ± 3.56
12 h	246.82 ± 32.32^{Bb}	48.71 ± 1.27	10.66 ± 1.26^{Aa}	5.67 ± 0.30	3.79 ± 0.08	32.01 ± 4.79	35.62 ± 3.65^{Aab}	21.23 ± 2.42	26.92 ± 2.54
0.8 mg L^{-1} propofol									
1 h	71.70 ± 15.35^{Ac}	48.03 ± 3.81	7.32 ± 0.43^{Ab}	5.66 ± 0.19	3.41 ± 0.26	29.04 ± 2.69	26.40 ± 2.39^{Aa}	27.80 ± 1.43	28.28 ± 2.46
6 h	166.38 ± 39.41^{Bb}	44.27 ± 4.34	7.34 ± 0.79^{Ab}	5.15 ± 0.17	3.85 ± 0.31	18.01 ± 1.60	20.73 ± 1.98^{Aa}	27.25 ± 1.93	23.09 ± 2.44
12 h	200.28 ± 18.84^{Ca}	38.13 ± 2.32	9.18 ± 1.64^{Aa}	5.78 ± 0.24	3.47 ± 0.13	24.55 ± 2.39	47.80 ± 4.70^{Bb}	21.01 ± 1.48	26.91 ± 2.11

Values are mean ± SE. Different upper case letters indicate significant difference between times within the same concentration and different lower case letters indicate significant difference between concentrations at a given sampling by two-way ANOVA and Tukey's test or the Scheirer–Ray–Hare extension of the Kruskal–Wallis test and the Nemenyi test. No significant differences were observed in glucose, protein, ammonia, urea, ALT or AST

AP alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

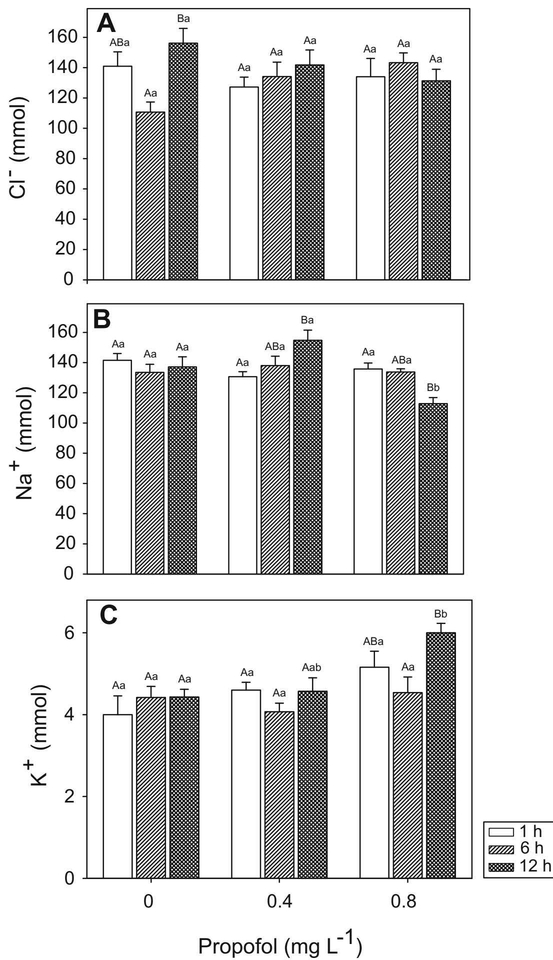

Fig. 1 Plasma levels of Cl^- (**a**), Na^+ (**b**) and K^+(**c**) in *Rhamdia quelen* subjected to 0, 0.4 or 0.8 mg L^{-1} propofol for 1, 6 or 12 h. Values are mean ± SE. *Different upper case letters* indicate significant difference between times within the same concentration and *different lower case letters* indicate significant difference between concentrations at a given sampling by two-way ANOVA and Tukey's test

still scarce; hence, the lack of physiological evidence as to justify the reduction in the RBC nucleus of anaesthetized silver catfish.

Plasma analyses

Cortisol, glucose and lactate

One hour after being subjected to tank transfer, the fish in the control group showed the highest level of cortisol obtained in this investigation, 403.49 ± 48.51 ng mL^{-1}. The 1 h peak is in agreement with responses observed by Wagner et al. (2003), Bolasina (2006), Barcellos et al. (2012) and Koakoski et al. (2012). In comparison with control group, both sedative concentrations of the anesthetic influenced the dynamics of cortisol and prevented the hormone peak. Davis and Griffin (2004), Small (2004) and Gressler et al. (2012b) also observed that administration of anesthetics prevented cortisol increase in fish. In opposition to what was demonstrated by the current results, some authors state that a low concentration may actually act as a stressor because the level of nervous depression does not mitigate certain physiological stress responses (Iwama et al. 1989; Olsen et al. 1995; Maricchiolo and Genovese 2011).

It is well accepted that catecholamines and corticosteroids inhibit glycogen synthesis and stimulate gluconeogenesis in order to fuel homeostatic mechanisms activated during exposure to stressors (Wendelaar Bonga 1997; Sladky et al. 2001). Preceding works observed hyperglycemia along with a rise in cortisol (Davis and Griffin 2004; Maricchiolo and Genovese 2011; Filiciotto et al. 2012), while others did not (Cho and Heath 2000; Iversen et al. 2003; Matsche 2011). Likewise, in the present study, the increased cortisol in control group did not alter carbohydrate metabolism.

Although plasma glucose did not show the classic stress-induced catabolic response, the higher lactate observed at 1 and 6 h at 0 mg L^{-1} propofol in comparison with the highest concentration of the anesthetic showed the provision of a rapid energetic resource following the handling stress when sedation was not present. However, the low levels of the metabolite suggest that there was an initial activation of anaerobic metabolism contributing to ATP supply, but without deficit in oxygen or glycidic resources. Rotllant et al. (2001) and Small (2004) also described stress-related responses of lactate, what happened along with a rise in glucose. Stressful conditions are typically associated with elevated plasma lactate concentrations, for the anaerobic state caused by stress results in muscle glycogen and lactate breakdown, with some of the lactate being released into circulation (Barton and Iwama 1991). In opposition to the present findings, anesthetics have been accountable for lactate rises (Molinero and Gonzalez 1995; Olsen et al. 1995; Iversen et al. 2003; Wagner et al. 2003). As Iwama et al. (1989) explained, lactate increases in blood when insufficient oxygen is

available for aerobic cell metabolism, what could be due to reduced ventilation and circulation, common side effects of several anesthetics.

Total protein, ammonia and urea

Depending on the type of stressor imposed to fish, cortisol may have an effect on protein and amino acids metabolism (Conceição et al. 2012). In the current research, the level of total protein was not affected by the experimental conditions. Barcellos et al. (2003), on the other hand, detected an effect of harvesting on total protein, indicating the possible use of such compound as substrate for the gluconeogenesis observed in the study. Laidley and Leatherland (1988) and Matsche (2011), in turn, registered a significant increase in plasma protein in fish subjected to anesthesia comparing to control; the latter authors indicated this shift as a result of RBC destruction.

Ammonia accounts for the greatest fraction of nitrogenous waste in teleost fish, followed by urea (Kajimura et al. 2004). Both waste products were examined but none presented any difference between the groups, corroborating the observed absence of stress-induced protein utilization in this study.

AP, ALT and AST

Propofol has hepatic as well as extra-hepatic clearance routes in mammals (Mather et al. 1989; Matot et al. 1993). In fish, nonetheless, there are no studies assessing propofol metabolism, but the increased AP within 12 h of exposure to 0.8 mg L^{-1} propofol may be due to hepatic hyperactivity in order to metabolize the drug. In keeping with the present work, some investigations indicated no changes in AST or ALT following anesthesia (Velisek et al. 2005a, 2009). Barcellos et al. (2003) reported increased AP and AST in stressed *R. quelen*, indicating these elevations as a result of the regulation of hepatic metabolism promoted by the increased cortisol levels, what was not observed in this study.

Cl^-, Na^+ and K^+

Hydromineral disturbance typically arises from stress in fish (Barton and Iwama 1991; Wendelaar Bonga 1997). At 0 mg L^{-1} propofol, the concentration of Cl^- was significantly higher at 12 compared to 6 h, what may have been due to a shift of Cl^- from intracellular to extracellular space (McDonald and Robinson 1993).

Following propofol administration, there were slight changes in Na^+ and K^+ concentrations which were related to both efflux and influx. Some authors state that anesthesia triggered a decline in Cl^- (Davis and Griffin 2004), Cl^- and Na^+ (Gressler et al. 2012b) and K^+ (Davidson et al. 2000) levels. Enhanced respiratory demands may arise from anesthesia, and, along with increased oxygen uptake, there is increased diffusive ion and water movements across the gill (Becker et al. 2012).

Conclusion

The findings of this study suggest that the use of propofol at the concentration of 0.4 mg L^{-1} is suitable for *R. quelen* transport. No major or irreversible damage was observed through the evaluated indices, what implies that the anesthetic preserved the physiology of the fish during short-, medium- and longtime exposure.

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