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Use of MS-222 (tricaine methanesulfonate) and propofol (2,6-diisopropylphenol) as anaesthetics for the tetra *Astyanax altiparanae* (Teleostei, Characidae)

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Abstract

The aim of this study was to evaluate the anaesthetic effect of MS-222 and propofol and determine their optimal concentrations for safe handling of the tetra *Astyanax altiparanae* in the laboratory. The fish were separated by length into three classes: I (1.5–5.0 cm), II (5.1–8.0 cm) and III (greater than 8.1 cm). Pilot tests were performed to evaluate the appropriate anaesthetic concentrations for inducing the five possible anaesthetic stages: I – sedation; II – light anaesthesia; III – deep anaesthesia; IV – surgical anaesthesia; and V – spinal collapse. After defining the maximum and minimum concentrations required to induce stage IV anaesthesia, the animals were exposed to five intermediate concentrations (n = 10 fish/concentration) of each anaesthetic for 15 min. The animals were then transferred to clean water to evaluate the time required for recovery. In addition, blood glucose levels were measured for class II and class III fish subjected to the previously defined ideal concentrations for each of the tested anaesthetics (n = 10 fish/treatment). Both evaluated substances are suitable to anaesthetize *A. altiparanae*. The optimal MS-222 concentration was 90 mg L^{-1}, and this result was similar for all three size classes. The optimal propofol concentrations for inducing surgical anaesthesia in the size classes I, II and III were 0.22, 0.23 and 0.27 respectively.

Keywords: fish, handling, model species, synthetic anaesthetic

Introduction

The use of fish as a model for laboratory tests has become increasingly popular in recent decades (DeTolla, Srinivas, Whitaker, Andrews, Hecker, Kane & Reimschuessel 1995; Jenkins, Bart, Bowker, Bowser, MacMillan, Nickum, Rachlin, Rose, Sorensen, Warkentine & Whitledge 2014). In certain countries, fish are the third-most commonly used animal group in scientific experiments (Overturf 2009). However, routine activities in laboratory tests and in aquaculture in general expose fish to a variety of stressors with a significant potential to affect their physical condition and well-being during activities related to biometrics, transport, gametes and blood collection, individual marking and identification and surgical procedures, among others (Barton 2000; Lima, Ribeiro, Leite & Melo 2006; Ashley 2007).

A number of substances have been used to anaesthetize fish to minimize the adverse effects of various handling procedures. Natural substances such as the essential oils of peppermint and clove have been widely used for this purpose (Taylor & Roberts 1999; Harper 2003; Palić, Herolt, Andreasen, Menzel & Roth 2006). However, the most frequently used anaesthetics are synthetic, such as quinaldine sulphate (2-methylquinoline), benzocaine (ethyl p-aminobenzoate), 2-phenoxyethanol (Inoue, Hackbarth & Moraes 2004; Velasco-Santamaría, Palacios-Ruiz & Cruz-Casallas 2008; Bertozi Júnior, Diemer, Neu, Bittencourt, Boscolo & Feiden 2014) and, more recently, propofol and MS-222 (tricaine methanesulfonate), which is the most

commonly used synthetic anaesthetic worldwide (Kreiberg 2003; Rombough 2007; Sneddon 2012; Topic Popovic, Strunjak-Perovic, Coz-Rakovac, Barisic, Jadan, Persin Berakovic & Sauerborn Klobucar 2012).

MS-222 is supplied as a white crystalline powder, and its main properties include high solubility in water and rapid induction and full recovery of animals subjected to anaesthesia (Hseu, Yeh, Chu & Ting 1998; Roberts 2009; Readman, Owen, Murrell & Knowles 2013). MS-222 has been used on freshwater and marine fish (Lemm 1993; Hseu *et al.* 1998; Roubach, Gomes & Val 2001; Sladky, Swanson, Stoskopf, Loomis & Lewbart 2001; Welker, Lim & Yildirim-Aksoy 2007; Ross & Ross 2008; Zahl, Kiessling, Samuelsen & Olsen 2010; Gholipour, Mirzargar, Soltani, Ahmadi, Abrishamifar, Bahonar & Yousefi 2011; Stockman, Weber, Kass, Pascoes & Paul-Murphy 2012; Ribeiro, de Melo, do Espirito Santo, de Souza e Silva, Santos & Luz 2013; Gressler, Riffel, Parodi, Saccol, Koakoski, DaCosta, Pavanato, Heinzmann, Caron, Schmidt, Llesuy, Barcellos & Baldisserotto 2014; Nordgreen, Tahamtani, Janczak & Horsberg 2014). Moreover, it is the only anaesthetic approved for use by the US Food and Drug Administration (FDA 1997; Carter, Woodley & Brown 2011; Delbon & Ranzani-Paiva 2012).

The first report on the anaesthetic efficacy of propofol (2,6-diisopropofol) was published in 1973 and focused on a rat experiment. Kay and Rolly (1977) were the first researchers to use propofol as an anaesthetic agent in humans. According to Miller and Eriksson (2009), propofol is currently the anaesthetic most commonly used for the induction and maintenance of anaesthesia and sedation in higher vertebrates, and it is also widely used as a human anaesthetic (Andrews, Leslie, Sessler & Bjorksten 1997).

Propofol has also been used as an anaesthetic in various aquatic organisms, such as the bamboo shark *Chiloscyllium plagiosum* (Miller, Mitchell, Heatley, Wolf, Lapuz, Lafortune & Smith 2005), bottlenose dolphin *Tursiops truncatus* (Howard, Finneran & Ridgway 2006), turtle *Caretta caretta* (MacLean, Harms & Braun-McNeill 2008), blue crab *Callinectes sapidus* (Quesada, Smith & Heard 2011) and bullfrog *Lithobates catesbeianus* (Cardoso 2012). The efficacy of propofol for safe anaesthesia in fish has been demonstrated in recent studies, such as that of Fleming, Heard, Floyd and Riggs (2003) with sturgeon *Acipenser oxyrinchus*; Peyghan, Papahn, Nadaf and Ebadi (2008) with grass carp *Ctenopharyngodon idella*; Gressler, Parodi, Riffel, Costa and Baldisserotto (2012) with catfish *Rhamdia quelen* and Valença-Silva, Braz, Barreto, Salvadori and Volpato (2014) with tilapia *Oreochromis niloticus*.

Propofol is commercially available as a whitish aqueous emulsion at a concentration of 1% containing 10% soybean oil, 2.25% glycerol and 1.2% purified egg yolk lecithin as surfactant (Massone 1999; Miller & Eriksson 2009; Meyer & Fish 2011). This compound has become popular because of its short anaesthetic action, rapid recovery, safety and minimal side effects (Sawyer 2008; Gomułka, Wlasow, Szczepkowski, Misiewicz & Ziomek 2014).

Gholipour and Ahadizadeh (2013), argued that the efficacy and safety of any anaesthetic agent may vary according to the species, life stage and environmental conditions. These authors note that only a limited number of studies have evaluated the efficacy of propofol in fish and suggest that additional studies should be performed to establish the appropriate operating conditions and comparative advantages of using propofol to induce anaesthesia relative to other anaesthetics used in fish.

The tetra *Astyanax altiparanae* is an important biological model. Nevertheless, only benzocaine was evaluated to promote anaesthesia in this species (Gimbo, Saita, Gonçalves & Takahashi 2008). This study aims to investigate the use of MS-222 and propofol as anaesthetics for the handling of the tetra *A. altiparanae* under laboratory conditions and define the recommended concentrations for anaesthesia at the stage IV for various size classes.

Materials and methods

The experiments were performed at the Laboratory for Research on Aquatic Organisms (Laboratório de Pesquisa com Organismos Aquáticos – LAPOA) of the Combined Group for Aquaculture and Environmental Studies (Grupo Integrado de Aquicultura e Estudos Ambientais – GIA), which is located in the Division of Agricultural Sciences, Federal University of Paraná (Universidade Federal do Paraná – UFPR) in Curitiba, Paraná (PR), Brazil.

Origin and maintenance of fish

The 450 *A. altiparanae* used in the experiments were obtained from CEASA (Centrais de Abastecimento

do Paraná S/A), a commercial company based in Curitiba (PR), Brazil. Upon receipt, the animals were gradually acclimated to the laboratory water temperature and pH conditions. Then, they were transferred to three circular tanks that contain internal systems for biological filtration and were filled with 800 L of water and maintained under continuous aeration. The temperature was controlled using a digital thermostat (Steck-TIC-17RGTi, Brazil) and maintained between 24.0 and 26.0°C. The animals were fed *ad libitum* twice daily (8:00 and 18:00 hours) with commercial feed containing 45% crude protein.

Biometrics

Before the experiment all fish were classified according to size through individual weighing on a digital precision balance (Shimadzu-AY220; Shimadzu, Tokyo, Japan) and measuring (total length) using a manual calliper (Vonder-200 mm/0.05 mm; Vonder, Curitiba/PR, Brazil). The animals were separated by length into three distinct classes: I: fish from 1.5 to 5.0 cm; II: fish from 5.1 to 8.0 cm; III: fish longer than 8.0 cm. The duration of this measuring procedure was 40 ± 29 s (median ± SD). Animals rested by 10 days before exposure to anaesthetics. Biometrics were also performed after exposure to anaesthetic immediately before transferring the animals to recovery tanks.

Observation system

Before testing began, fish that had been previously selected by size class were collected randomly from maintenance tanks and transferred to the observation aquariums, where they remained for 3 days before beginning the experiments. The observation system consisted of 24 rectangular glass aquariums (30 × 30 × 40 cm) filled with 25 L of water and 0.2 fish L^{-1}. The aquariums were interconnected by a water recirculation system and biological and mechanical filters and maintained under constant aeration. After the adjustment period, individuals were subjected to 8 h of fasting and then exposed to the anaesthetic treatments.

The following physical and chemical parameters of water in the recirculating systems were monitored and controlled for daily during the experiments: pH (6.99 ± 0.41; mean ± SD) using a digital pH meter (AZ-86505; AZ Instrument, Taichung City, Taiwan); temperature (25.3 ± 1.69°C); dissolved oxygen (6.18 ± 0.77 mg L^{-1}) and oxygen saturation percentage (75.46 ± 8.48) using a digital oximeter (YSI 550A; YSI, Yellow Springs, OH, USA); dissolved CO_2 (1.73 ± 0.72 mg L^{-1}) using titration with 0.02 N sodium hydroxide solution (APHA, 2005a); and nitrogen concentration in the form of total ammonia (0.16 ± 0.4 mg L^{-1}) using the indophenol method (APHA, 2005b). The samples were then read on a bench top spectrophotometer (Spectronic Instruments, Rochester, NY, USA). Water hardness was obtained by Eriochrome Black indicator followed by ethylenediaminetetraacetic acid titration (113.00 ± 33.56 mg L^{-1} of $CaCO_3$). Pilot tests evaluated and confirmed that pH and water hardness were similar when compared before, during and after experiments with anaesthetics, remaining stable.

Determination of effective anaesthetic concentrations

Stock solutions (10%) were prepared by dissolving each anaesthetic in distilled water, and the respective solutions were maintained under refrigeration in amber flasks. The stock solutions were then diluted using water from the fish maintenance system to create the test solutions, which were then placed in 1 or 2 L beakers along with the fish.

The anaesthetic effects of MS-222 and propofol on *A. altiparanae* were evaluated in two steps: (i) pilot experiments intended to determine the concentrations required to obtain all five possible anaesthetic stages, and (ii) tests to determine the concentrations required to obtain the stage IV with minimal risk to animal survival.

The pilot experiments followed the method proposed by Pedrazzani and Ostrensky (2014). A fish was placed in a glass container with the respective anaesthetic at the desired concentration. After 15 min of exposure, the fish was assessed for behavioural changes that are characteristic of each anaesthetic stage. Depending on the response, a new and higher concentration was prepared, and a new fish was exposed individually to the respective anaesthetic. This procedure was repeated successively until the dosages were sufficient to induce all anaesthetic stages: I – sedation; II – light anaesthesia; III – deep anaesthesia; IV – surgical anaesthesia and V – spinal collapse. Thus, the pilot experiments were performed using the minimum number of animals necessary to define the effective concentration ranges for each

anaesthetic. Concentrations that induced most animals to anaesthetic stage IV without causing death were considered safe.

For the experiments to determine the concentrations required for surgical anaesthesia stage IV, five concentrations of each anaesthetic, which were defined by the results of the pilot experiments, were tested on each size class (Table 1).

Class I and II fish were anesthetized in 1 L beakers, class III fish were anesthetized in 2 L beakers (n = 10 fish/concentration/anaesthetic), and the animals were individually immersed in the test solutions for 15 min (anaesthetic induction phase). Another group of 10 fish were subjected to the same procedure for the same period in water that did not include anaesthesia (control).

During the induction phase, the times required for the fish to reach the desired anaesthetic stages were timed and recorded. The animals were then sexed based on the dimorphic roughness that occurs on the anal fin of males and is absent in females (Andrade, Menin & Ribeiro 1984; Navarro, Silva, Ribeiro-Filho, Calado, Rezende, Silva & Santos 2006). Next, the fish were transferred to beakers containing water without anaesthesia (recovery phase). The animals were considered recovered when they returned to the upright position and began to swim regularly. The time required for recovery was timed and recorded.

After the phases of induction and anaesthetic recovery, the fish were transferred to the maintenance aquariums and monitored for another 48 h for possible mortality, and their feeding behaviour was recorded.

Blood collection and analysis of blood glucose levels

Class II and class III fish were individually subjected to the previously defined optimal concentrations for each of the tested anaesthetics (n = 10/class/anaesthetic). The control fish were subjected to the same handling procedure but were not exposed to any exogenous substance. The anaesthesia induction procedure was similar to the procedure described above. After this period, the fish were subjected to venipuncture in the caudal region using 3 mL syringes and 0.55 × 20 mm needles to collect approximately 0.5 mL blood. Blood glucose levels were assessed using a digital glucometer (FreeStyle Lite®; Abbott Laboratories, North Chicago, IL, USA) and analytical strips (OneTouch Select Life Scan Inc., Milpitas, CA, USA). Class I fish had insufficient blood volume to obtain samples for analysis; therefore, they were not analysed. Only three individuals of class II and no fish of class III died during blood collection. The alive animals were then transferred to a observation tank, where they were monitored for 48 h.

Table 1 Concentrations of MS-222 and propofol evaluated for their anaesthetic effect on the distinct size classes of the tetra *Astyanax altiparanae*

Compound (mg L^{-1})	Class		
	I	II	III
MS-222	70; 75; 80; 85; 90	50; 60; 70; 80; 90	50; 60; 70; 80; 90
Propofol	0.21; 0.22; 0.23; 0.24; 0.25	0.21; 0.22; 0.23; 0.24; 0.25	0.24; 0.25; 0.26; 0.27; 0.28

Statistical analysis

A preliminary analysis of data normality was performed using the Shapiro–Wilk test. As the data did not fit the normal Gaussian curve, the anaesthetic induction and recovery times for the tested compounds were compared statistically using the Mann–Whitney and Kruskal–Wallis tests. All of the tests were performed using a 95% confidence interval with the software Statsoft Statistica™ version 10.0 (Statsoft Inc., Tulsa, OK, USA).

Results

Concentrations and anaesthetic effects

Even the lowest tested concentrations of both MS-222 and propofol were sufficient to induce anaesthetic stages I and II in all individuals. In all cases, the concentrations that enabled some or all of the organisms subjected to the tested compounds to reach anaesthetic stages III and IV were identified. In class III, none of the fish reached anaesthetic stage V using propofol. In the other classes, at least one animal reached this stage but never more than 30% of the tested specimens. None of the fish died during the experiment (Table 2). Behavioural changes were not observed in the treatment without anaesthetic. None of the fish of any size class died within 48 h after the anaesthetic recovery period.

Influence of individual weight on anaesthetic induction and recovery times

Anaesthetic induction and recovery times as a function of individual weight and tested concentrations are shown in Figure 1. Tests with MS-222 revealed that the weight of the animals and tested concentrations had little effect on the time of anaesthetic induction to the surgical stage. Anaesthetic recovery time was not affected by the weight of the animals; however, there was a clear influence of the tested concentration on animal recovery time. In general, two groups were observed: one group involved concentrations of less than 70 mg L^{-1} and had a shorter recovery time; and the other group involved concentrations between 70 and 90 mg L^{-1} and had a twofold recovery time.

For propofol, a minor influence of concentration and greater influence of individual weight were observed for the time required to reach the surgical stage, with individuals heavier than 25 g having a shorter induction time. However, the pattern was reversed for anaesthetic recovery time, with the weight of the fish having little influence on the recovery time, which was heavily influenced by the concentration of propofol.

There was no significant correlation between anaesthetic induction and recovery times for fish submitted to either MS-222 ($P = 0.16$) or propofol ($P = 0.59$).

Optimal concentrations of each anaesthetic

Only the anaesthetic concentrations that achieved all of the following established criteria with fish of each size class were selected and comparatively evaluated: (i) most tested individuals should reach the stage IV; (ii) no more than 30% of tested individuals could reach the anaesthetic stage V. Of the

Table 2 Concentrations and anaesthetic effects achieved with *Astyanax altiparanae* in size classes I (1.5–5.0 cm), II (5.1–8.0 cm) and III (longer than 8.0 cm) and the respective number of individuals that reached each anaesthetic stage

Anaesthetic	Size class	Concentration (mg L^{-1})	Anaesthetic stage					Deaths
			I	II	III	IV	V	
MS-222	I	70	10	10	0	0	0	0
		75	10	10	1	0	0	0
		80	10	10	3	0	0	0
		85	10	10	4	1	0	0
		90	10	10	10	7	1	0
	II	50	10	10	0	0	0	0
		60	10	10	3	0	0	0
		70	10	10	2	0	0	0
		80	10	10	8	3	0	0
		90	10	10	10	7	1	0
	III	50	10	10	0	0	0	0
		60	10	10	1	0	0	0
		70	10	10	4	0	0	0
		80	10	10	10	9	0	0
		90	10	10	10	10	1	0
PROPOFOL	I	0.21	10	10	10	10	0	0
		0.22	10	10	10	9	0	0
		0.23	10	10	10	10	0	0
		0.24	10	10	10	10	0	0
		0.25	10	10	10	10	2	0
	II	0.21	10	10	8	7	0	0
		0.22	10	10	9	9	0	0
		0.23	10	10	9	9	0	0
		0.24	10	10	9	8	0	0
		0.25	10	10	10	10	3	0
	III	0.24	10	10	0	0	0	0
		0.25	10	10	3	0	0	0
		0.26	10	10	9	9	0	0
		0.27	10	10	10	7	0	0
		0.28	10	10	10	9	0	0

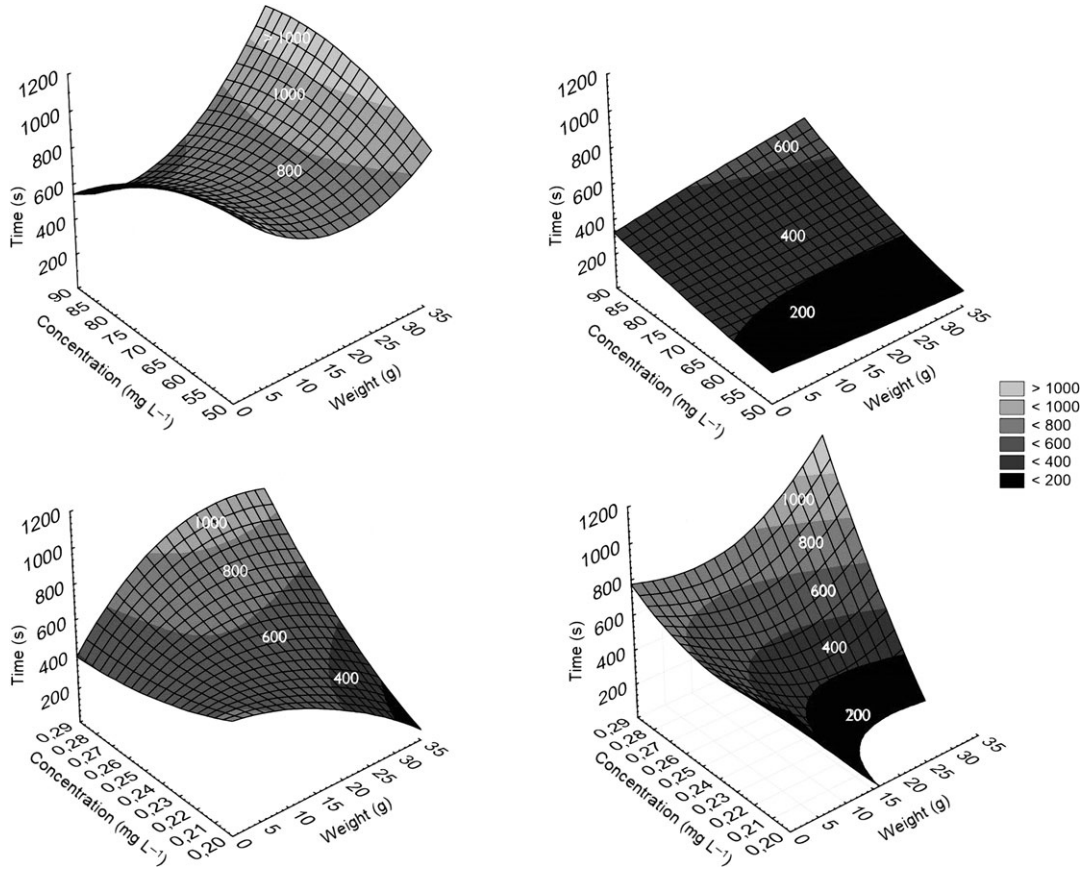

Figure 1 Time required for anaesthetic induction to the surgical stage (left) and anaesthetic recovery time (right) relative to the concentration and weight of *Astyanax altiparanae* subjected to MS-222 (top) and propofol (bottom).

five tested concentrations, these prerequisites were met at MS-222 concentrations ranging from 80 to 90 mg L^{-1} depending on the fish size class. For propofol, the prerequisites were met at concentrations ranging between 0.21 and 0.28 mg L^{-1}. Class I fish exposed to MS-222 had shorter anaesthetic induction times and longer recovery times at a concentration of 90 mg L^{-1} compared with 85 mg L^{-1}. For propofol, differences were observed between the effective concentrations only for the time of anaesthesia induction ($P < 0.05$; Fig. 2).

A similar pattern was observed for class II fish; however, differences ($P > 0.05$) were not observed between the times required for induction of fish exposed to propofol (Fig. 3). For class III fish, there were no significant differences ($P > 0.05$) in the anaesthetic induction or recovery times for any of the effective concentrations of each anaesthetic (Fig. 4).

The recommended concentrations of each anaesthetic for the distinct size classes of *A. altiparanae* were then established based on these results. The lowest possible concentration that could obtain the desired effect was always used. The expected time to reach the stage IV of anaesthesia and recovery for each of the recommended concentrations are presented in Table 3.

Blood glucose levels

No significant differences were observed for blood glucose levels or according to the sex of the individuals in size classes II and III that were subjected to the different anaesthetic compounds at previously defined optimal concentrations (Table 4).

Discussion

Induction should occur quickly in fish, preferably within 3–5 min, to minimize hyperactivity reactions or stress (Noga 1996; Ross & Ross 2008). Recovery should also be quick, preferably within

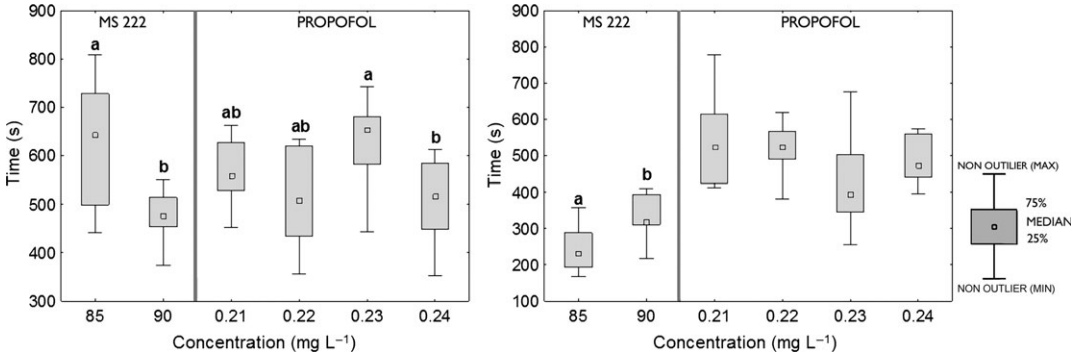

Figure 2 Anaesthetic induction (left) and recovery (right) times for *Astyanax altiparanae* of size class I (1.5–5.0 cm), which were subjected to MS-222 and propofol and reached the surgical stage of anaesthesia. Different letters indicate significant differences relative to the induction and recovery times for the tested concentrations of the same anaesthetic.

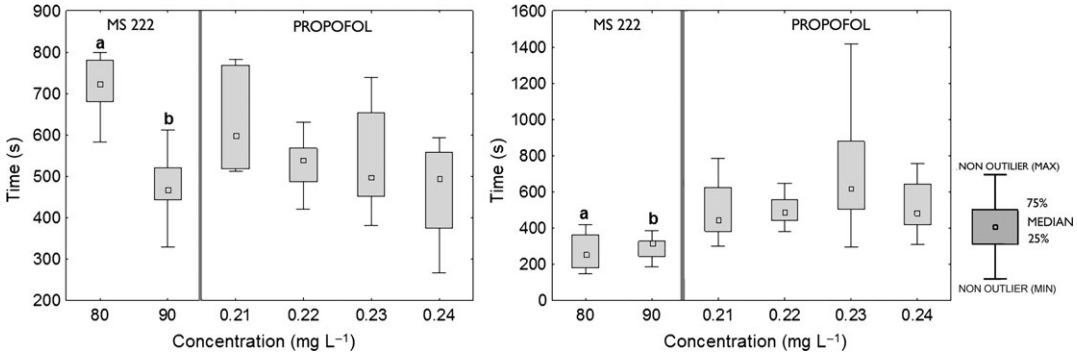

Figure 3 Anaesthetic induction (left) and recovery (right) times for *Astyanax altiparanae* of size class II (5.1–8.0 cm) subjected to MS-222 and propofol that reached the surgical stage of anaesthesia. Different letters indicate significant differences, when comparing the induction and recovery times for the tested concentrations of the same anaesthetic.

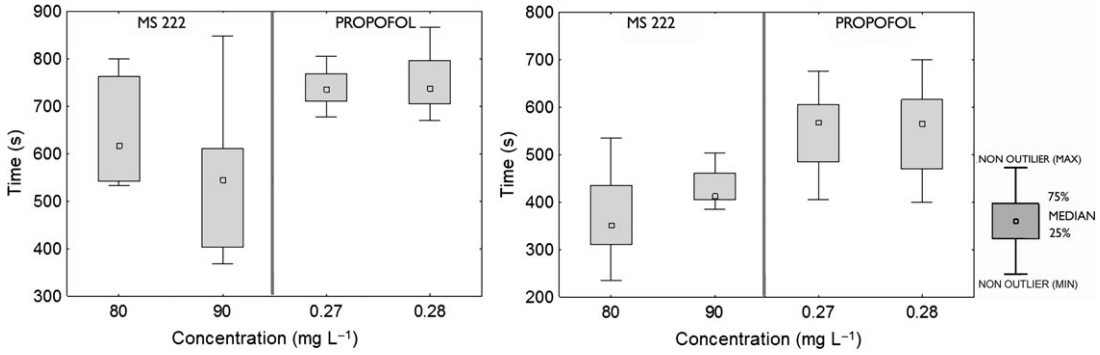

Figure 4 Anaesthetic induction (left) and recovery (right) times for *Astyanax altiparanae* of size class III (>8.1 cm) subjected to MS-222 and propofol that reached the surgical stage of anaesthesia. Different letters indicate significant differences relative to the induction and recovery times for the tested concentrations of the same anaesthetic.

5–10 min after the animals are transferred to water free of anaesthetic (Marking & Meyer 1985; Roubach & Gomes 2001).

The results obtained here suggest that the same concentration of MS-222 (90 mg L^{-1}) can be used as an anaesthetic for *A. altiparanae* regardless of

Table 3 Optimal concentrations and expected induction and recovery times (s) (median ± 50%) for size classes I, II and III of *Astyanax altiparanae* subjected to MS-222 and propofol

Anaesthetic	Size class	Concentration (mg L^{-1})	Expected induction time (s)	Expected recovery time (s)
MS-222	I (1.5–5 cm)	90	476 (454–514)	320 (311–394)
	II (5.1–8.0 cm)	90	469 (443–521)	313 (243–325)
	III (>8.1 cm)	90	545.5 (403–611)	413.5 (405.5–460)
Propofol	I (1.5–5 cm)	0.22	508 (434–620.5)	523 (490–566)
	II (5.1–8.0 cm)	0.23	497 (452–653)	617 (501–877)
	III (>8.1 cm)	0.27	737 (710–767)	568 (485–605)

Table 4 Mean blood glucose level of the tetra *Astyanax altiparanae* of different sexes and size classes (II: 5.1–8.0 cm; and III: >8.1 cm) subjected to MS-222 and propofol at the optimal concentrations for each size class

Anaesthetic	Parameter	Variable	*n*	Blood glucose (mg dL^{-1})	*P**
MS-222	Sex	F	11	53.7 (± 16.2)†	0.32
		M	9	69.8 (± 19.4)	
	Class	II	10	71.5 (± 22.6)	0.08
		III	10	50.4 (± 15.9)	
Propofol	Sex	F	11	65.8 (± 20.8)	0.05
		M	9	50.4 (± 16.8)	
	Class	II	10	65.9 (± 22.0)	0.08
		III	10	61.9 (± 16.4)	
Control	Sex	F	13	59.6 (± 16.5)	0.93
		M	7	63.1 (± 23.9)	
	Class	II	10	66.6 (± 21.1)	0.96
		III	10	55.1 (± 17.4)	
Analysis	*P*				
Anaesthetics	0.95				
Sex	0.09				
Class	0.32				

*Probability calculated using the non-parametric Kruskal–Wallis test.
†Values in brackets represent the standard error of the respective means.

animal size class. This value is consistent with recommendations for *Pagellus bogaraveo* of 70 mg L^{-1} (Maricchiolo & Genovese 2011); *Brycon cephalus* (Roubach *et al.* 2001) and *Puntius denisonii* of 100 mg L^{-1} (Mercy, Malika & Sajan 2013).

Roubach, Gomes, Fonseca and Val (2005) and Zahl, Kiessling, Samuelsen and Hansen (2011) postulated that because smaller fish have less gill surface, they should require lower anaesthetic concentrations. This trend was observed in this study only for the animals of size classes I and III that were exposed to propofol. However, the trend is not sufficient to explain the differences between the propofol concentrations (0.22, 0.23 and 0.27 mg L^{-1}, for size classes I, II and III respectively) recommended here for *A. altiparanae* and concentrations (2.5 to 6.99 mg L^{-1}) recommended for *Rhamdia quelen* (Gressler *et al.* 2012) and *Carassius auratus* (Gholipour & Ahadizadeh 2013). The specimens used in all these studies were of similar size; therefore, it indicates that the difference is species specific.

It is important to note that in this study, the concentrations recommended for both MS-222 and propofol produced the desired anaesthetic effects and presented a wide safety margin, because none of the animals died from exposure to those concentrations or during the subsequent 48 h.

According to Park, Hur, Im, Seol, Lee and Park (2008), the exposure time (in addition to the concentration) is essential for anaesthetic effectiveness because prolonged exposure may increase the mortality rates in fish. In this study, MS-222 yielded an induction time to the stage IV of anaesthesia of 5.6, which is 26.0% lower than induction time by propofol. The difference was even greater for anaesthetic recovery time and ranged from 27.2% to 49.2% depending on the size class. This indicates a clear advantage of MS-222 over

propofol, especially in cases where animal handling should be performed as quickly as possible.

However, in specific cases, such as for procedures that require longer anaesthesia time, which occur in induced reproduction or surgery, a longer period of anaesthesia may be beneficial and necessary (Prince & Powell 2000). Thus, propofol is potentially more suitable for such cases.

Another advantage of propofol over MS-222 is that it can be used in injectable form, which was described by Fleming *et al.* (2003), Peyghan *et al.* (2008) and Gomułka *et al.* (2014). However, because the injectable form requires physical restraint of the animal and can cause pain, possible injuries and stress, this advantage is relative and may only be viable for larger fish.

The evaluation of different blood parameters can be an important tool in determining optimal concentrations of various anaesthetics and may indicate the stress level of fish during handling (Feng, Zhuang, Zhang, Kynard, Shi, Duan, Liu & Huang 2011). However, the difficulty of obtaining significant amounts of blood from individuals of *A. altiparanae*, especially those in class I, limited the blood parameter analysis to glucose.

Blood glucose has become a commonly analysed parameter in studies of fish anaesthesia. Roubach *et al.* (2001), observed changes in the glycaemic pattern of juvenile Red-tailed Brycon (*Brycon cephalus*) exposed to MS-222 concentrations of 200 and 300 mg L^{-1}. Welker *et al.* (2007), subjected *Ictalurus punctatus* to a twofold higher MS-222 concentration than what was used in this study, and the fish also exhibited changes in blood glucose levels after exposure. Those studies revealed that high MS-222 concentrations can cause stress in animals and consequently alter the blood glucose level. However, the increase in glucose levels resulting from exposure to anaesthetic agents can also be described as a result of greater release of catecholamines into the bloodstream because of hypoxia caused by suppression of fish respiration during anaesthesia or as an adaptive response to the stressor (Iwama, McGeer & Pawluk 1989; Fabbri, Capuzzo & Moon 1998; Pankhurst 2011).

Gressler, Sutili, DaCosta, Parodi, DaSilva, Koakoski, Barcellos and Baldisserotto (2014), subjected juvenile catfish *Rhamdia quelen* to concentrations of 0.4 and 0.8 mg L^{-1} of propofol, which are relatively close to those used in this study, and found no differences in blood glucose levels. However, Gomułka *et al.* (2014) evaluated the haematological parameters of individuals of European whitefish (*Coregonus* sp.) that were anesthetized with 5 mg L^{-1} of propofol and concluded that there were differences in glucose levels after treatments. Those researchers claimed that procedures that increase stress in the fish resulted in higher blood glucose and consequently gluconeogenesis; however, they noted that it is impossible to determine the intensity or type of handling that significantly promoted such changes. According to Takahashi, de Abreu, Biller and Urbinati (2008), factors such as fish size, sex, and species and the type, intensity and severity of the stressor may also contribute to changes in circulating blood glucose levels. However, such factors must be further analysed before conclusions can be drawn regarding the action of propofol on the blood homoeostasis of fish anesthetized with this agent.

In this study, however, all of the animals were exposed to the same handling conditions during testing and no differences between the sexes or size classes were observed in terms of blood glucose levels for any of the anaesthetics. This suggests that both MS-222 and propofol provide results that are satisfactory and appropriate for anaesthesia in *A. altiparanae*. In this case, the choice of product will depend on the methods by which the animal will be handled and should consider the characteristics of the anaesthetic induction and recovery periods provided by each anaesthetic as well as factors such as cost and ease of procuring the product.

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