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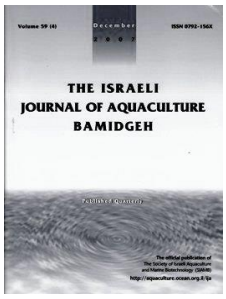


# Efficacy of Tricaine Methanesulfonate (MS-222) as an Anesthetic Agent for Short-term Anesthesia in Juvenile Yellow Perch (*Perca flavescens*)

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**Keywords:** Yellow perch; Anesthesia; MS-222

**Abstract**

The present study was conducted to assess an optimal dose of tricaine methansulfonate (MS-222) for rapid anesthesia and recovery in juvenile Yellow perch *Perca flavescens*. Yellow perch (≈7.0 g/fish) exposed to increasing concentrations of MS-222 (100, 150, 200, 250 and 300 mg/L) showed significant ($P<0.05$) decreased anesthetic induction and increased recovery time with increasing MS-222 dose. Among the doses tested, the 250 mg/L MS-222 dose showed the shortest anesthetic induction and recovery times. Blood glucose and hematocrit levels were significantly ($P<0.05$) lower in juvenile yellow perch exposed to the 250 mg/L MS-222 dose than the other MS-222 doses tested. Juvenile yellow perch (about 9.7 g/fish) exposed to 250/mg MS-222 combined with bicarbonate buffer at three ratios (w/w: 1:0.5, 1:1 and 1:2) showed significantly ($P<0.05$) reduced anesthesia induction and recovery times. The shortest times of anesthesia induction and recover were observed in treatments using the 1:1 MS-222 to buffer ratio. Analysis on serum biochemical parameters (e.g., osmolality, glucose, albumin, calcium, alanine transaminase, total protein and calcium) showed that use of sodium bicarbonate with MS-222 did not have any significant effect on the above measurements compared with those determined in fish exposed to MS-222 only. Anesthesia induction time and recovery time of yellow perch were significantly affected by body sizes. These results suggest that a brief exposure to a 250 mg/L MS-222: sodium bicarbonate dose may be optimal for minimizing handling disturbance in juvenile yellow perch.

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## Introduction

Anesthetics are an integral part of farmed fish production and experimental protocols as they allow fish to be handled easily during procedures, however, they are known to induce a stress response during procedures including transport, grading, sorting, tagging, artificial reproduction procedures, and surgery, etc. There are several anesthetic agents that have been used in research and routine aquaculture procedures to immobilize fish and minimize their stress responses (Neiffer and Stamper, 2009). Among the many types of anesthetics, tricaine methanesulphonate (MS-222) is the most commonly used anesthetic and the only one approved by the U.S. Food and Drug Administration (1997) for use on aquatic organisms. MS-222 is absorbed across the gills (and the skin in some species), biotransformed in the liver and probably kidney, and cleared primarily through the gills, with additional metabolites eliminated in urine and bile (Neiffer and Stamper 2009). An investigation in Nile tilapia *Oreochromis niloticus* has demonstrated that MS-222 did not induce primary DNA damage in Nile tilapia under both *in vivo* and *in vitro* conditions (Barreto et al., 2007). In terms of genotoxicity, the use of this important anesthetic in aquaculture can be considered safe for use.

In recent years, more attention has been given to establishing optimal doses for the use of various anesthetics in finfish species. It is well known that responses to the same anesthetic can vary considerably within and among various teleost species and it is inappropriate to extrapolate optimal anesthetic concentrations for different species (Husen and Sharma 2016). For example, inappropriate concentrations of an anesthetic may lead to adverse effects such as stress and its related physiological hazards to a particular fish species, or even death. Therefore, characterization of the effective dose for a specific anesthetic and a specific species, is a necessary practice (Chambel et al., 2015).

An important body of information is lacking in that there is no published study available that defines any recommended MS-222 concentrations for use in yellow perch *Perca flavescens*. Yellow perch is an ecologically- and commercially-important fish species in North America, and of great potential value to the aquaculture industry in the Midwestern United States and Canada (Malison, 2000;Brown et al., 2009). The supply of yellow perch from the Great Lakes has decreased, but demand for perch remains high (Malison, 2000; Marsden and Robillard 2004). One impediment to yellow perch culture and management has been limited understanding of the species regarding basic physiological parameters in response to handling and disturbances. Yellow perch is known to display high sensitivity to common management stressors (including netting, transportation, and weighing). We hypothesize that application of an optimal dose of MS-222 will benefit yellow perch in recovering from handling stress. Thus, the objective of this study was to assess an optimal dose of MS-222 as a means of minimizing handling stress on juvenile yellow perch. Efficacy of the anesthetic agent MS-222 was investigated for juvenile yellow perch, based on its effect on time to anesthesia status, recovery from anesthesia, and blood biochemical parameters.

## Materials and Methods

*Experimental animals and husbandry*

Yellow perch juveniles were produced at the School of Freshwater Science, University of Wisconsin-Milwaukee (UWM), Wisconsin, USA. The fish were kept in circular fiberglass tanks with bottom center drains (60 cm diameter, 60 cm height) and about 450 L water volume supplied with 6 L/min of degassed and dechlorinated municipal water. Water temperature and dissolved oxygen were continuously monitored by a computer (Water Management Technologies, Baton Rouge, LA) and maintained at 22 ± 0.5°C. Water pH was 8.0 ± 0.1; ammonia nitrogen was less than 0.01 mg/L, and dissolved oxygen maintained > 7 mg/L, respectively. The same water source and water quality were maintained for different tests conducted in this study. Fish were maintained on a 12L:12D photoperiod. Fish were fed a commercial diet (EXTR 450 1.6-mm sinking pellet, Rangen Inc., Idaho, USA). The proximate composition of the commercial feed was: moisture 6.5%, protein 44.5%, crude fat 15.0%, crude fiber 5.0%, and ash 10.0%. Fish were fed twice daily (08:30 and 16:00) at about 3% feeding rate. All fish were fasted for 24 h prior to the start of each trial. Fish maintenance and treatment were conducted in

accordance with an animal protocol approved by the Animal Care and Use Committee, University of Wisconsin, Milwaukee, USA.

*Anesthetic agent*

MS-222 (Tricaine-S, Western Chemical, Inc., WA, USA) was measured in a milligram-scale by digital balance (Fisher Science Education ALF104, Thermo Fisher Scientific Inc., MA, USA). Different doses of MS-222 were added directly into fresh aerated municipal water to achieve desired test concentrations. MS-222 has been reported to reduce pH when dissolved in and thus it is recommended to have MS-222 buffered with sodium bicarbonate ($NaHCO_3$) to maintain pH (Popovic et al. 2012).

*Procedure for fish anesthesia and recovery*

Prior to each test, approximately 20 fish were transferred into a white plastic bucket (20 L) with holes drilled at the bottom. The bucket was immersed in the maintenance tank to allow the fish acclimate to the environment for 30 min. Individual fish were transferred from the acclimation bucket to a separated testing bucket containing aerated anesthetic solution. The bucket used for acclimation and testing were the same color and in the same size (diameter 28 cm and a height of 35 cm). The time period for each fish to reach anesthetic stage was recorded when the fish were transferred into the testing bucket filled with 5 L of MS-222 solution with or without buffer. Following anesthesia (criteria outlined below), fish were immediately transferred into a recovery tank. The recovery process was observed in a circular fiberglass tank (45 cm diameter, 50 cm height, *ca.* 30 L water volume) supplied with 1.0 L /min of water with constant aeration. The water temperature was 22$^{o}$ C in the testing bucket and recovery tank.

*Criteria for Determination of Anesthesia and experimental approach*

The time for anesthesia induction was recorded from fish exposure to anesthetic water solution; effective anesthesia period was defined as the time when an experimental fish was placed in the testing bucket until the time when its opercular movement ceased (Dong et al., 2017). When an experimental fish reached this stage of anesthetization, it was immediately removed from the testing bucket and placed into the recovery tank. The recovery time was the difference from period when an anesthetized fish was placed into a recovery tank until it fully recovered from anesthetization with normal swimming and maintenance of full equilibrium. The anesthesia and recovery times were recorded for each individual subject using a stopwatch. The optimal concentration of MS-222 was defined as the minimum concentration that leads to the shortest times to anesthesia, and recovery from anesthesia.

*Trial 1: Effect of different MS-222 doses on anesthesia induction and recovery times*

To evaluate the effects of different MS-222 concentrations on yellow perch juveniles, one hundred fish with similar body size (6.84±1.08 g/fish, 8.82±0.49 cm/fish; mean±SEM) were randomly selected and gently transferred from the acclimation buckets to the testing buckets by dip netting. Then, 20 fish were individually exposed to one of the five MS-222 concentrations: 100, 150, 200, 250 and 300 mg/L and induction and recovery times were measured and recorded for each fish. Following full recovery, fish were exposed to an overdose of MS-222 and netted to determine body weight and body length.

*Trial 2: Effect of different MS-222 doses on blood levels of glucose and hematocrit*

Ninety fish with similar body size (11.13±1.84 g/fish, 10.30±0.64 cm/fish; mean±SEM) were used in this test. Fish were transferred following the same protocol described in Trial 1. Fifteen fish were individually exposed to one of the six treatments, including a control group without anesthetic, and five MS-222 concentrations as outlined for trial I. The control fish were immediately killed by decapitation and represent a zero-stress state for purposes of this study.

After the fish were euthanized, or anesthetized, the tail of fish was severed to obtain whole blood for measuring glucose and HCT levels. Whole blood glucose level was measured by using a Glucose Meter (CVS/pharmacy TM, AgaMatrix, Inc., NH, USA) and disposable test strips (CVS/Pharmacy TM advanced glucose meter test strips, AgaMatrix, Inc., NH, USA). Portable blood glucose meters have been used to measure glucose concentrations in a variety of commercially-important finfish (Trushenski et al., 2010;

Trushenski et al., 2012). To determine hematocrit (HCT) values, two micro HCT tubes for each fish (Heparinized glass, Iris Sample Processing, CA, USA) containing blood samples were centrifuged in micro-hematocrit centrifuge (Crit Spin®, Model M961-22, IRIS Sample Processing, MA, USA) for 2 min. The tubes were then placed on a micro HCT capillary tube reader (AtatApin®, IRIS Sample Processing, MA, USA) for the determination of HCT values.

*Trial 3: Effect of MS-222: buffer ratio on anesthesia induction and recovery time*

Based on trial 2, the concentration of MS-222 at 250 mg/L was optimal in terms of the combined times for anesthesia induction and recovery, as well as its effects on measured hematological parameters. To assess the effects of buffering, eighty fish with similar body size (9.64±1.37 g/fish, 9.96±0.50 cm/fish; mean±SEM) were used to evaluate the ratio of MS-222: buffer ($NaHCO_3$) on anesthesia induction and recovery times. The ratios of MS-222 (250 mg/L) to buffer were 1:0.0, 1:0.5, 1:1, and 1:2. Twenty fish per treatment were exposed to the above anesthetic:buffer combinations using the same procedures as described in trial I.

*Trial 4: Effect of buffered MS-222 and non-buffered MS-222 on biochemical parameters of serum*

This trial was conducted to compare the effects of 1:1 buffered MS-222:sodium bicarbonate (250 mg/L ) on serum biochemical parameters of yellow perch. Thirty fish (body weight 47.1±8.5g/fish, mean±SEM) were transferred to 57 L polypropylene tanks with 5 fish per tank, which were equipped with flow-through water (22 ºC). Fish were acclimated overnight to these conditions. On the following day, each treatment was randomly allocated to three replicate tanks. Fish from each tank were then transferred to a bucket containing non-buffered MS-222 solution or buffered MS-222. Blood was collected via caudal puncture of the hemal arch using non-heparinzed syringes after the fish was fully anesthetized. Blood samples were allowed to clot for 4 h on ice and then blood samples were centrifuged at 4,000 x g for 20 minutes at 4°C to collect serum samples. Serum was stored at -80°C until use. Serum biochemistry parameters were determined using the Abaxis VetScan VS2 Veterinary Chemistry Analyzer (Union City, CA, USA). For each sample, 100 microliters of serum were used to determine the following parameters using a disposable Comprehensive Diagnostic Rotor (part number #500-0038): albumin (ALB, g/L), alkaline phosphatase (ALP, U/L), alanine transaminase (ALT, U/L), total bilirubin (TBIL, μmol/L), calcium (Ca, mEq/L), inorganic phosphorous (P, mmol/L, glucose (GLU, (mmol/L), sodium $Na^+$ (mmol/L), total protein (TP, g/L), and globulin (g/L). Previous works in teleosts have demonstrated the suitability of the VS2 platform (and other point-of-care devices) for clinical chemistry use in finfish (Densmore and Panek 2013; Stoot et al., 2014; Floyd-Rump et al., 2017). Serum osmolality (mOsm/kg) was measured in triplicate using a vapor pressure osmometer (Vapro 5520, Wescor, Logan, UT, USA).

*Trial 5: Effect of body weight on anesthesia induction and recovery times*

To assess the effect of different body weight of yellow perch on anesthesia induction and recovery times with MS-222, fifty fish from three size-groups (6.42±1.11 g/fish, 12.66±2.14 g/fish, and 19.21±1.98 g/fish) of juvenile yellow perch were used. Animals were anesthetized using 250 mg/L buffered MS-222 (1:1). Experimental operations were the same as those described in trial I.

*Statistical Analyses*

All data were presented as means ± standard error of the mean (SEM). When necessary, data were power transformed to ensure normality and homogeneity of variance between groups. All the data were subjected to one-way ANOVA using SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) and presented as means ± SEM. The differences among the mean values were analyzed by Duncan's multiple-range test and the mean values were considered significantly different when $P$ value was $< 0.05$. Non-linear regression was accomplished using Excel (v. 2016, Microsoft, Redmond, WA, USA).

## Results

*Effect of MS-222 concentrations on anesthesia induction and recovery times*

As shown in Table 1, anesthesia induction time of juvenile yellow perch varied significantly ($P<0.05$) with MS-222 concentrations. Specifically, induction time decreased with the increasing anesthetic concentration up to 250 mg/L. Recovery time also varied

significantly (P<0.05) with MS-222 concentrations. The recovery time of the 200 mg/L group was the highest (P<0.05) among all concentration groups and lowest (P<0.05) in the 100 and 150 mg/L groups.

**Table 1.** Effect of different MS-222 concentrations on anesthesia induction and recovery times of juvenile yellow perch

| *Concentration (mg/L)* | *Anesthesia time (s)* | *Recovery time (s)* | *Body weight (g/fish)* | *Body length (cm/fish)* |
|---|---|---|---|---|
| 100 | 541.10 ± 36.87$^{w}$ | 75.45 ± 9.10$^{z}$ | 6.85 ± 0.71 | 8.94 ± 0.31 |
| 150 | 267.80 ± 35.35$^{x}$ | 80.20 ± 10.33$^{z}$ | 7.07 ± 1.17 | 8.72 ± 0.58 |
| 200 | 181.95 ± 26.91$^{y}$ | 103.15 ± 13.94$^{x}$ | 6.79 ± 0.94 | 8.82 ± 0.49 |
| 250 | 94.80 ± 12.68$^{z}$ | 90.60 ± 15.44$^{y}$ | 7.09 ± 1.31 | 9.02 ± 0.51 |
| 300 | 84.70 ± 17.257d$^{z}$ | 93.50 ± 18.53$^{y}$ | 6.98 ± 1.07 | 8.79 ± 0.49 |

Values (mean±SEM, n=20 per group) in the same column with different lowercase letters are significantly different (P<0.05).

Analysis based on polynomial regression showed that MS-222 at a concentration of 253 mg/L resulted in the shortest induction time ($Y= 0.0202X^2 – 10.229X + 1355.3$, $R^2 = 0.9648$) under the current testing conditions (Figure 1). Fish exposed to 250 mg/L MS-222 appeared to display the shortest combined time of anesthesia induction and recovery.

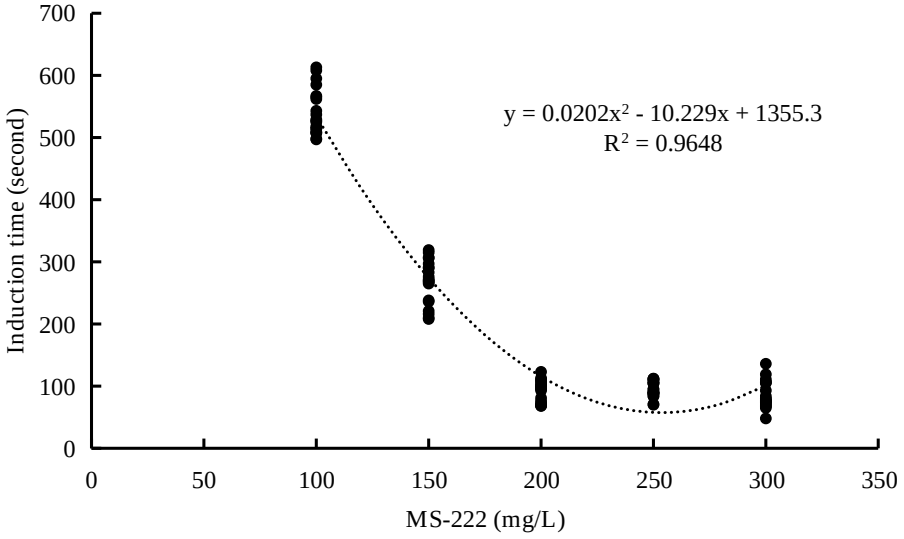


**Figure 1.** Non-linear regression analysis of dose-response effect of MS-222 anesthesia induction time shown in Table 2. Estimation of the MS-222 concentration that caused the shortest induction time to anesthesia for yellow perch juvenile. The estimated concentration of MS-222 = 253 mg/L

*Effect of MS-222 concentration on blood glucose and HCT levels*

The levels of glucose and HCT decreased with the increase of MS-222 concentrations (Table 2).

**Table 2.** Effect of different MS-222 concentrations on blood glucose and hematocrit (HCT) levels of juvenile yellow perch

| *Concentration (mg/L)* | *Glucose (mmol/L)* | *HCT (%)* | *Body weight (g/fish)* | *Body length (cm/fish)* |
|---|---|---|---|---|
| 0 | 4.9 ± 1.2$^{z}$ | 36.40 ± 3.54$^{z}$ | 10.63 ± 1.13 | 10.21 ± 0.55 |
| 100 | 14.2 ± 2.1$^{x}$ | 53.50 ± 6.09$^{w}$ | 11.14 ± 1.49 | 10.30 ± 0.51 |
| 150 | 8.9 ± 1.8$^{y}$ | 48.77 ± 3.61$^{x}$ | 11.22 ± 2.32 | 10.32 ± 0.79 |
| 200 | 4.9 ± 0.7$^{z}$ | 45.97 ± 4.10$^{xy}$ | 11.13 ± 2.30 | 10.30 ± 0.78 |
| 250 | 4.8 ± 0.7$^{z}$ | 46.57 ± 3.62$^{xy}$ | 11.39 ± 1.90 | 10.37 ± 0.65 |
| 300 | 4.7 ± 0.7$^{z}$ | 43.33 ± 4.08$^{y}$ | 11.26 ± 1.93 | 10.34 ± 0.66 |

HCT=hematocrit.

Values (mean±SEM, n = 15 per group) in the same column with different lowercase letters are significantly different (P<0.05).

Glucose levels of 100 mg/L and 150 mg/L MS-222 groups were significantly ($P<0.05$) elevated above the levels observed in the control group and the 200, 150, and 300 mg/L MS-222 groups. Glucose levels of other three highest MS-222 groups were not significantly different from one another or the control group ($P>0.05$). The lowest blood glucose level was seen in fish exposed to 262 mg/L MS-222 concentration ($Y = 0.0004X^2 – 0.2099X + 559.53$, $R^2 = 0.8752$) based on polynomial regression analysis (Figure 2). HCT levels of juvenile yellow perch were significantly ($P<0.05$) affected by the MS-222 concentrations used. HCT levels were lowest in the control, 200, 250, and 300 mg/L MS-222 concentration groups and significantly ($P<0.05$) elevated in the 100 and 150 mg/L MS-222 concentration groups.

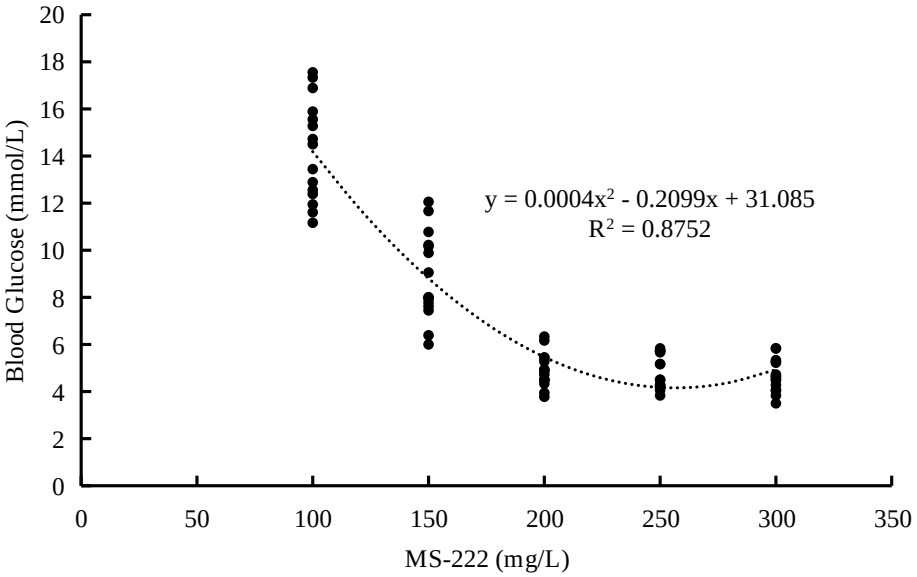


**Figure 2.** Non-linear regression analysis of dose-response effect of blood glucose levels shown in Table 2. Estimation of the MS-222 concentration that caused the lowest blood glucose in yellow perch juvenile. The estimated concentration of MS-222 = 262 mg/L.

*Effect of MS-222:buffer ratio on anesthesia induction time, recovery time, and biochemical parameters in serum*

The MS-222: buffer ($NaHCO_3$) ratio had significant effects on anesthesia and recovery times of juvenile yellow perch ($P<0.05$). The time for anesthesia induction and recovery was highest in the non-buffered MS-222 group ($P<0.05$) and times remained elevated in the 1:0.5 ratio group (Table 3). In contrast, anesthesia induction and recovery times were significantly ($P<0.05$) lower in the 1:1 and 1:2 MS-222: buffer ratio groups as compared with other groups tested.

**Table 3.** Effect of MS-222 (250 mg/L): bicarbonate buffer ratio on anesthesia and recovery times of juvenile yellow perch

| *Ratio of MS-222 to Buffer* | *Anesthesia time (s)* | *Recovery time (s)* | *Body weight (g/fish)* | *Body length (cm/fish)* |
|---|---|---|---|---|
| No buffer | 170.40 ± 29.03$^x$ | 155.75 ± 35.14$^x$ | 10.15 ± 1.29 | 10.04 ± 0.45 |
| 1:0.5 | 142.60 ± 21.02$^y$ | 129.40 ± 18.55$^y$ | 9.86 ± 1.23 | 9.84 ± 0.44 |
| 1:1 | 100.5 ± 12.26$^z$ | 100.45 ± 18.01$^z$ | 9.27 ± 1.13 | 9.93 ± 0.52 |
| 1:2 | 109.80 ± 11.75$^z$ | 110.15 ± 25.68$^z$ | 9.46 ± 1.61 | 10.06 ± 0.62 |

Values (mean±SEM, n=20 per group) in the same column with different lowercase letters are significantly different ($P<0.05$).

Furthermore, there was no significant difference in measured serum biochemical parameters of yellow perch exposed to MS-222 or buffered MS-222 (Table 4).

**Table 4.** Effect of MS-222 (250 mg/L) without and with buffer (1:1 ratio) on measurements of general biochemical analysis of serum from yellow perch juvenile

| *Items* | *MS-222 Group* | *MS-222+Buffer Group* |
|---|---|---|
| Osmolality(mOsm/kg) | 299 ± 0.8 | 298 ± 0.8 |
| Glucose(mmol/L) | 9 ± 0.5 | 8 ± 0.7 |
| Albumin(g/L) | 26 ± 0.5 | 26 ± 0.7 |
| ALP(U/L) | 91 ± 3.4 | 88 ± 3.5 |
| ALT(U/L) | 677 ± 17.0 | 677 ± 17.5 |
| TBIL(Umol/L) | 5 ± 0.1 | 5 ± 0.3 |
| Calcium(mEq/L) | 6.5 ± 0.04 | 6.5 ± 0.08 |
| P(mmol/L) | 2.7 ± 0.1 | 2.7 ± 0.1 |
| Na(mmol/L) | 135 ± 0.4 | 136 ± 0.8 |
| TP(g/L) | 50 ± 0.8 | 50 ± 1.0 |
| Globulin(g/L) | 23 ± 0.5 | 23 ± 0.6 |

ALP=alkaline phosphatase; ALT=Alanine transaminase; TBIL=total bilirubin; P=inorganic phosphorous; Na=sodium; TP= total protein.
MS-222 group, n=15 fish; MS-222 + buffer group, n = 15 fish. Values (not SEM values) are rounded to the nearest whole number. Values (mean±SEM) in the same row are not significantly different (P>0.05) between treatment groups.

*Effect of body weight on anesthesia induction and recovery times*

Yellow perch exposed to the anesthesia solution containing MS-222 and buffer at a ratio of 1:1, were shown to have increased anesthesia induction time and recovery time in response to their body weight (Table 5, P<0.05). Although recovery time increased with body weight, there was no significant difference between the 12.66±2.14 g/fish and the 19.21±1.98 g/fish groups.

**Table 5.** Effect of body weight of juvenile yellow perch on anesthesia and recovery times (1:1 ratio of MS-222: bicarbonate buffer, 250 mg/L)

| *Body weight(g/fish)* | *Anesthesia time(s)* | *Recovery time(s)* | *Body length(cm/fish)* |
|---|---|---|---|
| $6.42 \pm 1.11^{c}$ | $102.82 \pm 19.45^{z}$ | $105.42 \pm 21.72^{y}$ | $8.58 \pm 0.76^{z}$ |
| $12.66 \pm 2.14^{b}$ | $155.18 \pm 39.32^{y}$ | $123.20 \pm 30.15^{x}$ | $10.62 \pm 0.61^{y}$ |
| $19.21 \pm 1.98^{a}$ | $191.10 \pm 40.01^{x}$ | $131.06 \pm 28.51^{x}$ | $12.04 \pm 0.43^{x}$ |

Values mean±SEM, n=20 per group) in the same column with different lowercase letters are significantly different (P<0.05).

## Discussion

*Effect of MS-222 concentrations on anesthesia induction and recovery times*

In the present study, anesthesia induction time of yellow perch juveniles decreased with the increase in the concentrations of MS-222 used. This observation is in agreement with previous studies, which showed that induction time decreased inversely according to the concentration of anesthetic agent in other fish species (Pawar et al., 2011; Ghanawi et al., 2013). Longer recovery periods with increasing doses of MS-222 up to 200 mg/L were also observed in juvenile yellow perch from this study, suggesting that the fish may require extended periods to excrete the chemical for recovery. However, the recovery time tended to decrease when the yellow perch were exposed to MS-222 at higher concentration of 250 or 300 mg/L. This finding was consistent with the study of Husen and Sharma (2016) who reported that the recovery time of 100 mg/L MS-222 group was significantly longer than those of fish exposed to 75 mg/L and 125 mg/L MS-222; whereas recovery times of fish exposed to 75 and 125 mg/L were similar. This indicated that a suboptimal concentration of MS-222 could cause a longer time needed to attain full anesthesia and thus the fish remain excited from handling and become stressed. This idea is further supported by the elevated blood glucose level in the yellow perch exposed to 100 mg/L MS-222. By contrast, a higher concentration of MS-222 may anesthetize fish quickly and reduce their hyperactivity or excitement easily. Therefore, the interaction time for the fish with MS-222 would be shortened when exposed to a higher concentration of MS-222. This may partially explain the short recovery observed in fish

exposed to MS-222 at 250-300 mg/L. Similarly, a dose-independent recovery time had been documented in juvenile Senegalese Sole *Solea senegalensis* anaesthetized with MS-222 (Weber et al., 2009). Furthermore, independence of the recovery time was suggested to be related to the amount of anesthetic absorbed by the fish gill surface; therefore, after the fish attained a level of equilibrium with the anesthetic, this equilibrium is maintained until anesthetic is removed (Weyl et al., 1996).

*Effect of MS-222 concentration on blood glucose and HCT levels*

Response of blood glucose concentrations varied due to the concentration of MS-222 with elevated levels in the yellow perch exposed to 100-150 mg/L MS-222, compared with control levels, and lower in the fish exposed to 200-300 mg/L. MS-222 is thought (at the proper concentration) to block activation of the hypothalamo-pituitary-interrenal (HPI) axis, which is associated with the stress response in fish. Failure to suppress activation of the HPI axis during stress can result in a release of cortisol, which in turn causes various secondary stress responses, including increases in circulating levels of glucose and hematocrit (Popovic et al., 2012; Ostrensky et al., 2016). Furthermore, it was found that the anesthetics themselves could be a stressor and produce stress in fish even in buffered solutions (Cho and Heath 2000).

The blood glucose has been known to be a physiological variable and serves as a surrogate indicator for the stress response in teleosts (Wagner et al., 2002). Increases in blood glucose levels can be a result of catecholamine release during brief periods of handling and anesthesia in teleosts, including yellow perch (Ostrensky et al., 2016). In the present study, yellow perch exposed to higher concentrations (≥ 200 mg/L) of MS-222 were not stressed significantly because their blood glucose levels remained at the control levels. However, an increased blood glucose level was detected in the yellow perch exposed to 100-150 mg/L MS-222. This finding was similar to the observations reported for several species of marine fish exposed to 80-100 mg/L of MS-222 (Thomas and Robertson, 1991). In juvenile Chinook Salmon *Oncorhynchus tshawytscha*, anesthetization with 200 mg/L MS-222 did not prevent an early and progressive rise in blood glucose (Congleton, 2006), which is in conflict with observed results in yellow perch. This indicates that the stress response to anesthesia may not only depend upon concentration but may also be species-specific as suggested by Thomas and Robertson (1991). The normal blood glucose concentration detected in yellow perch exposed to MS-222 at a concentration ≥ 200 mg/L suggested that these concentrations of MS-222 could alleviate the stress caused by anesthesia agent or by disturbance of the fish before they reached a state of full anesthesia.

The HCT levels of yellow perch exposed to MS-222 were higher than in the control group, but the HCT tended to decrease with the increased concentrations of MS-222. The increased HCT was probably due to the occurrence of erythrocyte swelling as suggested by Soivio and co-workers (1977) who reported the increased HCT values in Rainbow Trout *Oncorhynchus mykiss* with all the anesthetics used. Similarly, a severe impact of MS-222 on hematological indices was reported in Siberian Sturgeon *Acipenser baerii* probably due to the swelling and destruction of erythrocytes caused by MS-222 (Gomulka et al., 2008). On the other hand, response of HCT and blood glucose to MS-222 treatment does not always follow a predictable pattern. MS-222 was shown to cause an increase of HCT value and hemolysis in some cyprinid fish, but their blood glucose levels (as a stress indicator) were not affected by the anesthesia (Hattingh, 1977). This is comparable to what we observed in yellow perch exposed to different concentrations of MS-222. While MS-222 is effective in reducing stress associated with confinement and handling, there are indications that anesthesia may in itself induce a stress response, to some extent, thus altering blood HCT values (Zahl et al., 2012). Consequently, it is still unclear whether the induction of HCT in the yellow perch from the current study was attributable to handling and/or to anesthesia-induced stress.

Regression analyses of the data from this study indicates that the most effective, and less stressful, concentration of MS-222 for juvenile yellow perch occurred within the range of 250-262 mg/L for this anesthetic. This is supported by the shortest anesthesia induction time, relatively short recovery period, and the normal blood glucose level (a secondary indicator of stress) detected in the fish exposed to MS-222.

*Effect of MS-222: buffer ratio on anesthesia induction time, recovery time and biochemical parameters in serum*

MS-222 is acidic and may cause acid-induced stress in fish by lowering the pH of the water (Popovic et al., 2012). It has also been reported that the use of non-buffered MS-222 may cause serious epidermal, gill and corneal damage in fish (Davis et al., 2008). To overcome this problem, buffering or neutralizing the water acidity with sodium bicarbonate has been proposed (Popovic et al., 2012). In the present study, the beneficial effect of sodium bicarbonate was shown when MS-222 was added with sodium bicarbonate at the ratio of 1:1 or 1:2, which resulted in a shorter induction and reduced recovery times than the fish exposed to non-buffered MS-222 or to a lower (1:0.5) ratio. The reduced anesthesia induction and recovery times indicated that stress might be alleviated with the buffer administration. In addition, water quality parameters, such as temperature, pH, salinity, and hardness, can affect metabolic rate, acid-base regulation, osmoregulation and ion regulation. These factors can also influence the pharmacodynamics or efficiency of MS-222. Therefore, the buffering protocols for different fish species, or fish being treated in different water quality, should be optimized for experimental and husbandry purposes (Carter et al., 2011). On the other hand, at the optimal concentration of MS222 (250 mg/L) no difference in serum biochemical parameters (including the glucose level) were measured in fish anesthetized with or without buffer. This suggests that the secondary stress response was not influenced by the presence of sodium bicarbonate buffer under the current testing conditions.

*Effect of body weight on anesthesia induction and recovery times*

The importance of fish body size for response to anesthesia is limited and available information is inconsistent. Results from the current study demonstrated that anesthesia induction and recovery times corresponded to the size of yellow perch examined. This finding is similar to results presented by Dong et al. (2017), who reported that anesthesia time significantly increased with the increased body weight of largemouth Bronze Gudgeon *Coreius guichenoti*. Similar observations have been reported in two other finfish species (Houston et al., 1976; Zahl et al., 2012). Solubilized anesthetics enter the bloodstream through the gills, skin, and accessory respiratory organs (Neiffer and Stamper 2009). Thus, effective concentration is related to the gill area and body weight ratio, which can vary considerably among fish species. Small fish generally have a large surface area, thinner skin, and higher metabolic rates than larger fish. Thus, uptake and excretion of MS-222 through the skin for a smaller fish is more efficient, leading to fast anesthesia and recovery in a smaller fish (Neiffer and Stamper 2009), whereas larger individuals (lower metabolic rate and less surface area) generally require a greater concentration of anesthetic and extended time of recovery than smaller individuals (Weyl et al., 1996). These hypotheses can partly explain the different effects of body weight on both induction and recovery times of juvenile yellow perch observed in this study.

Under the specified experimental conditions used in this study, we observed anesthesia induction time to decrease with increasing concentrations of MS-222 in juvenile yellow perch. However, recovery time was not affected a manner similar to anesthesia time with increasing anesthetic concentration. An adequate MS-222 concentration could alleviate handling induced stress, based on blood glucose and hematocrit responses observed in this study. Furthermore, addition of sodium bicarbonate, at a ratio of 1:1, does not seem to influence the general serum clinical biochemistry measurements under the current testing conditions. For short periods of anesthesia, it is recommended that a ratio of MS-222 to buffer ($NaHCO_3$) at 1:1 should be used, and a dose of 250 mg/L MS-222 may be applied to shorten the anesthesia induction and recovery times in juvenile yellow perch. However, since we observed an effect body weight on anesthesia and recovery times, individual practitioners and researchers should verify effectiveness of MS-222 : buffer combinations for the size of fish being anesthetized.

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**References**

**Barreto R. E., Gontijo M.M.C A., Alves-de-Lima R.O., Raymundi V.C., Pinhal D., Reyes V.A.V., Volpato G.L., and Salvadori D.M.F.,** 2007. MS222 does not induce primary DNA damage in fish. *Aquacult. Int.,* 15(2):163-168.

**Brown T.G., Runciman B., Bradford M.J., Pollard S.,** 2009. A biological synopsis of yellow perch (*Perca flavescens*). Canadian Manuscript Report of Fisheries and Aquatic Sciences, 2883:28. Available at: https://pdfs.semanticscholar.org/a2b8/a5270b79294bdcaf74b4c4df4dddd95a3a03.pdf

**Carter K.M., Woodley C.M., Brown R.S.,** 2011. A review of tricaine methanesulfonate for anesthesia of fish. *Rev. Fish Biol. Fisher.*, 21(1):51-59.

**Chambel J., Pinho R., Sousa R., Ferreira T., Baptista T., Severiano V., Mendes S., Pedrosa R.,** 2015. The efficacy of MS-222 as anaesthetic agent in four freshwater aquarium fish species. *Aquac. Res*., 46(7):1582-1589.

**Cho G.K., Heath D.D.,** 2000. Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquac. Res*., 31(6):537-546.

**Congleton J.L.,** 2010. Stability of some commonly measured blood-chemistry variables in juvenile salmonids exposed to a lethal dose of the anaesthetic MS-222. *Aquac. Res*., 37(11), 1146-1149.

**Davis M.W., Stephenson J., Noga E.J.,** 2008. The effect of tricaine on use of the fluorescein test for detecting skin and corneal ulcers in fish. *J. Aquat. Anim. Health*, 20(2):86-95.

**Densmore C.L., Panek F.M.,** 2013. Effects of depletion sampling by standard three-pass pulsed DC electrofishing on blood chemistry parameters of fishes from Appalachian streams. *N. Am. J. Manag.*, 33(2):298-306.

**Dong C., Pan L., He D., Xie J.J., Tang H.Y., Yang Z., Wang X.M., Yang S.R.,** 2017. The efficacy of MS-222 as anesthetic agent in largemouth bronze gudgeon *Coreius guichenoti*. *N. Am. J. Aquacult.,*79(1):123-127.

**Floyd-Rump T.P., Horstmann-Dehn L.A., Atkinson S., Skaugstad C.,** 2017. Effect of *Ichthyophonus* on blood plasma chemistry of spawning Chinook salmon and their resulting offspring in a Yukon River tributary. *Dis. Aquat. Organ.*, 122(3):223-236.

**Ghanawi J., Monzer S., Saoud I.P.,** 2013. Anaesthetic efficacy of clove oil, benzocaine, 2-phenoxyethanol and tricaine methanesulfonate in juvenile marbled spinefoot (S*iganus rivulatus*). *Aquac. Res*., 44(3):359-366.

**Gomulka P., Własow T., Velíšek J., Svobodová Z., Chmielinska E.,** 2008. Effects of eugenol and ms-222 anaesthesia on siberian sturgeon acipenser baerii brandt. *Acta Veterinaria Brno*, 77(3):447-453.

**Hattingh J.,** 1977.The effect of tricaine methanesulphonate (MS-222) on the microhaematocrit of fish blood. *J. Fish Biol.*, 10:453-455.

**Husen M., Sharma S.,** 2016. Anaesthetic efficacy of MS-222 and AQUI-S® in advanced size fry of rohu, *Labeo rohita*, (Hamilton-Buchanan). *Aquac. Res*., 47(8):2496-2505.

**Malison J.A.** 2000. A white paper on the status and needs of yellow perch aquaculture in the North Central Region. Pages 17. In North Central Regional Aquaculture Center, Michigan State University, East Lansing, MI. Available at: https://www.ncrac.org/files/biblio/YellowPerchWhitePaper112103.pdf

**Marsden J. E., Robillard S.R.,** 2004. Decline of yellow perch in southwestern Lake Michigan, 1987-1997. *N. Am. J. Manag.*, 24(3):952-966.

**Neiffer D.L., Stamper M.A.,** 2009. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR J.,* 50(4):343-360.

**Ostrensky A., Pedrazzani A.S., Vicente A.L.,** 2016. Use of MS-222 (tricaine methanesulfonate) and propofol (2, 6-diisopropylphenol) as anaesthetics for the tetra *Astyanax altiparanae* (Teleostei, Characidae). *Aquac. Res*., 47(11):3477-3488.

**Pawar H.B., Sanaye S.V., Sreepada R.A., Harish V., Suryavanshi U., Ansari Z.A.,** 2011. Comparative efficacy of four anaesthetic agents in the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852). *Aquaculture*, 311(1):155-161.

**Popovic N.T., Strunjak-Perovic I., Coz-Rakovac R., Barisic J., Jadan M., Persin Berakovic A., Sauerborn Klobucar R.,** 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *J. Appl. Ichthyol.*, 28(4):553-564.

**Soivio A., Nyholm K., Huhti M.,** 1977. Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout, Salmo gairdneri. *J. Fish Biol.*, 10(1):91-101.

**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 portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conserv. Physiol.*, 2(1):1-21.

**Thomas P., Robertson L.,** 1991. Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate and metomidate. *Aquaculture*, 96(1):69-86.

**Trushenski J., Schwarz M., Takeuchi R., Delbos B., Sampaio L.A.,** 2010. Physiological responses of cobia Rachycentron canadum following exposure to low water and air exposure stress challenges. *Aquaculture*, 307(1):173-177.

**Trushenski J.T., Bowker J.D., Mulligan B.L., Gause B.R.,** 2012. Induction, recovery, and hematological responses of largemouth bass to chemo-and electrosedation. *N. Am. J. Aquacult.,* 74(2):214-223.

**United States Food and Drug Administration, Center for Veterinary Medicine**, 1997. ANADA 200-226 Tricaine-S - origianl approval. FOIA Drug Summaries. U.S. Food and Drug Administration, Washington, D.C. Available at: https://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm132992.htm

**Wagner E., Arndt R., Hilton B.,** 2002. Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide. *Aquaculture*, 211(1):353-366.

**Weber R.A., Peleteiro J.B., Martín L.O.G., Aldegunde M.,** 2009. The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis Kaup* 1858). *Aquaculture*, 288(1):147-150.

**Weyl O., Kaiser H., Hecht T.,** 1996. On the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for goldfish, *Carassius auratus* (L.), at different temperatures and concentrations. *Aquac. Res.,* 27(10):757-764.

**Zahl I.H., Samuelsen O.B., Kiessling A.,** 2012. Anaesthesia of farmed fish: implications for welfare. *Fish Physiol. Biochem.*, 38(1):201-218.