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RESEARCH PAPER

Effects of Tricaine as an Anaesthetics on Goldfish, *Carassius Auratus* (Linnaeus 1758) at Different Salinities and Concentrations

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Abstract

The present experiment was designed to determine the effects of tricaine methanesulfonate (MS-222) as an anaesthetic on goldfish *Carassius auratus* at five different salinities and MS-222 concentrations. Goldfish (230±25 mg and 24.07±5.59 mm) were exposed to 150, 200, 300, 400, and 500 mg l^{-1} MS-222 concentrations at 0, 8, 12, 14, and 16 ppt of salinities. Even though, a lot of literature exist about the anesthetic usage on foodfish species, not much information seems to be available on ornamental fish aquaculture. Five tricaine methanesulfonate concentrations for each salinity were used to anesthetize goldfish and recorded their induction and recovery times. The necessary time to make anesthesia on fish relied on concentration intensity and salinity. When exposed to any of the concentrations, fish achieved a deep state of anesthesia (range of induction time 0.13 and 8.90 min). It is recommended that ideal concentration of MS-222 was 200 mg l^{-1} at 12 ppt to reduce fish mortality and stress.

Keywords: Anesthetic, *Carassius auratus*, goldfish, salinity, tricaine methansulfonate.

Farklı Tuzluluk ve Konsantrasyonlarda Tricaine'nin Bir Anestezik Olarak Japon Balıkları, *Carassius auratus* (Linnaeus 1758) Üzerine Etkileri

Özet

Bu çalışmada, tricaine metansülfonat (MS-222)'ın beş farklı tuzluluk ve konsantrasyonda bir anestezik olarak japon balıkları üzerine etkileri saptanmıştır. Japon balıkları (230±25 mg ve 24,07±5,59 mm) 150, 200, 300, 400 ve 500 mg l-1 MS-222 konsantrasyonlarına ve 0, 8, 12, 14 ve 16 ppt tuzluluk değerlerine maruz bırakılmıştır. Yemeklik balıklarda anestezik kullanımı üzerine bir çok literatür olmasına rağmen, süs balıkları yetiştiriciliği üzerine fazla bir bilgi bulunmadığı görülmektedir. Her bir tuzluluk için beş tricaine metansülfonate konsantrasyonu japon balıklarını anestezi yapmakta kullanıldı ve anestezi ve iyileşme süreleri kayıt edildi. Anestezi için gerekli zaman, konsantrasyon yoğunluğuna ve tuzluluk değerelerine göre değişmiştir. Her bir konsantrasyon maruziyetinde, balıklar derin anesteziye girdiler (anestezi süresi aralığı 0,13-8,90 dak). Balık ölümlerini ve stresi azalmak için ideal MS-222 konsantrasyonun 12 ppt tuzlulukta 200 mg l-1 olduğu tavsiye edilmiştir.

Anahtar Kelime: *Carassius auratus*, Japon balığı, tuzluluk, tricaine metansülfonat.

Introduction

Anesthesia is important to reduce stress and injury damage during handling procedures (measuring and weighing ,grading, tagging, vacination, live transport, blood sampling, biopsies of gonads, gamet collection, etc.) in aquaculture (Coyle *et al*., 2004; Mylonas *et al*., 2005). Several anesthetics are commonly used for fishes such as tricaine metansulfonate (MS-222), phenoxyethanol, quinaldin, benzocain, clove oil and metomidate (Mercy *et al*., 2013). There is a lot of considerations to decide which anesthetics is suitable to use for fish. It replies on efficiency, monetary value, accessibility, facility of use, safety to fish, human being, ambience (Mylonas *et al*., 2005). MS-222 provides all of these considerations. It also has been demonstrated to be effective and have been widely used with many species of fish (Hseu *et al*., 1998; Carter *et al*., 2011; Popovic *et al*., 2012; Mercy *et al*., 2013; Lepic *et al*., 2014; Mazik and Simco, 2014).

Lower doses of anesthetics are for light sedation. Higher doses are used for rapid and deep anesthesia. However, response to anesthetics relies on the

species, weight of organism, condition of animal and environmental factors (salinity, temperature, etc.) (Sneddon, 2012). In our study, we tried to see how salinity change affects the responses to anesthetics (MS-222). The aim of the study was to expose goldfish to five MS-222 concentrations (150, 200, 300, 400 and 500 mg l^{-1}) at 0, 8, 12, 14 and 16 ppt of salinities and to assess induction time, recovery time and survival for each concentration and salinity and find out whether addition of salt to tricaine affects the induction time or not.

Materials and methods

The experiments were done, using goldfish commercially collected from Aydın, Turkey pet shops. Water quality parameters were pH 7.95, EC 1300 ms cm^{-1}, ammonia 0.25 mg l^{-1}, nitrite 0.04 mg l^{-1}, alkalinity 595 mg l^{-1}, total hardness 775 mg l^{-1}. Fish average weight and length (mean±SD; n=5) were 230±25 mg and 24.07±5.59 mm, respectively. Fish had been starved for 24 h before the experiment. For tricaine methansulfonate (Sigma), stock solution (0.4 %, 100 ml) with 1 M Tris-Cl (pH 9.0) and working solution (0.2 %, 100 ml) were prepared. Application was undertaken in an aerated 250 ml beaker. Fish was exposed 150, 200, 300, 400, 500 mg l^{-1} concentrations of MS-222 for 0, 8, 12, 14, 16 ppt salinities at 25.6 °C and pH 7.94 until anaesthesia stage of 3 for induction and recovery stage of 3 for recovery times were writen down for each concentration. After recovery, fish were arranged to maintenance aquarium and were observed for 48 h for untoward effects. Experiment was performed on five fish in duplicate for each tricaine concentration (n=5).

The induction time was recorded for each fish when fish lost total equilibrium, its operculum rate ceased and fish did not answer to presure on its body (SIII). Anesthetized each fish was weighed and measured. After that, fish was placed into the freshwater being same temperature. Recovery time was registered when fish swimed in a normal style (RIII) (Table 1).

Statistical Analysis

For statistical analysis, differences between tricaine concentrations and salinity concentrations were examined using SSPS (version 18). Induction, recovery times and survival were compared for each salinity and concentrations. The data are presented as mean±SD. Analysis of variance and Duncan's multipe range tests were used to test for significant differences. Level of significance established in all tests was $P < 0.05$.

Results

In this study, induction and recovery times at each salinity and concentration were given in the Table 2. The induction time of *Carassius auratus* decreased with increasing concentrations of MS-222. The induction time was less than three minutes for a dose of 300 mg l^{-1} at freshwater and 8 ppt and 200 mg l^{-1} at 14 and 16 ppt. In addition of this, 200 mg l^{-1} of concentration at 12 ppt was considered as the best effective concentration of MS-222 for the induction of anesthesia in *C .auratus*. At 200 mg l^{-1} in 12 ppt of salinity, the time to reach a complete anesthesia (stage III) (2.24±0.95 min) was significantly different ($P<0.05$) from the others (Table 2). At higher concentrations, the time taken to reach stage III decreased, but the recovery time was dose-independent. In freshwater, recovery time was longer than that in all of saltwater. At 16 ppt of salinity and >150 mg l^{-1} of tricaine, fish recovered quickly. It is demonstrated a negative relation between induction time and tricaine concentration for every salinity. Survival after anesthesia did not differ significantly among trials. No death took place during anesthetization procedure and after 24 hours. Survival was excellent for all trial.

As concentration and salinity increased, induction time decreased (Figure 1). It is also showed inverted relation between recovery time and concentration at all salinities. Fish recovery decreased as concentration and salinity increased (Figure 2).

Discussion

Number of studies are presented about anesthetics for fisheries and some of them are about efficiency of anesthetics (Munday and Wilson, 1997; Lemm, 1993; Keene *et al*., 1998; Waterstrat, 1999; Walsh and Pease, 2002; Mylonas *et al*., 2005; Tsantilas *et al*., 2006; Pawar *et al*., 2011; Weber *et al*., 2009; Hseu *et al*., 1998; Mercy *et al*., 2013). Some of them about effects of anesthesia on the basis of haemotological indices (Tort *et al*., 2002; Small, 2003; Wagner *et al*., 2003; Holloway *et al*., 2004; King *et al*., 2005; Bystriansky *et al*., 2006; Congleton, 2006; Gomulka *et al*., 2008; Lepic *et al*., 2014). Lack of study is on ornamental fish. Few studies have been presented (Massee *et al*., 1995; Weyl *et al*., 1996; Pramod *et al*., 2010).

The lowest effective doses of MS-222 were 300 mg $^{-1}$ at 0 and 8 ppt and 200 mg l^{-1} at 12, 14, 16 ppt. All of these doses developed induction and recovery times of less than 3 and 5 min respectively. An induction time of 3 min or less, with complete recovery in 5 min is evaluated acceptable for fish handling (Hseu *et al*., 1998; Weber *et al*., 2009). 12 ppt and 200 mg l^{-1} of MS-222 is the ideal concentration to use in goldfish husbandry operations.

There are some criterias for an ideal anesthetic in aquaculture. Ideal anesthetic should be less than 3 min for anesthesia and its recovery should be within 5 min (Mylonas *et al*., 2005). It must be non toxic to fish and users, leave no residues and be not expensive

Table 1. Stages of induction and recovery in fish (Coyle et al., 2004; Kucuk, 2010; King et al., 2005; Mercy et al., 2013)

Stages of Induction (S)	Description	Behavior/Response
I	Sedation	Slight loss of reactivity to external stimuli; operculum rate slightly decreased; equilibrium normal
II	Anesthesia	Partial loss of muscle tone ; swimming erratic; increased operculum rate; reactivity only to strong tact
III	Deep anesthesia	Total loss of muscle tone and equilibrium; slow but regular operculum rate; loss of spinal reflexes
IV	Death	Breathing and heart beat stop; eventual death
Stages of Recovery (R)	Desciption	Behavior/Response
I	Deep anesthesia	No body movements but opercular movements start
II	Anesthesia	Regular opercular movements and body movements start
III	Sedation	Equilibrium regained with preanesthetic appearance

Table 2. Induction time, recovery time, induction rage and survival of goldfish in five different salinities and tricaine concentrations (Mean ± SD, n=5)

Salinity (ppt)	Tricaine Conc. (mg l^{-1})	Induction Time (min)	Recovery Time (min)	Induction rage (min)	Survival (%)
0	150	$5.02\pm2.00_{AB,a}$	$5.30\pm0.89_{A,\ a}$	2.00-7.08	100±0.00
	200	$3.86\pm1.45_{AB,b}$	$5.13\pm1.02_{A,\ a}$	1.32-6.07	100±0.00
	300	$1.11\pm0.43_{AB,\ c}$	$4.04\pm0.84_{A,\ b}$	0.50-2.00	100±0.00
	400	$0.36\pm0.05_{AB,\ c}$	$3.73\pm0.57_{A,\ bc}$	0.30-0.46	100±0.00
	500	$0.21\pm0.06_{AB,\ c}$	$3.16\pm0.56_{A,\ c}$	0.13-0.30	100±0.00
8	150	$5.14\pm1.74_{A,\ a}$	$3,18\pm1.19_{B,\ a}$	2.35-8.01	100±0.00
	200	$3.99\pm1.13_{A,\ b}$	$2.97\pm0.62_{B,\ a}$	2.00-5.58	100±0.00
	300	$1.47\pm1.11_{A,\ c}$	$2.73\pm0.65_{B,\ a}$	0.40-4.41	100±0.00
	400	$0.34\pm0.05_{A,\ d}$	$2.71\pm0.85_{B,\ a}$	0.27-0.41	100±0.00
	500	$0.38\pm0.24_{A,\ d}$	$2,63\pm0.43_{B,\ a}$	0.24-1.06	100±0.00
12	150	$5.47\pm1.79_{ABC,\ a}$	$3.34\pm0.91_{B,\ a}$	3.93-8.90	100±0.00
	200	$2.24\pm0.95_{ABC,\ b}$	$3.09\pm1.11_{B,\ ab}$	1.08-3.59	100±0.00
	300	$0.80\pm0.39_{ABC,\ c}$	$2,82\pm0.55_{B,\ ab}$	0.30-1.35	100±0.00
	400	$0.40\pm0.22_{ABC,\ c}$	$2,34\pm0.73_{B,\ bc}$	0.25-1.00	100±0.00
	500	$0.37\pm0.13_{ABC,\ c}$	$1.99\pm0.49_{B,\ c}$	0.20-0.55	100±0.00
14	150	$5.61\pm1.81_{BC,\ a}$	$2.64\pm1.24_{C,\ a}$	2.83-8.76	100±0.00
	200	$2.34\pm1.37_{BC,\ b}$	$2,11\pm1.08_{C,\ ab}$	0.36-4.33	100±0.00
	300	$0,42\pm0.26_{BC,c}$	$2,06\pm0.45_{C,\ ab}$	0.25-1.13	100±0.00
	400	$0,43\pm0.11_{BC,\ c}$	$1.93\pm0.63_{C,\ ab}$	0.26-0.56	100±0.00
	500	$0,32\pm0.05_{BC,\ c}$	$1.58\pm0.46_{C,\ c}$	0.24-0.40	100±0.00
16	150	$5.65\pm1.83_{D,\ a}$	$2.40\pm1.16_{D,\ a}$	2.49-8.68	100±0.00
	200	$1,22\pm0.48_{D,\ b}$	$1,64\pm0.55_{D,\ b}$	0.44-2.04	100±0.00
	300	$0,35\pm0.08_{D,\ c}$	$1.53\pm0.40_{D,\ b}$	0.23-0.46	100±0.00
	400	$0,31\pm0.08_{D,\ c}$	$1.50\pm0.46_{D,\ b}$	0.22-0.43	100±0.00
	500	$0,27\pm0.03_{D,\ c}$	$1.27\pm0.57_{D,b}$	0.22-0.33	100±0.00

Upper case for salinity.
Lower case for tricane concentration.

(Mylonas *et al*., 2005). In this study, 300 mg l^{-1} 0f MS-222 caused for induction time in 1.11 and 1,47 min at 0 and 8 ppt of water. As salinity increases, 200 mg l^{-1} of MS-222 induced fish at 12, 14, and 16 ppt (2.24, 2.34 and 1.22 min). Hseu *et al*. (1998) found effective concentration of MS-222 (100 mg l^{-1}) for goldlined sea bream. Goldfish compared goldlined sea bream is more tolerant to MS-222. Salt water buffered water to keep from MS-222 acidification. That is why high concentration of MS-222 did not affect goldfish such as at >200 mg l^{-1}. Other studies verified salt water aleviated MS-222 effects in striped mullet (Sylvester, 1975), cod (Mattson and Riple, 1989) and atlantic halibut (Malmstrom *et al*., 1993). Tomasso *et al*. (1980) used anesthetics + salt combination (25 mg l^{-1} of MS-222 + 10 ppt of salt) for handling of hybrid striped bass. Anesthetics (MS-222) + salt (0, 8, 12, 14, 16 ppt) was tested to anesthetize goldfish in this work. Increasing salt in this combination reduced the effective concentration

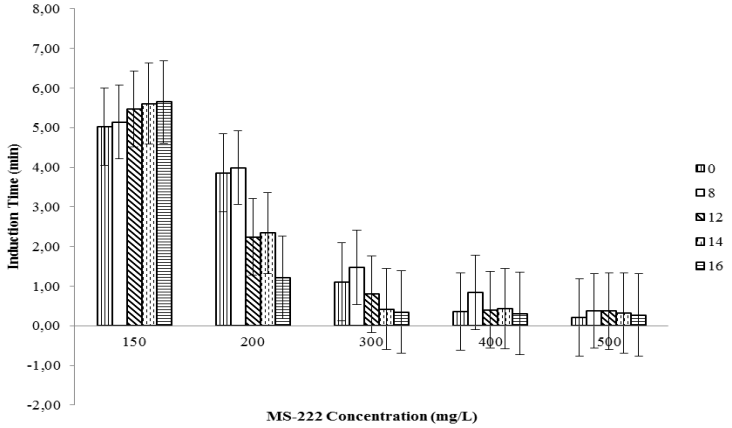

Figure 1. Mean±SD of induction time of goldfish immersed to anesthesia.

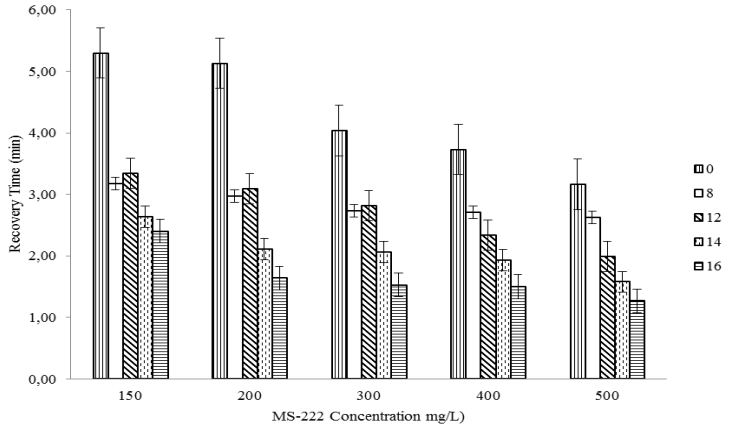

Figure 2. Mean±SD of recovery time of goldfish immersed to anesthesia.

of MS-222.

Induction times reduced significantly with the increases in anesthetic concentration that are compatible with previous studies (Mercy *et al*., 2013; Mylonas *et al*., 2005; Pramod *et al*., 2010; Pawar *et al*., 2011). But recovery times were dose independent. Weber *et al*. (2009) also found that recovery time of tricaine was self- directed from concentrations.

MS-222 is more often used in aquaculture. Because it is approved by the Food and Drug Administration (FDA) and agreed with all criteria of ideal anesthetics (Munday and Wilson, 1997; Hseu *et al*., 1998; Weber *et al*., 2009; Kucuk, 2010; Pramod *et al*., 2010; Pawar *et al*., 2011).

Salt increased survival, reduced plasma corticol and glucose and osmoregulatory disfunction in striped bass (Mazik *et al*., 1991; Mazik and Simco, 2014). In our study, survivals were full (100%) in all concentrations and salinities.

Hseu *et al*. (1998) compared five anesthetics (quinaldine, quinate, MS-222, benzocaine and 2-phenoxyethanol) to goldlined sea bream, although they mentioned that use of MS-222 was the most expensive anesthetics. However, this trial costed low price. Because less volume of water was needed for handling of ornamental fish.

Conclusion

The most effective dose and salinity was 200 mg l^{-1} at 12 ppt (2.24 min) for anesthesia. As salinity and concentration increased, induction time decreased. Goldfish resistant to MS-222 and salt at those levels.. Survival was excelent at high concentration and salinities. After adding salt to water, goldfish get anesthesia at lower concentration (200 mg l^{-1} at 16 ppt in 1.22 min). Induction time was 3,86 min at 200 mg l^{-1} in freshwater. As a result of that, MS-222 + salt combination is carried out quickly for goldfish anesthesia. It is recommended that 200 mg/L of MS-222 at 12 ppt can be use in goldfish aquaculture practice.,

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