African Journal of Biotechnology Vol. 9(5), pp. 755-759, 1 February 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Efficacy of tricaine on *Peocilia latipinna* at different temperatures and concentrations

Semra Küçük

Department of Fisheries, Faculty of Agriculture, Adnan Menderes University, 09100, Aydin, Turkey.
E-mail: skucuk@adu.edu.tr.

Accepted 11 December, 2009

The present experiment was designed to determine the effects of tricaine methanesulfonate as an anaesthetic for juvenile sailfin silver molly *Poecilia latipinna* at three different temperatures and anaesthetic concentrations. Silver molly (230 ± 25 mg and 24.07 ± 5.59 mm) were exposed to 150, 200, and 250 mg l^{-1} tricaine concentrations at 20, 25, and 30°C. Even though, a body of literature exist about the anaesthetic usage on foodfish species, not much information seems to be available on ornamental fish aquaculture. In the experiment, three tricaine methanesulfonate concentrations for each temperature were used to anaesthetize silver molly and recorded their induction, recovery times and survival. The necessary time to make anaesthesia on fish depended on concentration intensity and water temperature rise. When exposed to any of the concentrations, fish achieved a deep state of anaesthesia with induction times of 0.18 - 1.63 min and recovered fast in 1.53 - 2.08 min. To maximize safety and reduce fish mortality and stress, the minimum concentration producing desirable anesthetic effects was 150 mg l^{-1} at all three temperature.

Key words: Anesthetic, tricaine, *Poecilia latipinna*, temperature.

INTRODUCTION

This investigation of the suitability of tricaine methanesulfonate (MS-222) as a fish anaesthetic was prompted by the need for a safe anaesthetic for use in research on the environmental requirements of sailfin silver molly, *Poecilia latipinna* (Lesueur, 1821). This freshwater species (Cyprinodontiformes: Poeciliidae) is currently under evaluation for ornamental fish. Molly fish is com-mon in two species as *P. latipinna* and *Poecilia sphenops*.

In fisheries research and aquacultural operations, anaesthetics are necessary to minimize stress and reduce physical injury of fish during various handling procedures such as weighing, measuring, tagging, sampling, size grading, spawning of broodstock, transporting, vaccinating and surgery (Ross and ross, 1999; Coyle et al., 2004, Mylonas et al., 2005). When choosing an anaesthetic for a particular purpose, several considerations may be taken such as availibility, ease of use, effectiveness, physiological perturbations, cost, nature of the study and safety for the fish, consumers, user and the environment.

A number of different anesthetics have been used for aquaculture applications. Tricaine methanesulfonate (MS-222), Benzocaine, Quinaldine, 2-Phenoxyethanol, Metomidate, and Etomidate are widely used drugs for inhalation anaesthesia (Weyl et al., 1996; Ross and Ross, 1999; Mylonas et al, 2005). Styrylpyridine, 2 - amino- 4 -phenylthiazole, amylobarbitone, chloral hydrate, chlorbutanol, chloroform, clove oil, ether, lilnocaine, methyl pentynol, propoxate, quinalbarbitone, sodium cyanide, tertiary amyl alcohol, tertiary butyl alcohol, tribromoethanol, and urethane are less widely used drugs. Some plant extracts such as Derris (the active compound is rotenone) and Tephrosia (the active compound is tephrosin) are used for anaesthetic agent as well (Ross and Ross, 1999; Coyle et al., 2004).

MS-222 is chemically called tricaine methanesulfonate and commercially sold as Tricaine-S or Finquel. It looks like a white crystalline powder and dissolved easily in water. But it lowers water pH and caused acidic condition to fish. It should be buffered with baking soda (sodium bicarbonate) or tris-Cl (pH 9) to hold water pH in 7. One of the main handicap to use tricaine is that plasma cortisol level increased in deeply anesthetized fish (Davis and Griffin, 2004; Cho and Heath, 2000; Small, 2003). Induction time is as short as 15 s and is not suggested to use higher than 250 mg l^{-1} for warm water fish. Recovery

Table 1. Stages of anesthesia and recovery in fishes, from King et al. (2005).

Stages of anesthesia	Description
I	Loss of equilibrium
II	Loss of gross body movements but continued opercular movement
III	Same as stage II but opercular movement ceases
Stages of Recovery	**Description**
I	No body movements but opercular movements start
II	Regular operculum movements and body movements start
III	Equilibrium regained with preanesthetic appearance

Table 2. Induction time (S3), induction range, recovery time (R3) and recovery range in minutes and survival of molly exposed to tricaine at three temperatures (Mean ± SD, n = 5).

Temp. (°C)	Tricaine conc. (mg l^{-1})	Induction time	Induction range	Recovery time	Recovery range	Survival (%)
20	150	1.63 ± 0.97 $^{A, a}$	0.43 - 4.16	1.89 ± 0.56^{a}	1.24 - 3.24	100
	200	0.80 ± 0.33 $^{B, b}$	0.36 - 1.29	1.82 ± 0.50^{a}	1.26 - 3.00	100
	250	0.69 ± 0.39 $^{C, b}$	0.34 - 1.43	1.59 ± 1.08^{a}	0.50 - 5.05	100
25	150	0.50 ± 0.26 $^{D, bc}$	0.26 - 1.20	1.82 ± 0.42^{a}	1.30 - 2.33	100
	200	0.42 ± 0.21 $^{E, c}$	0.21 - 1.06	1.53 ± 0.71^{a}	0.47 - 3.26	100
	250	0.30 ± 0.10 $^{F, c}$	0.16 - 0.50	1.70 ± 0.40^{a}	1.24 - 2.24	100
30	150	0.30 ± 0.10 $^{G, cd}$	0.15 - 0.49	1.67 ± 0.40^{a}	1.15 - 2.27	100
	200	0.19 ± 0.04 $^{H, cd}$	0.12 - 0.25	1.76 ± 0.67^{a}	1.10 - 3.58	100
	250	0.18 ± 0.04 $^{H, cd}$	0.13 - 0.30	2.08 ± 0.97^{a}	1.18 - 4.28	100

*Upper case for tricaine concentration.
*Lower case for temperature.

and equilibrium can be regained in few minutes. MS-222 displays a good safety margin in fish such as effective concentration and maximum safe concentration. Safe margin constricts when temperature arises and becomes smaller for small fish. It is more effective in warm water with low hardness. After exposure, MS-222 is excreted by kidney with urine within 24 h. It is approved for use in aquaculture in the U.S. and U.K. It (MS-222) has 21 day withdrawal time, required only for marketing fish.

In this study, sailfin silver molly were exposed to three MS-222 concentrations (150, 200, and 250 mg l^{-1}) at 20, 25 and 30°C temperatures to evaluate induction time, recovery time and survival for each concentration.

MATERIALS AND METHODS

The experiments were undertaken, using juvenile sailfin silver molly were grown at Aquarium Unit of Adnan Menderes University Agricultural Faculty, in Aydin, Turkey. Parameters of water source were pH 7.55, DO 6.25 mg l^{-1}, EC 1316 µs cm^{-1}, ammonia 0.22 mg l^{-1}, nitrite 0.07 mg l^{-1}.alkalinity 600 mg l^{-1} and total hardness 780 mg l^{-1}. Fish average weight and length (mean ± SD) were 230 ± 25 mg and 24.07 ± 5.5 mm, respectively. Fish had been starved for 24 h prior to the experiment and tricaine methanesulfonate (sigma) stock solution (0.4%, 100 ml) buffered with 1 M Tris-Cl (pH 9.0) and working solution (0.2%, 100 ml) were prepared. Application was done in an aerated 250 ml beaker placed into 9 L aquaria. Fish was exposed to 150, 200, and 250 mg l^{-1} concentrations of MS-222 at 20, 25, and 30°C water temperatures until anesthesia stage of 4 for induction time and recovery stage of 2 for recovery time. The induction and recovery times were recorded for each temperature and concentration. Following recovery, fish were placed to maintenance aquarium and were observed for 48 h for adverse effects. Experiment was fulfilled on five fish in triplicate (n = 15).

The time of induction was recorded for each fish when fish lost total equilibrium, its operculum rate stopped and fish did not response to pressure on its body (S3). Thereafter, the time of recovery was writen down when fish started swimming in a normal manner (R3) after putting it into the freshwater being identical temperature (Table 1). Anaesthesied each fish was weighed and measured its length.

For statistical analysis, differences between tricaine concentrations and temperatures were examined using analysis of covariance induction time, recovery time, operculum rate, body weight and body length in SAS. Tukey's multiple range was used to compare the means. In all statistical analysis, $P < 0.05$ was considered significant.

RESULTS

In this study, induction and recovery times of each concentration and temperature were given in Table 2. At 20°C, the induction times of anaesthesic effect in fishes

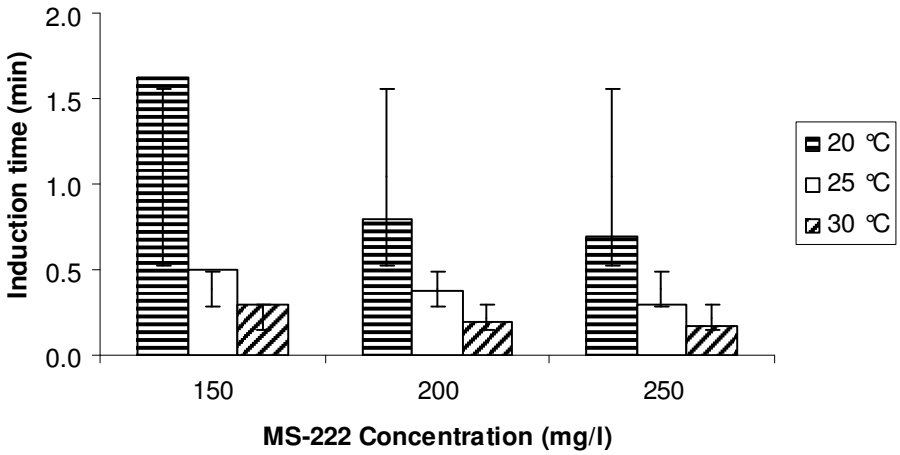

Figure 1. Mean ± SD of induction time of sailfin silver molly submitted to anaesthesia.

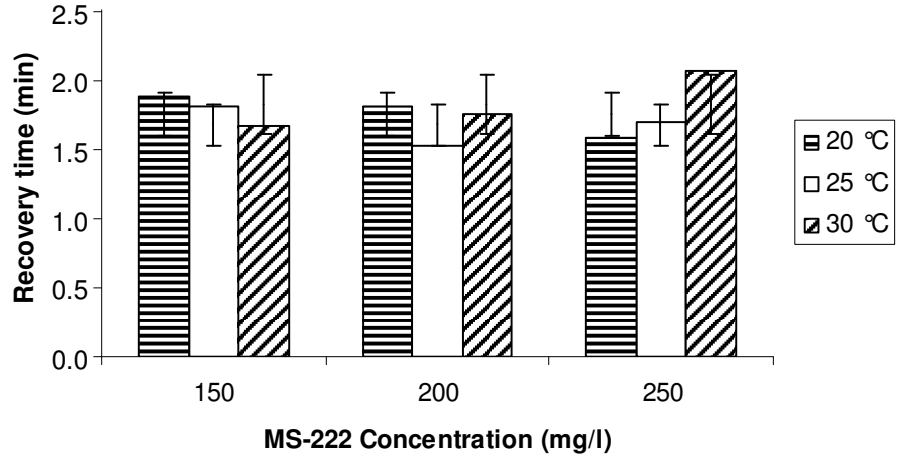

Figure 2. Mean ± SD of recovery time of sailfin silver molly submitted to anaesthesia.

exposed to 150, 200, and 250 mg l^{-1} concentrations of MS-222 were significantly different. They demonstrated 1.63, 0.80, and 0.69 min, respectively. At 25°C, three concentrations had significantly different induction times. They became 0.50, 0.42, and 0.30 min. At 30°C, 150 mg l^{-1} only was significantly different from those of fish exposed to 200 and 250 mg l^{-1} of MS-222. The induction times were 0.30, 0.19, and 0.18 min, respectively. When the temperature difference among concentration is considered, 150 mg l^{-1} at 20°C was significantly different from the others. It is obvious that the induction time of the anaesthesic effect of MS-222 reduced while temperature and concentration increased during the experiment. Recovery time did not show significant difference among concentration and temperature groups. It indicated that 150 mg l^{-1} concentration of MS-222 is enough for anesthesia at 20°C and higher temperature. Survival after anesthesia did not differ significantly among groups. No fish died during trials.

Figure 1 showed a negative relation between concentration and induction time of the anaesthesic effect of MS-222 at all three temperatures. When concentration and temperature increased, induction time reduced.

Figure 2 demonstrated the positive relationship between concentration and recovery time. Fish recovery became long and mortality risk increased when concentration arised.

MS-222-exposed fish had over 100/min operculum rate in all groups. There was no difference among groups.

DISCUSSION

There is plenty of studies on the use of anesthetics in aquaculture. Some studies tested efficacy of commonly used anesthetics and compared them (Lemm, 1993; Munday and Wilson, 1997; Keene et al., 1998; Waterstrat, 1999; Walsh and Pease, 2002; Mylonas et al., 2005;

Table 3. Anesthetic agent of tricaine methanesufonate used in various fish.

Name	Scientific Name	Reference
Red drum Goldfish	*Sciaenops ocellatus*, *Carassius carassius*	Massee et al., 1995
Striped bass	*Morone saxatilis*	Lemm, 1993
Coral fish	*Pomacentrus amboinensis*	Munday and Wilson, 1997
Goldlined sea bream	*Sparus sabra*	Hseu, et al., 1998
Chinook salmon	*Oncorhynchus tshawytscha*	Cho andHeath, 2000
Red pacu	*Piaractus brachypomus*	Sladky et al., 2001
Catfish	*Ictalurus punctatus*	Small, 2003
Trout	*Oncorhynchus mykiss*	Gilderhus and Marking, 1987
		Pirhonen and Schreck, 2003
		Wagner et al., 2003
		Holloway et al., 2004
		Bystriansky et al., 2006
Black sea bass	*Centropristis striata*	King et al., 2005
Hybrid striped bass	*Morone chrysops* X *M. saxatilis*	Davis and Griffin, 2004

Tsantilas et al., 2006), while some of them investi-gated how anesthetics influence blood chemistry of fishes (Tort et al., 2002; Wagner et al., 2003; Holloway et al., 2004; King et al., 2005; Bystriansky et al., 2006; Con-gleton, 2006). Most of the studies have concluded on farmed fish (Gilderhus and Marking, 1987; Lemm, 1993; Munday and Wilson, 1997; Hseu et al., 1998; Waterstrat, 1999; Cho and Heath, 2000; Walsh and Pease, 2002; Small, 2003; Tsantilas et al., 2006; Pirhonen and Schreck, 2003). But a few attempt was demonstrated about ornamental fish (Massee et al., 1995; Weyl et al., 1996). In the present study, molly one of the widespread aquarium fish was tested.

Anaesthetics are needed for easy handling, sorting, measuring, transporting and surgical procedures in aqua-culture and fisheries to facilitate application procedure. The most often used ones include MS-222, 2-phenoxy-ethanol, quinaldine, benzocaine and metomidate. A suitable anaesthetic may vary. It may depend on stress, environmental factors, fish condition and species. Generally, an ideal anesthetic ougth to induce anesthesia quickly in less than 3 min, permit a fast recovery in 5 min or less, produce no poison to fish and cause no hazard to human and be inexpensive (Mylonas et al., 2005). In this study, 150 mg l^{-1} of MS-222 was determined ideal con-centration for three temperatures and induced anesthesia on fish within ≤ 1.63 min and fish regained its equilibrium within ≤ 2.08. No fish died during the experiment and researhers incurred no irritation. In fact, trial was conducted in low-cost due to being ornamental fish. Lemm (1993) found that 200 mg l^{-1} (18°C) of tricain caused 1.86 min of induction time and 200 mg l^{-1} (23°C) produced 1.83 min of induction time, with recovery times of 5.79 (18°C) and 11.78 (23°C) min, respectively at pH 7.0 - 7.8, alkalinity 292 and hardness 342 mg l^{-1}. In this study, closely related results was observed with 150, 200 and 250 mg^{-1} concentrations of MS-222 at 20°C when considering induction range although our alkalinity and hardness were two fold of it. However, in recovery range, the same situation was not encountered. At 20°C, 250 mg l^{-1} of MS-222 displayed a recovery range of 5.05 min. This difference of recovery may be as a result of the fact that molly (230 mg) are quite smaller than striped bass (300 - 1500 g) in size.

Even though a number of researchers have recently tried to study in order to replace MS-222 with 2-phenoxy-ethanol, clove oil, carbon dioxide, Aqui-S, or quinaldine (Davis and Griffin, 2004; Walsh and Pease, 2002; Mylonas et al., 2005; Pirhonen and Schreck, 2003), the anesthetics, MS-222, was more often chosen for applications in aquaculture due to legal use and also because it met the ideal anesthetic requirements (such as short time for induction and recovery, commercial availibity and facileness of preparation). MS-222 was tested on a number of fish species in Table 3.

Conclusion

Various anesthetics are used for aquaculture applica-tions, but MS-222 is the only approved one by the US and UK. There are not much information about ane-sthetic usage in ornamental fish. Here, it is observed that induction time was significantly different in three tempe-ratures and three concentrations except 200 and 250 mg l^{-1} at 30°C. it is suggested that the minimum tricain concentration be 150 mg l^{-1} at the three temperature (≥20 ºC) for safe use.

REFERENCES

Bystriansky JS, LeBlanc PJ, Ballantyne JS (2006). Anaesthetization of arctic charr *Salvelinus alpinus* (L.) with tricaine methanesulphonate or 2-phenoxyethanol for immediate blood sampling. J. Fish Biol. 69:

613-621.
Cho GK, Heath DD (2000). Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha*. Aquac. Res. 31: 537-546.
Congleton JL (2006). Stability of some commonly measured blood-chemistry variables in juvenile salmonids exposed to a lethal dose of the anaesthetic MS-222. Aquac. Res. 37: 1146-1149.
Coyle SD, Durborow RM, Tidwell JH (2004). Anaesthetics in aquaculture, SRAC Publication no 3900.
Davis KB, Griffin BR (2004). Physiological responses of hybrid striped bass under sedation by several anesthetics, Aquaculture. 233: 531-548.
Holloway AC, Keene J, Noakes DG, Moccia RD (2004). Effects of clove oil and MS-222 on blood hormones profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum, Aquac. Res. 35: 1025-1030.
Hseu JR, Yeh SL, Chu YT, Ting YY (1998). Comparison of efficacy of five anesthetics in goldlined sea bream, *Sparus sarba*. Acta Zool. Taiwan. 9: 35-41.
Gilderhus PA, Marking LL (1987). Comparative efficacy of 16 anaesthetic chemicals on rainbow trout. N. Am. J. Fish. Manage. 7: 288-292.
Keene JL, Noakes DLG, Moccia RD, Soto CG (1998). The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 29: 89-101.
King W, Hooper B, Hillsgrove S, Benton C, Berlinsky D (2005). The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquac. Res. 36: 1442-1449.
Lemm CA (1993). Evaluation of five anaesthetics on striped bass, Resource publication. U.S. Fish. Wildl. Serv. p. 196.
Massee KC, Rust MB, Hardy RW, Stickney RR (1995). The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish, Aquaculture. 134: 351-359.
Munday PL, Wilson SK (1997). Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish, J. Fish Biol. 51: 931-938.
Mylonas CC, Cardinaletti G, Sigelaki I, Polzonetti-Magni A (2005). Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus auratus*) at different temperature. Aquaculture. 246: 467-481.
Pirhonen J, Schreck CB (2003). Effects of anaesthesia with MS-222, clove oil and CO_2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). Aquaculture. 220: 507-514.
Ross LG, Ross B (1999). Anaesthetic and Sedative Techniques for Aquatic Animals, second edition, Blackwell publishing, Oxford.
Sladky KK, Swanson CR, Stoskopt MK, Loomis MR, Lewbart GA (2001). Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). Am. J. Vet. Res. 62: 337-342.
Small B (2003). Anaesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anaesthetized channel catfish *Ictalurus punctatus*, Aquaculture. 218: 177-185.
Tort L, Puigcerver M, Crespo S, Padros F (2002). Cortisol and haematological response in sea bream and trout subjected to the anaesthetics clove oil and 2-phenoxyethanol. Aquac. Res. 33: 907-910.
Tsantilas H, Galatos AD, Athanassopoulou F, Prassinos NN, Kousoulaki K (2006). Efficacy of 2-phenoxyethanol as an anaesthetic for two size classes of white sea bream, *Diplodus Sargus* L. and sharp snout sea bream, *Diplodus puntazzo* C. Aquaculture. 253: 64-70.
Wagner GN, Singer TD, McKinley RS (2003). The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res. 34: 1139-1146.
Walsh CT, Pease BC (2002). The use of clove oil as an anaesthetic for the longfinned eel, *Anguilla reinhardtii* (Steindachner). Aquac. Res. 33: 627-635.
Waterstrat PR (1999). Induction and recovery from anaesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. J. World Aquac. Soc. 30: 250-255.
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: 757-764.