www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 13: 51-56 (2013) DOI: 10.4194/1303-2712-v13_1_07

Comparative Efficacy of Three Anesthetic Agents on Juvenile African Catfish, *Clarias gariepinus* (Burchell, 1822)

Fatih Öğretmen[1], Kaya Gökçek[2,*]

[1] Muğla Sıtkı Koçman University, Faculty of Fisheries and Aquaculture, Muğla, Turkey.
[2] Mustafa Kemal University, Marine Sciences and Technology, İskenderun, Turkey.

* Corresponding Author: Tel.: :+90.534 4150716; Fax: +90.362 6141877;
E-mail: kayagokcek@yahoo.com

Received 22 June 2012
Accepted 15 December 2012

Abstract

In this study, the efficacy of three anaesthetic agents (clove oil, 2-phenoxyethanol and eugenol) was compared in captive-bred African catfish, *Clarias gariepinus* (Burchell, 1822). The lowest effective concentrations based on the efficacy criteria of complete anesthesia induction within 180 s and recovery within 300 s were determined to be 50 mg L^{-1} (induction 193±62 s and recovery time 251±32 s) for clove oil, 750 µl L^{-1} (induction 145±24 s and recovery time 174±10 s) for 2-phenooxyethanol, and 50 mg L^{-1} (induction 197±29 s and recovery time 310±17 s) for eugenol. The onset of individual phases of anesthesia and recovery times depended significantly on the concentration of the anaesthetic used ($P<0.05$). An inverse exponential relationship was observed between concentrations of anaesthetic and induction time, whereas exponential relationships were observed between concentrations and recovery times for all anaesthetic agents evaluated. The final conclusion of this study, clove oil is the most suitable agent for juvenile African catfish from the three anesthetics tested.

Keywords: Clove oil, 2-phenoxyethanol, eugenol, induction time, recovery time.

Karabalık, *Clarias gariepinus* (Burchell, 1822) Jüvenilleri Üzerine Üç Anestezik Maddenin Etkinliğinin Karşılaştırılması

Özet

Bu çalışmada, üç anestezik maddenin (karanfil yağı, fenoksiethanol ve eugenol) etkinliği kültür şartları altında yetiştirilmiş Karabalık'larda karşılaştırılmıştır. Etkin en düşük doz kriteri olan 180 s içinde bayılma ve 300 s içinde ayılma hali karanfil yağında 50 mg L^{-1} (bayılma 193±62 s ve ayılma 251±32 s), fenoksiethanolde 750 µl L^{-1} (bayılma 145±24 s ve ayılma 174±10 s) ve eugenolde 50 mg L^{-1} (bayılma 197±29 s ve ayılma 310±17 s) dozlarında elde edilmiştir. Bireysel olarak bayılma ve ayılma hallerinin başlangıcı, kullanılan anestezik maddenin dozuna bağlı olarak istatistiki açıdan farklı bulunmuştur ($P<0,05$). Anestezik madde konsantrasyonu ile bayılma süresi arasında ters bir üssel ilişki tespit edilmiş, ancak anestezik madde konsantrasyonu ile ayılma süreleri arasındaki ilişki üssel olarak ölçülmüştür. Bu çalışmanın sonucunda, kullanılan üç anestezik maddeden Karabalıklar için en uygun olanın karanfil yağı olduğu söylenebilir.

Anahtar Kelimeler: Karanfil yağı, fenoksiethanol, eugenol, bayılma süresi, ayılma süresi.

Introduction

Fish are routinely handled during the activity of stripping, weighing, selection, broodstock management and treatments for and against fish diseases. Thus, anesthetics play very important role in aquaculture sector. Anesthetics reduce activity in fish and general anesthesia occurs which ends in a total loss of consciousness. Reflex activity is lost entirely and skeletal muscle tone is also reduced (Mc Farland, 1960). Overdose or overexposure during treatments reduces breathing and results in low oxygen saturation in blood and ultimately in respiration and circulation disorders (Tytler and Hawkins, 1981).

The most commonly used anesthetics in aquaculture are MS-222 (tricaine methane sulphonate), benzocaine (ethly-p-aminobenzoate), methomidate, clove oil, eugenol and 2-phenoxyethanol (ethylen glycol monophenyl ether) (Velisek *et al*., 2006, 2011). Currently, only MS-222 is licensed for use in food fish in the USA and the European Union. Also, Aqui S has a huge usage ratio in Australia and Japan. However, compounds such as 2-phenoxyethanol, clove oil, eugenol and benzocaine

have been evaluated experimentally, and are being used in non-food fish and in research (Coyle *et al*., 2004).

Choosing an appropriate anesthetic depends mainly on its effectiveness in immobilizing fish with good recovery rates (Gilderhus and Marking, 1987; Burka *et al.*, 1997). An ideal anesthetic should possess several attributes such as non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery (Treves-Brown, 2000). It is often advisable to identify the lowest effective concentration of different anesthetics in a specified species, as the responses to the same anesthetic may vary considerably among different species (Pawar *et al.*, 2011).

The African catfish or sharptooth catfish, *C. gariepinus* (Burchell, 1822), is an omnivorous and fast growing fish species. Because of high value as food in all over the world, many of commercial fish farms, government fisheries stations and institutions produce this species extensively and intensively. Although reproductive biology and the hatchery techniques were well studied, the effect of commercially used anesthetics on African catfish is still unknown. Given the growing interest in the culture of African catfish and lack of detailed practical information on the administration of anesthetics, the overall aim of present study was to determine induction and recovery times of three most common fish anesthetic agents (clove oil, 2-phenoxyethanol and eugenol) that could be efficiently use in African catfish under controlled conditions.

Material and Methods

Experimental Animals

African catfish juvenils were produced from local broodstock in captivity in the aquaculture department of Mustafa Kemal University, Antakya, Hatay, Turkey. Two-month old fingerlings (average length 10.6±1.6 cm and weight 6.5±3.0 g) were transferred to the production unit of National States of Hydraulic Works Department in Adana and held for 2-week acclimatization period before the study began.

Anesthetic Agents

The anesthetic agents 2-phenoxyethanol, eugenol (Sigma Aldrich Chemic, Germany) (PE), and clove oil (67% eugenol) (Biopont, Budapest, Hungary) were used for the present study. Concentrations of the anesthetic agents were prepared a few minutes before the experiments. Since clove oil and eugenol do not dissolve in water (Woody *et al*., 2002) they were initially diluted in ethanol (ratio of clove oil/eugenol to ethanol 1:9). PE was initially mixed with water in a reagent bottle and then stirred to disperse the chemical to form small droplets before adding to anesthetic test aquarium.

Induction and Recovery Stages of Anesthesia

The efficacy of three anesthetic agents in fingerling African catfish was assessed by testing several concentrations of each anesthetic. Choice of minimum and maximum concentrations of each anesthetic was based on previously published information for teleosts (Gomes *et al*., 2001; Weber *et al*., 2009). The following concentrations of each agent were evaluated; clove oil (25, 50, 75, 100 and 125 mg L^{-1}), PE (250, 500, 750, 1000 and 1250 µl L^{-1}) and eugenol (25, 50, 75, 100 and 125 mg L^{-1}). Seven individuals were exposed to five concentrations of each anesthetic totaling 105 individuals. Experiments were prepared in triplicate to verify findings.

After two weeks of acclimation, the fish were netted from rearing fiberglass tanks and transferred to the holding aquarium (300 L) filled with fresh and aerated water in the laboratory conditions. Fish were netted and transferred individually to the 20 L aquarium containing different concentrations of anesthetic solutions. The induction and recovery time for all anesthetics was measured under same experimental conditions using a digital stopwatch. Water quality was monitored by measuring dissolved oxygen and temperature (oxygen meter; YSI 550A), pH (pH meter; YSI 100), conductivity (conductivity meter; YSI EC300) in the tanks and were detected 8.55±0.86 mg/L, 22°C, 7.84±1.26 and 390 mS/cm, respectively.

Changes in the physiological status of the anesthetized fish were assessed in four consecutive stages for induction and three stages for recovery described by Theinpoint and Niemegeers (1965) with little modifications based on the behavioral response of African catfish (Gullian and Villanueva, 2009) (Table 1).

Statistical Analysis

A Kruskal-Wallis test was used to assess the differences in induction and recovery times of

Table 1. Signs and stages of anesthesia in African catfish, *C. gariepinus* (modified from Theinpoint and Niemegeers, 1965)

Induction stages
I 1: Loss of balance, partial inhibition of reactions to external stimuli
I 2: Total loss of equilibrium. Fish still react to strong stimuli
I 3: Total loss of reflexes and movement. Fish lay on bottom of the tank
Recovery stages
R 1: Start of movement. Fish still lay on bottom of the tank
R 2: Regular breathing. Reaction to strong stimuli. Irregular balance
R 3: Total recovery of equilibrium. Reaction to slight stimuli. Normal swimming

different concentrations of the same anesthetic agent (Zar 1999). Non-linear regression analyses were used to establish the relationship between dosage and induction time, as well as dosage and recovery time. Significance difference was tested and represented $P<0.05$. All results were processed and analyzed with the SPSS computer program (SPSS Systems for Windows, Version 13.0).

Results

Stages of Anesthesia

Significant differences ($P<0.05$) in the induction and recovery stages at different concentrations of the three anesthetic agents were identified for African catfish (Table 2). Induction times decreased significantly with increasing concentrations for all the anesthetic agents evaluated. On the other hand, recovery times increased with increasing concentrations of anesthetic agents ($P<0.05$).

Induction and Recovery in Relation to Concentration

A significant correlation was observed between anesthetic concentration and induction time for all tested anesthetic agents ($P<0.05$), whereas scatter plots yielded an inverse exponential relationship (Figure 1). The regression equations of times to reach I3 and concentrations (c) of three anesthetic agents in African catfish were $I3=131344\ e^{-1,0435c}$ ($R^2=0.89$) for clove oil, $I3=533.92\ e^{-0.0017c}$ ($R^2=0.92$) for PE, and $I3=453{,}98e^{-0.0177c}$ ($R^2=0.93$) for eugenol. Similarly, a significant correlation ($P<0.05$) was observed between anesthetic concentration and times to reach R3 for all anesthetic agents, whereas scatter plots showed exponential relationships (Figure 1). The regression equations established for recovery time and concentrations were $R3=0.0015\ c^{1.7999}$ ($R^2=0.95$) for clove oil, $R3=20{,}563\ e^{0.0027c}$ ($R^2=0.89$) for PE, and $R3=187\ c^{0.0102}$ ($R^2=0.96$) for eugenol.

Post-Treatment Survival

African catfish reared in post-treatment tanks recovered well after the anesthetic experiment. No mortality was observed during post-treatment period.

Discussion

Anti-stress agents form an integral component of modern day aquaculture (Pawar *et al*., 2011). Biological factors include species, the stage of life cycle and age, size and weight, lipid content, body content and disease status. All these factors affect the metabolic rate and therefore the pharmacokinetics of the anaesthetic compound (Iversen *et al*., 2003). Environmental factors including temperature and pH also affect the metabolic rate in fish, in addition to changing the uptake across the gills, and therefore increase or decrease the efficacy of an anaesthetic

Table 2. Induction and recovery times (s) for African catfish anaesthetized with five concentrations of three anaesthetic agents. Data are presented as mean±sd

Clove oil	Concentrations (mg L^{-1})				
Stages	25	50	75	100	125
I 1	111 ± 62^{a}	68 ± 20^{b}	45 ± 19^{bc}	36 ± 12^{bc}	29 ± 5^{c}
I 2	171 ± 106^{a}	101 ± 23^{b}	60 ± 16^{bc}	52 ± 12^{bc}	39 ± 4^{c}
I 3	615 ± 68^{a}	193 ± 62^{b}	109 ± 33^{c}	91 ± 19^{c}	64 ± 8^{c}
R 1	150 ± 26^{a}	165 ± 29^{a}	186 ± 42^{a}	285 ± 44^{ab}	238 ± 95^{bc}
R 2	186 ± 21^{a}	189 ± 28^{a}	204 ± 54^{a}	271 ± 45^{b}	316 ± 84^{b}
R 3	225 ± 16^{a}	251 ± 32^{a}	301 ± 18^{b}	375 ± 26^{c}	488 ± 24^{d}
PE	Concentrations (µl L^{-1})				
Stages	250	500	750	1000	1250
I 1	104 ± 27^{a}	69 ± 20^{b}	36 ± 10^{c}	34 ± 4^{c}	32 ± 7^{c}
I 2	177 ± 75^{a}	176 ± 30^{a}	63 ± 13^{b}	49 ± 8^{b}	42 ± 7^{b}
I 3	353 ± 17^{a}	256 ± 39^{b}	145 ± 24^{c}	84 ± 6^{d}	75 ± 9^{d}
R 1	7 ± 5^{a}	34 ± 21^{b}	44 ± 6^{b}	86 ± 11^{c}	193 ± 20^{d}
R 2	9 ± 8^{a}	67 ± 20^{b}	69 ± 9^{b}	177 ± 17^{c}	365 ± 42^{d}
R 3	30 ± 15^{a}	120 ± 12^{b}	174 ± 10^{c}	361 ± 18^{d}	508 ± 21^{e}
Eugenol	Concentrations (mg L^{-1})				
Stages	25	50	75	100	125
I 1	142 ± 48^{a}	85 ± 22^{b}	37 ± 9^{c}	36 ± 6^{c}	27 ± 14^{c}
I 2	235 ± 92^{a}	145 ± 35^{b}	61 ± 13^{c}	60 ± 11^{c}	40 ± 17^{c}
I 3	302 ± 36^{a}	197 ± 29^{b}	109 ± 18^{c}	79 ± 14^{cd}	52 ± 11^{d}
R 1	163 ± 24^{a}	218 ± 28^{ab}	251 ± 65^{bc}	295 ± 61^{c}	317 ± 112^{c}
R 2	197 ± 23^{a}	268 ± 28^{bc}	315 ± 63^{cd}	331 ± 74^{cd}	361 ± 117^{d}
R 3	234 ± 17^{a}	310 ± 17^{b}	441 ± 12^{c}	552 ± 32^{d}	657 ± 53^{e}

In all lines, means with different superscripts are significantly different from each other ($P<0.05$).

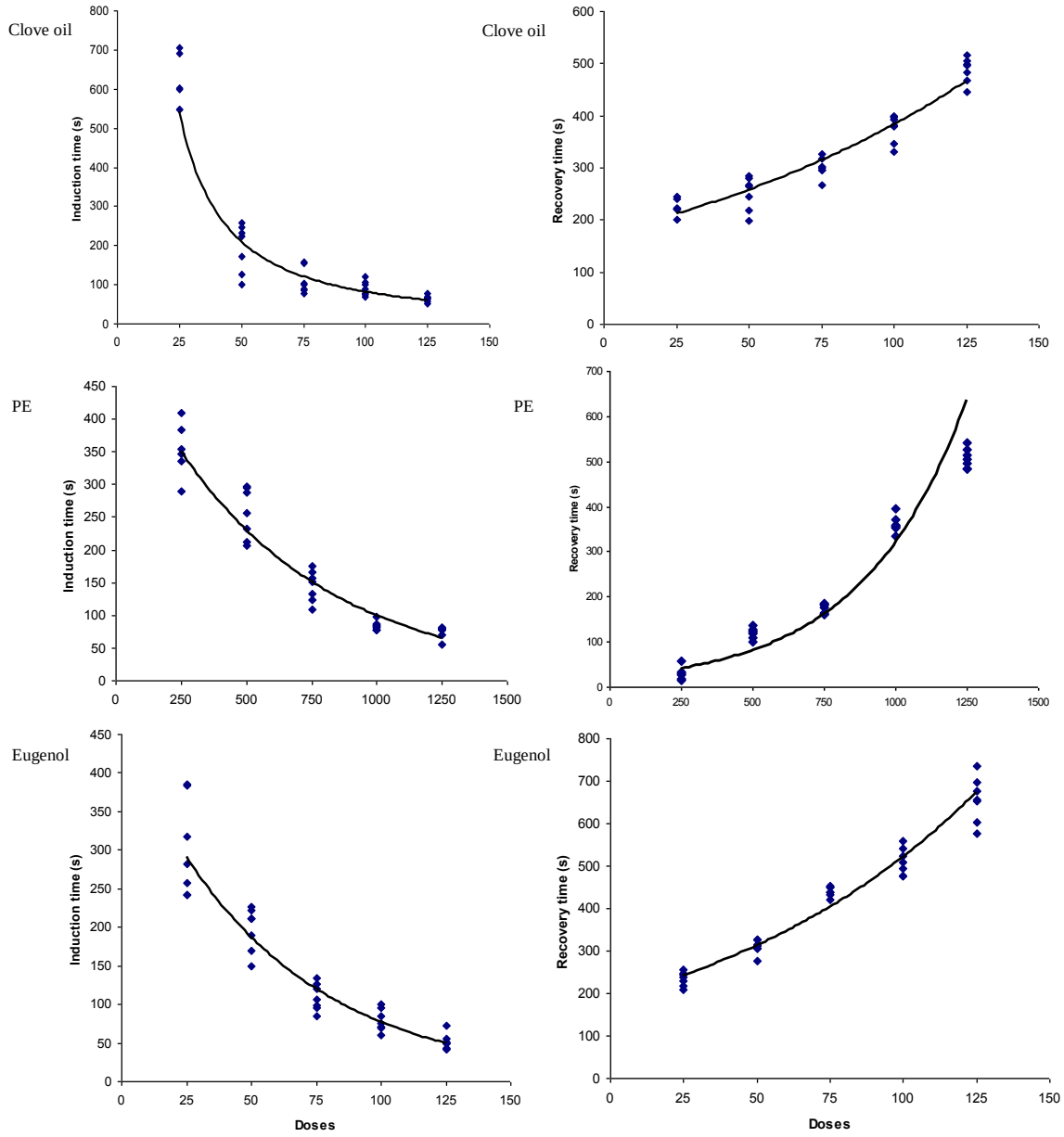

Figure 1. Induction and recovery times (s) relation to anaesthetic concentrations for African catfish (n=7 for each trial)

agent (Burka *et al*., 1997; Ross and Ross, 1999).

In the present study, the induction times decreased significantly with the increasing clove oil, 2-phenoxyethanol and eugenol concentrations ($P<0.05$). The results are in agreement with previous studies in teleost fish (Mattson and Riple, 1989; Hseu *et al*., 1998; Mylonas *et al*., 2005; Gullian and Villanueva, 2009; Weber *et al*., 2009; Heo and Shin, 2010). On the other hand, recovery times increased with increasing concentrations of anaesthetic in fingerling African catfish. Prolonged recovery with increased anaesthetic dosage has been reported in sockeye salmon (Woody *et al*., 2002) and cobia (Gullian and Villanueva, 2009). However, decreasing recovery times with an increase in concentration of clove oil and 2-phenoxyethanol for European sea bass and gilthead seabream has been reported by Mylonas *et al*. (2005). The explanation put forward by these authors is that with the highest concentration the fish is not contact with the anaesthetic for long, which allow faster recovery (Pawar *et al*., 2011). Also, differences in the physiological responses of fish to the anaesthetic agents also influence this trend (Weber *et al*., 2009).

According to Marking and Meyer (1985), the anaesthetic agent is considered effective if it produces a complete induction within 180 s and recovery with 300 s for fish. In this study, application of clove oil at concentration of 50 mg L^{-1}, 2-phenoxyethanol at concentration of 750 µl L^{-1}, and eugenol at concentration of 50 mg L^{-1} resulted in quick induction, total immobilization and fast recovery in

African catfish juvenils. Although higher concentrations of three anaesthetic agents achieved shorter induction times, aforementioned concentration were effective and presented a good margin of safety when compared against the above efficacy criteria. On the other hand, the clove oil used in the experiment was contained 67% of eugenol and the eugenol used in the experiment was pure (99%). Except lowest concentration of both anesthetics (25 mg L^{-1}), there was no significant differences between induction time of African catfish ($P>0.05$). With increasing eugenol concentrations, sedation and anesthesia induction times were reduced, but recovery times exhibited the opposite pattern with fish experiencing more rapid recovery when exposed to lower eugenol concentrations. The recovery times were significantly longer in eugenol than clove oil and 2-phenoxyethanol, except in lowest concentration ($P<0.05$). These results are in agreement with those found in other species anesthetized with eugenol or clove oil (Endo *et al*., 1972; Hikasa *et al*., 1986; Munday and Wilson, 1997; Keene *et al*., 1998; Woody *et al*., 2002; Iversen *et al*., 2003; Hoskonen *et al*., 2004). Because of longer recovery time especially in high concentrations of eugenol is not advisable for juvenile African catfish in short time applications to reduce working time and labor.

In many countries, the use of fish anesthetics is a matter of concern as there are no specific laws regulating their use (Pawar *et al*., 2011). Clove oil, 2-phenoxyethanol and eugenol have been extensively used as an anaesthetic agent in aquaculture of freshwater and marine fishes. Further studies on different life stages, gender, reproduction state and sizes, followed by assessments of the effects of anesthetics on haematological profile and respiration rate will advance our understanding of anesthesia of African catfish.

References

Burka, J.F., Hammell, K.L., Horsberg, T.F., Johnson, G.R., Rainnie, D.J. and Speare, D.J. 1997. Drugs in salmonid aquaculture. J. Vet. Pharmacol. Ther., 20: 333-349.

Coyle, S.D., Durborow, R.M. and Tidwell, H.J. 2004. Anaesthetic in Aquaculture: Southern Regional Aquaculture Center (SRAC) Publication, 3900. Texas, 6 pp.

Endo, T., Ogishima, K., Tanaka, H. and Ohshima, S. 1972. Studies on the anaesthetic eject of eugenol in some freshwater fishes. Bulletin of the Japanese Society of Scientific Fisheries, 38: 761-767.

Gilderhus, P.A. and Marking, L.L. 1987. Comparative efficacy of 16 anaesthetic chemicals on rainbow trout. N. Am. J. Fish Manag., 7: 288-292.

Gomes, L.C., Chiparri-Gomes, A.R., Lopes, N.P., Roubach, R. and Araujo-Lima, C.A.R.M. 2001. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. J. World Aqua. Sco., 32: 426-431.

Gullian, M. and Villanueva. J. 2009. Efficacy of tricaine methanesulphonate and clove oil as anaesthetics for juvenile cobia *Rachycentron canadum*. Aquaculture Res., 40: 852-860.

Heo, G.J. and Shin, G. 2010. Efficacy of benzocaine as an anaesthetic for Crucian carp (*Carassius carassius*). Vet. Anaesth. Analg., 37: 132-135.

Hikasa, Y., Takase, K., Ogasawara, T. and Ogasawara, S. 1986. Anaesthesia and recovery with tricaine methanesulfonate, eugenol and tripental sodium in the carp, *Cyprinus carpio*. Japanese Journal of Veterinary Science, 48: 341-351.

Hoskonen, P. and Pirhonen, J. 2004. Temperature ejects on anaesthesia with clove oil in six temperature-zone fishes. Journal of Fish Biology, 64: 1136-1142.

Hseu, J.R., Yeh, S.L., Chu, Y.T. and Ting, Y.Y. 1998. Comparison of efficacy of five anaesthetic goldlined sea bream, *Sparus sarba*. Acta. Zool., Taiwan, 9: 35-41.

Iversen, M., Finstad, B., Mac Kinley, R.S. and Eliiassen, R.S. 2003. The efficacy of metomidate, clove oil, Aqui-S and Benzoak as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts and their potential stress-reducing capacity. Aquaculture, 221: 549-566.

Keene, J.L.I., Noakes, D.L.G., Moocia, R.D. and Soto, C.G. 1998. The efficacy of clove oil as an anesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research, 29: 89-101.

Marking, L.L. and Meyer, E.P. 1985. Are better anesthetics needed in fisheries? Fisheries, 10: 2-5.

Mattson, N.S. Riple, T.H. 1989. Metomidate, a better anaesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol and phenoxyethanol. Aquauculture, 83: 89-94.

Mc Farland, W.N. 1960. The use of anaesthetics for the handling and the transport of fishes. California Fish Game, 46: 407-431.

Munday, P.L. and Wilson, S.K. 1997. Comparative efficacy of clove oil and other chemicals in anesthetization of *Pomacentrus amboinensis*, a coral reef fish. Journal of Fish Biology, 51:931-938.

Mylonas, C.C., Cardinaletti, G., Sigelaki, I. and Polzonetti-Magni, A. 2005. Comparative efficacy of clove oil and 2-phenoxyethanol as anaesthetics in aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus auratus*) at different temperatures. Aquaculture, 246: 467-481.

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

Ross, L.G. and Ross, B. 1999. Anesthetics and sedative techniques fro aquatic animals. Blackwell Sci., Oxford, 159 pp.

Theinpoint, D. and Niemegeers, C.J.E. 1965. 7464- a new potenet anesthetics in fish. International Zoo. Year Book, 5: 202-205.

Treves-Brown, K.M. 2000. Anaesthetics in applied fish pharmacology. Kluwer Academic Publishers, Dordrecht, Netherlands: 206-217.

Tytler, P. and Hawkins, A.D. 1981. Vivisection, anesthetics and minor surgery. In: A.D. Hawkins (Ed.) Aquarium Sys. Academic Press, New York: 247-278.

Velisek, J., Wlasow, T., Gomulka, P., Svobodava, Z., Novotny, L. and Ziomek, A. 2006. Effects of clove oil anaesthesia on catfish (*Siluris glanis* L.). Acta. Vet. Brno., 75: 99-106.

Velisek, J., Stara, A., Li, Z.H., Silovska, S. and Turek, J. 2011. Comparison of effects of four anaesthetics on blood chemical profiles and oxidative stress biomarkers in rainbow trout. Aquaculture, 310: 369-375.

Woody, C.A., Nelson, J. and Ramstad, K. 2002. Clove oil as an anaesthetic for adult sockeye salmon: field trails. Journal of Fish Biology, 60: 3450-347.

Weber, R.A., Peleterio, J.B., Garcia Martin, L.O. and 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: 147-150.

Zar, J.H. 1999. Bio-statistical Analysis, 4th Edition. Prentice Hall, New Jersey, 929 pp.