www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 11: 463-467 (2011) DOI: 10.4194/1303-2712-v11_3_17

Effect of Exposure Duration on Time to Recovery from Anaesthesia of Clove Oil in Juvenile of Russian Sturgeon

Bilal Akbulut[1,*], Eyüp Çakmak[1], Nilgün Aksungur[1], Yahya Çavdar[1]

[1] Central Fisheries Research Institute, Adil Yazar St., Kaşüstü, Yomra, Trabzon, Turkey.

* Corresponding Author: Tel.: +90.533 8180149; Fax: +90.462 3411152;
E-mail: bilbulut@yahoo.com

Received 27 September 2009
Accepted 7 April 2011

Abstract

The efficacy of clove oil as an anaesthetic was evaluated in Juvenile of Russian sturgeon (*Acipenser gueldenstaedtii*) under two experiments. Dose response examined using four anaesthetic concentrations varying from 0.22 to 0.90 g/L of clove oil and effect of exposure duration on the recovery time analysed for 5 and 10 min. Mean body length (cm), 24.9±1.37 and weight (g) 63.2±10.89, n=120, juvenile Russian sturgeon placed individually to the anaesthetic bath prepared with clove oil, and induction (I1: Sedation and I2: Loss of equilibrium) and recovery times (R1: Regain of equilibrium and R2: Sedation) recorded in seconds. Allowing fish remain in the anaesthesia after fish reaching anaesthesia stage, time to recovery from anaesthesia recorded. Fish exposed to clove oil failured to respond to external stimuli and reached to anaesthesia, sympocal stage in <3 min and recovered in <5 min at all doses except 0.22 g/L dose of clove oil. Exposure five and ten minute to anaesthesia at the 0.22, 0.45 and 0.90 g/L concentrations, fish recovered from anaesthesia in <10 min, except 0.90 g/L. The higher dose of clove oil resulted in significantly shorter induction time and prolonged recovery time. While correlation between induction time and dose determined as negative, r= -0.594 and a positive correlation has been found between dose and recovery time, r=0.422 (P<0.05). The study demonstrated that clove oil can be used as an effective anaesthetic measuring length and weight of juvenile Russian sturgeon and dose and exposure duration effect on time to recovery from anaesthesia of clove oil.

Keywords: Anaesthesia, clove oil, dose response, exposure duration, recovery time.

Karaca Mersinlerinin Karanfil Yağı ile Bayıltılmasında Anesteziye Maruz Kalma Süresinin Ayılmaya Etkisi

Özet

Karaca mersinlerinde (*Acipenser gueldenstaedtii*) karanfil yağının anestetik etkisi iki çalışma ile ölçülmüştür. Doz tepkisi 0,22'den 0,90 g/L'ye kadar değişen dört farklı konsantrasyonda ölçülmüş ve anesteziye 5 ve 10 dakika maruz kalmanın anesteziden kurtulmaya etkisi incelenmiştir. Ortalama boyları 24,9±1,37 cm ve ağırlıkları 63,2±10,89 g olan karaca mersinleri karanfil yağı ile hazırlanan bayıltıcı solüsyona tek tek yerleştirilmiştir. Her balığın bayılma (I1: hafif bayılma ve I2: tam bayılma) ve ayılma (R1: hafif ayılma ve R2: tam ayılma) süreleri saniye düzeyinde kayıt edilmiştir. Balıklar tam bayıldıktan sonra anestetik solüsyon içinde bekletilmiş ve ayılma süreleri kayıt edilmiştir. Karanfil yağına maruz kalan balıklar, 0,22 g/L hariç diğer dozlarda 3 dakikadan az sürede bayılarak dış uyarıya tepki vermemiş ve beş dakikadan az sürede ayılmışlardır. Anesteziye 5 ve 10 dakika maruz kalan balıklar 0,22, 0,45 ve 0,90 g/L dozlarda, 0,90 g/L doz hariç, 10 dakika içinde ayılmışlardır. Karanfil yağı dozu arttıkça bayılma süresi kısalmış ve ayılma süresi uzamıştır. Bayılma süresi ile doz arasında negatif r= -0,594 ve doz ile ayılma süresi arasında pozitif r=0,422 (P<0,05) ilişki bulunmuştur. Bu çalışma karanfil yağının karaca mersinlerinin boylarının ölçülmesi ve tartılmasında kullanılabilecek etkili bir anestetik olduğunu, doz ve maruz süresinin anesteziden kurtulma süresini etkilediğini göstermiştir.

Anahtar Kelimeler: anestezi, karanfil yağı, doza tepki, maruz süresi, ayılma süresi.

Introduction

Anaesthetics are often used in aquaculture, fisheries and biological researches as a way to minimize fish hyper-motility, which is a considerable source of injuries during handling procedures. The consequent damages from such accidents succumbed fish to increase the susceptibility to pathogens and infection diseases. Therefore, reducing fish motility by anaesthetics may decrease the undesirable

handling consequences (Ross and Ross, 2008).

Various handling procedures frequently expose fish to a variety of acute stressors that have the potential to negatively affect fish performance and survival (Barton, 1997; Barton, 2000; Iverson *et al*., 2003). It is necessary to minimize stress or mitigate the effects of its on fish (Berka, 1986) and to reduce physical injury, mortality, pain and immobilize animals (Summerfelt and Smith, 1990; Soto and Burhanuddin, 1995; Wagner *et al*., 2002).

Anaesthetics are used for measuring length and weight of fish (Soto and Burhanuddin, 1995), to minimize handling stress (Wagner *et al*., 2002) and aid to the handling of fish during practices that include tagging (Kennedy *et al*., 2007).

When choosing an anaesthetic, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment (Soto and Burhanuddin, 1995), and the choice may also depend on the nature of the experiment and species of fish (Summerfelt and Smith, 1990; Munday and Wilson, 1997).

A variety of anaesthetics including MS-222 (tricane methane sulfonate), 2-phenoxyethanol, isobutanol, methyl pentynol, xyaline, sodium becarbonate, pentobarbitol and clove oil have been used in fish culture (Marking and Meyer, 1985; Soto and Burhanuddin, 1995; Wagner *et al*., 2002; Altun *et al*., 2009; Uçar and Atamanalp, 2010).

Clove oil is actually a mixture of different compounds. The three significant active ingredients are eugenol, isoeugenol and methyleugenol. 'Clove oil' is 85 to 95% eugenol. Isoeugenol and methyleugenol make up 5 to 15% of the remaining ingredients (FDA, 2002).

Eugenol (4-allyl-2-methoxyphenol) is obtained from the buds, leaves and stems of clove tree (*Eugenia aromatica* or *caryophyllus*) has multiple uses mainly in dentistry and medicine as an antiseptic, analgesic and anaesthetic agent (Kramer, 1985; Karapmar and Aktuğ,1987; Pulla and Lokesh, 1992; Kamble and Patil, 2008; Ross and Ross, 2008).

The clove oil is a natural product, and is less expensive and more potent than other anaesthetics used in fish. In addition, it has been shown to be safe for humans (Miller *et al*., 1989) and the U.S. Food and Drug Administration has classified it as a generally considered as safe (GRAS) compound (Summerfelt and Smith, 1990). Gomulka *et al*. (2008) reported that the use of eugenol (0.075 ml/L) does not cause irreversible damage in Siberian sturgeon (*Acipenser baerii*).

Clove oil has been used as fish anaesthetic in many countries with economic advantages and no apparent toxic properties (Soto and Burhanuddin, 1995; Munday and Wilson, 1997; Woody *et al*., 2001). Despite the common use of anaesthetics in fish, there is little information about their influence on the sturgeon organism.

Current aquaculture of sturgeon is an economically viable means of sustainable, commercial caviar production. Mean rearing problems occurred in the handling and/or transport of sturgeon. Considering their size and value, importance of anaesthesia is by far highest. Hence, in this study efficiency of clove oil at different dose levels and effect of exposure duration onto recovery time from anaesthesia tested on the juvenile of Russian sturgeon.

Materials and Methods

Preparation of Anaesthetic Solutions

Clove oil in the 20 ml glass bottles is marketed in the herbalist for general human usage was preferred as anaesthetic. The density of clove oil is 0.916 kg/L (Karden, Turkey). The clove oil stock solution was prepared by dissolving clove oil with 95% ethanol (1:10 ratio of clove oil-ethanol) as described by Anderson *et al*. (1997) to facilitate mixing. Ethanol has no known anaesthetic properties on fishes at low doses (Anderson *et al*., 1997; Munday and Wilson, 1997).

The solution was then, diluted in 50 ml fresh water to achieve complete dissolution of the anaesthetic. The stock solution was newly made on a day-to-day basis and was kept in the dark at the ambient temperature. The clove oil, previously dissolved in ethanol and fresh water, were added to 15 L test bucket filled with 5 L test water 5-6 min prior to the introduce to the fish, in order to allow complete dissolution of the anaesthetic (Anderson *et al*., 1997).

To examine the effect of ethanol exposure, a group of 10 fish was transferred by net into a 30 L tank with 10 ml/L of ethanol and was observed for 2 hours. New anaesthetic bath prepared for each test.

Experimental Fish and Condition

Main fish stock was maintained in eighteen square tanks, provided with a flow-through supply of aerated freshwater at a temperature of 22-23°C in the recirculation system. The water temperature in the buckets was maintained constant during the experiments. Throughout the experiment, the temperature, pH and dissolved oxygen were recorded with oxygen probe (YSI 556, USA).

Dose Response

These experiments were conducted with juvenile Russian sturgeon in 15 L plastic buckets filled with 5 L of continually aerated fresh water at the appropriated temperature. Final doses of the test water adjusted to the 0.22, 0.45, 0.67 and 0.90 g/L.

Exposure Duration

The time to recovery from anaesthesia was

examined in juvenile Russian sturgeon exposed to the 0.22, 0.45 and 0.90 g/L doses of clove oil in the fresh water. To determine the effect of exposure duration to anaesthesia, fish allowed remaining in the anaesthetic for 5 and 10 minutes.

Measurement of Induction and Recovery Time

Measurement of induction to and recovery times from anaesthesia were modified from earlier studies (Table 1). Approximately 10 fish for per dose and exposure duration were captured from one of the 300 L tanks, placed in a 15 L bucket filled with 10 L fresh water and allowed to calm for 3-5 min. All tests fish placed individually in to the anaesthetic baths supplied with aeration. Two observers, one for each fish, were recording the time required to reach induction stages (I1, I2) of anaesthesia and recovery times described as stage R1 and R2 to the nearest second using a chronometer (Table 1). At the dose response experiment, when the fish reached syncopal stage (S), fish removed from anaesthetic bath by net. In case of exposure duration experiment, fish allowed to remain in the anaesthetic for 5 and 10 minutes. The body weights (g) to nearest 0.01 g and lengths (cm) to the nearest 0.1 cm of specimens were measured. After length and weight measurement, fish placed into the 50 L tank with filled 30 L continuously aerated fresh water. The recovery tank water was changed for every 10 fish to ensure that fish were always in contact with clean water. According to the Table 1, once recovered, fish grouped according to dose and exposure duration were transferred into 300 L tank and were monitored for survival and abnormal behaviour and no fish mortality was observed during experiment and next 5 days. The dose response experiment was repeated three times and the exposure duration experiment was repeated two times.

During experiments dissolved oxygen of all test water not reduced below 6.0 mg/L. Temperature, pH and dissolved oxygen were measured in the trails ranged from 22 to 23°C, 6.94 to 7.79 and 6.18 to 7.41 mg/L respectively.

Statistical Methods and Calculations

Statistical tests were carried out using SPSS 16.0 for Windows. One-way Analysis of Variances were performed to test effect of doses and Univariate analysis of variance (ANOVA) were performed to test for significant differences in recovery times due to the duration exposure time, dose, and the interaction between those two variables. Tukey's procedure was used to make subsequent pairwise comparisons. Significant differences were established 0.05 level for differences among the groups. Spearman test was used to determine correlation between dose and time to anaesthesia and recovery from anaesthesia.

Results

There was not significant difference for lengths ($P=0.241$, $P<0.05$,) and weights ($P=0.296$, $P<0.05$) of Russian sturgeon used within dose and exposure duration trails.

Dose Response

All fish exposed to 0.45, 0.67 and 0.90 g/L dose of clove oil at the experiment were anaesthetized reaching the syncopal (S) stage and recovered from anaesthesia. All fish used in the dose and exposure duration trails reached stage I1 as lost of equilibrium, but give reaction to external stimuli in 63.0±14.40 s. The fish used in the 0.22 g/L lost equilibrium, but not reached to the syncopal stage. At this dose all fish moved during measurement while the other doses the fish weight (g) and length (cm) were easily measured suggesting the syncopal stage of anaesthesia was sufficient to routine procedures such as biometry.

Once dose of anaesthesia increased induction time decreased, contrary this tendency recovery time from anaesthesia lengthened as it expected (Figure 1). Summary statistics of induction and recovery times at different doses of clove oil for juvenile of Russian sturgeon was given in the Table 2 that the values expresses as means±SEM., $P<0.05$, a, b and c symbols indicate difference between doses. Table 2 demonstrates the time required to reach the syncopal

Table 1. Stages of anaesthesia and recovery from anaesthesia employed as endpoints in the some study (modified from Schoettger and Julin, 1967; Summerfelt and Smith, 1990; Keene *et al*., 1998)

Phase of Anaesthesia	Stage	Description	Notable behaviour of fish
Induction	I1	Sedation	Fish swimming, reaction to external stimuli.
	I2	Loss of equilibrium	Swimming ability stops, Partial loss of equilibrium, reaction to external stimuli.
Syncopal	S	Unconscious	No movement, Loss of reflex activity, no reaction to external stimuli, slow and irregular opercular ventilation.
Recovery	R1	Regain of equilibrium	No movement and reaction to external stimuli
	R2	Sedation	Partial recovery of equilibrium, regular opercular ventilation, reaction to strong external stimuli, not swimming.
Normal	-	-	Recovery of equilibrium, fish swimming.

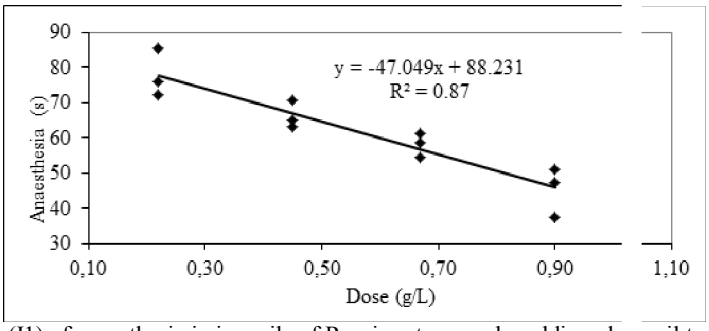

Figure 1. Induction time (I1) of anaesthesia in juvenile of Russian sturgeon by adding clove oil to the water.

Table 2. Time required to entire induction to and recovery from anaesthesia in the juvenile of Russian sturgeon (P<0.05, mean±SEM.)

Dose (g/L)	Weight (g)	Induction time (s)		Recovery time (s)	
		I1	I2	R1	R2
0.20	62.4±6.97	85.30±6.62^{a}	-	-	93.80±4.42^{a}
0.45	69.0±12.25	70.6±4.36^{ab}	144.7±15.69^{a}	67.90±4.96^{a}	119.7±10.31^{ab}
0.67	68.9±13.12	67.3±3.66^{bc}	119.7±10.31^{ab}	95.00±11.02^{ab}	154.9±7.32^{bc}
0.90	64.5±7.20	50.90±2.51^{c}	86.70±10.73^{b}	108.10±8.97^{b}	162.90±12.75^{c}

stage of anaesthesia and recovery time from anaesthesia. Time to anaesthesia were negatively (P<0.05, r=-0.594) and time to recovery form anaesthesia were positively (P<0.05, r= 0.422) related to doses.

Effect of Exposure Duration to Anaesthetics on Recovery Time

The time to recovery from anaesthesia was examined in the second experiment, the fish exposed for 5 and 10 min to the 0.22, 0.45 and 0.90 g/L doses of clove oil. The fish allowed to remain in the anaesthetic after reaching syncopal stage prolonged the time to recovery from anaesthesia. The increase in recovery time is in response to increasing exposure duration (Table 3). From analysis of data, it was observed that there were statistically significant relation between duration of exposure time and recovery time (P=0.002, P<0.05), but the joint effect of duration of exposure time and dose on recovery time (P=0.321, P>0.05) were not statistically significant.

The fish from all exposure recovered well after the anaesthetic experiment, and were feeding and behaving normally within 1 day. No mortality was observed in the following 5 days.

Discussion

Clove oil as an anaesthetic for fish needs to be assessed according to the criteria listed previously: efficacy, availability, easy of use, cost, and side effects on fish, humans and the environment. There is no simple definition of efficacy of anaesthetics in fish and many papers that were published regard efficacy (Iversen *et al*., 2003; Pirhonen and Schreck, 2003; Velisek *et al*., 2005; Mylonasa *et al*., 2005; Altun *et al*., 2009).

Taylor and Roberts (1999) examined time to loss of equilibrium to immobilization for rainbow trout exposed to clove oil from three sources and reported that means among sources were not significantly different. In this study, wildly used for human consumption clove oil preferred, because, it can be able to find easily and also is the cheapest for fish farmer and other user.

The use of clove oil may not be appropriate for some studies and very little is actually known of its effects on fish physiology (Anderson *et al*., 1997). But Cooke *et al*. (2004) examined the behavioural and physiological responses of largemouth bass (*Micropterus salmoides*) to a gradation of clove oil concentrations (0 to 20 mg/L) while exposed to truck transport. Wagner *et al*. (2002) compared three anaesthetics: tricaine methanesulfonate (MS-222), clove oil to determine physiological stress responses (plasma cortisol, glucose and chloride) of rainbow trout (*Oncorhynchus mykiss*) broodstock and reported that fish anesthetized with clove oil had significantly lower cortisol concentrations at 1 or 7 h post immersion than the other anaesthetics. Tort *et al*. (2002) tested clove oil as anaesthetic for haematology and stress indicators in the gilthead sea bream and rainbow trout and reported that clove oil does not block the cortisol response to stress; as happens with

Table 3. Effect of exposure duration to the recovery time (means±SEM in second)

Dose (g/L)	Exposure Duration (min)					
	5			10		
	R1	R2	R	R1	R2	R
0.22	-	-	122.4±13.69	-	-	198.0±47.33
0.45	90.2±10.89	165.3±16.01	255.5±24.99	168.0±41.84	359.1±93.23	527.1±134.60
0.90	245.2±22.95	381.1±38.11	626.3±57.54	334.2±21.09	463.6±20.87	797.8±35.10

other anaesthetics.

Taylor and Roberts (1999) examined efficacy of clove oil on juvenile and subadult white sturgeon (*Acipenser transmontanus*) and reported that the mean induction and recovery time for 1000 mg/L determined as 1.5 and 16.3 min respectively. Ross and Ross (2008) reported that increases in water temperature have a significant effect on the time induction and recovery time. When common carp and steelhead fry were anaesthetized clove oil, higher temperatures cause enhanced anaesthetic effects and shorter recovery times (Hikasa *et al*., 1986; Woolsey *et al*., 2004). This result is consistent with those of other published studies involving juvenile and adult fish (Hikasa *et al*., 1986; Anderson *et al*., 1997; Mylonasa *et al*., 2005). The mean induction data from the current study corroborate these data of Taylor and Roberts (1999), but time to recovery is shorter.

The temperature preferred in this study is optimal for sturgeon culture and recommended dissolved oxygen level is above 6.0 mg/L (Hochleithner and Gessner, 2001; Memiş *et al*., 2009).

The study present here indicates that the clove oil is an effective anaesthetic for measuring length and weight of juvenile Russian sturgeon. All test concentrations, 0.22, 0.45, 0.67 and 0.90 g/L, except 0.22 g/L dose, met the efficacy criteria specified by Marking and Meyer (1985) for handling within 3 min, recovery in 5 min and no mortality. The 0.22 g/L dose of clove oil is not suitable to use in the measurement of juvenile Russian sturgeon. But this dose can be used for transport or grading of Russian sturgeon.

Soto and Burhanuddin (1995) and Anderson *et al*. (1997) used short exposure times; when fish reached the desired level of anaesthesia, they were immediately returned to freshwater. In this study, fish exposed to clove oil for 5 and 10 min. Allowing to remain clove oil for 5 and 10 min after fish reaching anaesthesia, syncopal stage, effect on recovery time from anaesthesia. In the aquaculture industry, fish are rarely left in an anaesthetic solution for more than 10 min, so low doses of clove oil can provide a large temporal safety margin. Measuring length and weight of juvenile Russian sturgeon dose not take 5 or 10 minute, but it can be necessary to scrutinize of fish for rearing activities and/or other examinations.

During the experiments of this study no evident damages were observed for fish when they exposed to clove oil. However, further studies should be carried out to evaluate the metabolic effects of clove oil, and additional data are necessary in order to assess the effects on food intake, appetite and growth.

Acknowledgements

The study was carried out in the Central Fisheries Research Institute, RAS system. Russian sturgeon eggs were provided by FAO, TCP/TUR/3202 project.

Reference

Altun, T., Bilgin, R. and Danabaş, D. 2009. Effects of Sodium Bicarbonate on Anaesthesia of Common Carp (*Cyprinus carpio* L., 1758) Juveniles. Turkish Journal of Fisheries and Aquatic Sciences, 9: 29-31. doi:10.4194/trjfas.2009.005.

Anderson, W.G., McKinlay, R.S. and Colaveccia, M. 1997. The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management, 17: 301–307.

Barton, B.A. 1997. Stress in finfish: past, present and future-a historical perspective. In: G.K. Iwama, A.D. Pickering, J.P. Sumpter and C.B. Schreck (Eds.), Fish Stress and Health in Aquaculture, Society for Experimental Biology Seminar Series 62. Cambridge Univ. Press, Cambridge: 1-33.

Barton, B.A. 2000. Stress. In: R.R. Stickney (Ed.), Encyclopedia of Aquaculture. John Wiley and Sons, New York: 892-898.

Berka, R. 1986. The Transport of Live Fish: a Review. FAO Report. EIFAC Technical Paper 48. United Nations, Rome, 52 pp.

Cooke, S.J., Suski, C.D., Ostranda, K.G., Tufts, B.L. and Wahl, D.H. 2004. Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting large mouth bass (*Micropterus salmoides*). Aquaculture, 239: 509-529. doi:10.1016/j.aquaculture.

FDA. 2002. Guidance for Industry Status of Clove Oil and Eugenol for Anesthesia of Fish. Technical paper.

Gomulka, P., Wlasow, T., Velíšek, J., Svobodová, Z. and Chmielinska, E. 2008. Effects of Eugenol and MS-222 Anaesthesia on Siberian Sturgeon *Acipenser baerii* Brandt. Acta Vet. Brno, 77: 447-453.

Hikasa, Y., Katsuaki, T., Ogasawara, T. and Ogawara, S. 1986. Anesthesia and recovery with tricaine methanesulfonate, eugenol, and thiopental sodium in the carp, *Cyprinus carpio*. Japanese Journal of Veterinary Research, 48: 341–351. doi:10.2754/avb200877030447.

Hochleithner, M. and Gessner, J. 2001. The Sturgeons and

Paddlesfishes of the World: Biology and Aquaculture, Aqua Tech Publications, Kitzbühel, 100-206.

Iversen, M., Finstad, B., McKinlay, R.S. and Eliassen, R.A. 2003. The efficacy of metomidate, clove oil, AQUI-STM and Benzoak1 as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture, 221: 549-566. doi:10.1016/S0044-8486(03)00111-X.

Kamble, V.A. and Patil, S.D. 2008. Spice-Derived Essential Oils: Effective Antifungal and Possible Therapeutic Agents. Journal of Herbs, Spices & Medicinal Plants, 14(3): 129-143. doi:10.1080/10496470802598677.

Karapmar, M. and Aktuğ, S.E. 1987. Inhibitation of foodborne pathogens by thymol, eugenol, menthol and anethole. International Journal of Food Microbiology, 4:161-166.

Keene, J.L., Noakes, D.L.G., Moccia, R.D. and Soto, C.G. 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research, 29: 89-101. doi:10.1111/j.1365-2109.1998.tb01113.x.

Kennedy, B.M., Gale, W.L. and Ostrand, K.G. 2007. Evaluation of clove oil concentrations for use as an anesthetic durring field processing and passive integrated transponder implantation of juvenile steelhead. Northwest Science 81(2):147-154. doi: 10.3955/0029-344X-81.2.147.

Kramer, R.E. 1985. Antioxidants in clove. Journal American Oil Chemist's Society, 62: 111-113. doi:10.1007/BF02541505

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

Memiş, D., Ercan, E., Çelikkale, M.S., Timur, M. and Zarkua, Z. 2009. Growth and Survival Rate of Russian sturgeon (*Acipenser gueldenstaedtii*) larvae from fertilized eggs to artificial feeding. Turkish Journal of Fisheries and Aquatic Sciences, 9: 47-52.

Miller, K.W., Firestone, L.L., Alifimoff, J.K. and Streicher, P. 1989. Nonanesthetic alcohols dissolve in synaptic membranes without perturbing their lipids. PANS, 86(3): 1084-1087.

Munday, P.L. and Wilson, S.K. 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. Journal of Fish Biology, 51: 931-938. doi:10.1111/j.1095-8649.1997.tb01532.x.

Mylonas, C.C., Cardinaletti, G., Sigelaki, I. and 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 aurata*) at different temperatures. Aquaculture, 246: 467-481. doi:10.1016/j.aquaculture.2005.02.046.

Pirhonen, J. and Schreck, C.B. 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. doi:10.1016/S0044-8486(02)00624-5

Pulla, R.A.C. and Lokesh, B.R. 1992. Studies on spice prenciples as antioxidants in the inhabitation of lipid peroxidation of rat liver microsomes. Molecular and Cellular Biochemistry, 111: 117-124.

Ross, L.G. and Ross, B. 2008. Anaesthetic and Sedative Techniques for Aquatic Animals. Blackwell Puplishing, 3rd Edition, UK: 41-88.

Schoettger, R.A. and Julin, M. 1967. Efficacy of MS-222 as an anesthetic on four salmonids. Invest. Fish Contr., U.S. Dept. Int., 13: 1-15.

Soto, C.G. and Burhanuddin, S. 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). Aquaculture, 136: 149-152. doi:10.1016/0044-8486(95)01051-3.

Summerfelt, R.C. and Smith, L.S. 1990. Anaesthesia, surgery, and related techniques. C.B. Schreck and P.B. Moyle (Eds.), Methods for Fish Biology, American Fisheries Society, Bethesda, MD: 213-272.

Taylor, P.W. and Roberts, S.D. 1999. Clove oil: an alternative anaesthetic for aquaculture. N. Am. J. Aquac., 61: 150-155. doi: 10.1577/1548-8454(1999)061.

Tort, L., Puigcever, M., Crespo, S. and Padros, F. 2002. Cortisol and haematologycal respose in the sea bream and trout subjected to the anaesthetics clove oil and 2-phenoxyethanol. Aquaculture Research, 33: 907-910. doi:10.1046/j.1365-2109.2002.00741.x.

Uçar, A. and Atamanalp, M. 2010. The Effects of Natural (Clove Oil) and Synthetical (2-phenoxyethanol) Anesthesia Substances on Hematology Parameters of Rainbow Trout (*Oncorhynchus mykiss*) and Brown Trout (*Salmo trutta fario*). Journal of Animal and Veterinary Advances, 9(14): 1925-1933. doi:10.3923/javaa.2010.1925.1933

Velísek, J., Svobodová, Z. and Piaâková, V. 2005. Effects of Clove Oil Anaesthesia on Rainbow Trout (*Oncorhynchus mykiss*). Acta Vet. Brno., 74: 139-146.

Wagner, E., Arndt, R. and Hilton, B. 2002. Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anaesthetized with clove oil, tricaine methanesulfonate or carbon dioxide. Aquaculture, 211: 353-366. doi:10.1016/S0044-8486(01)00878-X.

Woody, C.A., Nelson, J. and Ramstad, K. 2001. Clove oil as an anaesthetic for adult sockeye salmon: field trails. Journal of Fish Biology, 60:2, 340-347. doi: 10.1111/j.1095-8649.2002.tb00284.x.

Woolsey, J., Holcomb, M. and Ingermann, R.L. 2004. Effect of Temperature on Clove Oil Anesthesia in Steelhead Fry. North American Journal of Aquaculture, 66:35-41. doi:10.1577/A03-008.