Turkish Journal of Fisheries and Aquatic Sciences 12: 667-673 (2012)
www.trjfas.org
ISSN 1303-2712
DOI: 10.4194/1303-2712-v12_3_15

Effect of Anaesthesia with Clove Oil and Benzocaine on Feed Intake in Siberian Sturgeon (*Acipenser baerii* Brandt, 1869)

Bilal Akbulut[1,]*, Eyüp Çakmak[1], Osman Tolga Özel[1], Nafiye Dülger[1]

[1] Central Fisheries Research Institute, Vali Adil Yazar St. No:14, Kaşüstü, Yomra, Trabzon, Turkey.

* Corresponding Author: Tel.: +90.462 3411053; Fax: +90.462 3411152;
E-mail: bakbulut@sumae.gov.tr

Received 19 December 2011
Accepted 23 July 2012

Abstract

The aim of this study wasto determine the optimal dose and the time required to reach a stable level of anaesthesia using clove oil and benzocaine on Siberian sturgeon fry (*Acipenser baerii* Brandt, 1869) with a mean length of 10.0±0.93 (7.2-12.0) cm and weight of 4.1±0.95 (2.1-6.4) g, and to compare the effects of them on feed intake after anaesthesia. In addition, the survival time and relationship between exposure duration and recovery time in 96 min with the optimal concentrations of these two anaesthetics were examined as part of the anaesthesia procedure. Effective concentrations (EC_{50}) were estimated to be 356 (330-381) mg/L of clove oil and 37 (33-40) mg/L of benzocaine for 3 minutes with 95% confidence limits. The recovery time with clove oil was longer than with benzocaine at 6, 12, 24 and 48 minutes ($P<0.05$). The mean lethal times (MLT_{50}) were estimated as 37.2 (5.1-69.3) min for clove oil and 63.0 (6.1-120.0) min for benzocaine with 95% confidence limits. Feed intake in fish treated with clove oil and benzocaine occurred within ten minutes, but the effect of both anaesthetics on feed intake continued for 4 h. However, it was determined that the effect of clove oil on feed intake was less than that of benzocaine.

Keywords: Siberian sturgeon, Anaesthesia, clove oil, benzocaine, feed intake.

Sibirya Mersini (*Acipenser baerii* Brandt, 1869)'nde Benzokain ve Karanfil Yağı Kullanımının Yem Alımına Etkisi

Özet

Bu araştırmanın amacı, Sibirya mersin balıklarının (*Acipenser baerii* Brandt, 1869) bayıltılması için en uygun karanfil yağı ve benzokainin dozlarını belirlemek, anesteziye maruz kalma süresinin ayılma süresine etkisini incelemek ve bu bayıltıcıların anesteziden sonra yem alımına etkilerini karşılaştırmaktır. Ayrıca, anestezi işleminin bir parçası olarak bu iki bayıltıcının en uygun konsantrasyonlarında 96 dakika içinde yaşama suresi ve anesteziye maruz kalma suresi ile ayılma suresi arasındaki ilişki incelenmiştir. Çalışmada ortalama boyları 10,0±0,93 (7,2-12,0) cm ve ağırlıkları 4,1±0,95 (2,1-6,4) g olan yavru balıklar kullanılmıştır. Bu balıkların karanfil yağı ve benzokain ile 3 dakika içinde bayılmaları için etkili konsantrasyonlar (EC_{50}) %95 güven sınırları ile sırasıyla 356 (330-381) mg/L ve 37 (33-40) mg/L olarak tahmin edilmiştir. Karanfil yağı ile bayıltılan balıkların ayılma suresi 6, 12, 24 ve 48 dakikalarda benzokainden daha uzun olmuştur ($P<0,05$). Karanfil yağı ve benzokain için ortalama ölüm süresi %95 güven sınırları ile sırasıyla 37,2 (5,1-69,3) ve 63,0 (6,1-120,0) dakika olarak tahmin edilmiştir. Karanfil yağı ve benzokain ile bayıltılan balıklar 10 dakika içinde yem almaya başlamış, fakat her iki anestezinin de yem alımına etkisi dört saate kadar devam etmiştir. Ama karanfil yağının yem alımına etkisinin benzokainden daha az olduğu belirlenmiştir.

Anahtar Kelimeler: Sibirya mersini, bayıltma, karanfil yağı, benzokain, yem alımı.

Introduction

In aquaculture, many anaesthetics have been used to immobilise fish. The most commonly used fish anaesthetics are tricaine methanesulphonate (MS-222), benzocaine, carbon dioxide, clove oil, AQUI-S, quinaldine, quinaldine sulphate, 2-phenoxyethanol, Metomidate and Etomidate (Marking and Meyer, 1985; Ross and Ross, 2008).

The ideal characteristics of anaesthetic agents have been remarked upon by numerous authors, usually when considering chemical methods (Marking and Meyer, 1985). In general, anaesthesia or sedation should be induced rapidly, preferably in less than 3 minutes, and with minimum accompanying hyperactivity or other stress (Ross and Ross, 2008). When selecting an anaesthetic for a particular purpose, the user may want to consider properties such as the convenience of use, safety for the fish, humans and the environment, effectiveness, physiological perturbations, economic aspects and commercial availability. In addition, the substance should be easily soluble in water or in a water-soluble solvent which can be used as a vehicle.

One of the most commonly used anaestheticsis clove oil, which is relatively new as a fish anaesthetic. In recent research, the use of clove oil for fish anaesthesia has gained popularity among other anaesthetics. It is extracted from the flowers, leaves and stalks of the clove tree (*Eugenia aromaticum* (i.e. *Syzygium aromaticum*) or *Eugenia caryophyllata* (Ross and Ross, 2008)). Its main active ingredients include eugenol (76.8-88.58%), eugenyl acetate (1.2-5.62%) and β-caryophyllene (1.39-17.4%) (Jirovetz *et al*., 2006; Chaieb *et al*., 2007). Eugenol has a multitude of properties which make it useful in a wide variety of applications, including as an antioxidant (Kramer, 1985; Pulla and Lokesh, 1992), as an antifungal agent (Kamble and Patil, 2008), as an antibacterial agent (Karapmar and Aktug, 1987; Kouidhi *et al*., 2010) and as an antiparasitic agent (Machado *et al*., 2011).

The International Union of Pure and Applied Chemistry (IUPAC) name for eugenol is 2-methoxy-4-prop-2-enyl-phenol. This compound has been used traditionally as a topical anaesthetic for toothaches, headaches and joint pain (Soto and Burhanuddin, 1995; Ross and Ross, 2008). Clove oil is also used as a food additive; as an organic substance, it does not require any withdrawal period and is readily available from health food stores.

Another widely used anaesthetic is benzocaine, which is the ethyl ester of p-aminobenzoic acid (PABA); it can be prepared from PABA and ethanol by Fischer esterification or via the reduction of ethyl p-nitrobenzoate. Its chemical name (IUPAC) is ethyl p-aminobenzoate or ethyl 4-aminobenzoate; it is a colourless crystal or a white, crystalline powder and is odourless. It is almost totally insoluble in water and must first be dissolved in either acetone or ethanol (Ross and Ross, 2008; WHO, 2009).

Several studies describe the physiological effects of different anaesthetics (Soivio *et al*., 1977; Cho and Heath, 2000; Wagner *et al*., 2002; Cooke *et al*., 2004; Uçar and Atamanalp, 2010) and their efficacy (Endo *et al*., 1972; Keene *et al*., 1998; Munday and Wilson, 1997; Peake, 1998; Anderson *et al*., 1997; Altun *et al*., 2009). There are plenty of studies available on the anaesthetic dosage of clove oil (Hikasa, *et al*., 1986; Woody *et al*., 2002; Woolsey *et al*., 2004; Akbulut *et al*., 2011a, 2011b; Feng *et al*., 2011) and benzocaine (Oswald, 1978; Mattson and Riple, 1989; Gomes *et al*., 2001) for pelagic fish species which are regarded as seeing-feeding fish, but comparative studies concerning the optimal dose and the effects of anaesthesia on feed intake for sturgeon species which forage on the bottom by touch with barbells (smelling-feeding fish species) are limited. Soto and Burhanuddin (1995) did, however, observe most rabbitfish (*Siganus lineatus*) feeding a few hours after anaesthesia with clove oil, Prince and Powell (2000) reported that adult rainbow trout (*Oncorhynchus mykiss*) fed actively 1 week after anaesthesia with clove oil, Pirhonen and Schreck (2003) stated that steelhead trout (*Oncorhynchus mykiss*) ate relatively well 4 h after anaesthesia with MS-222, clove oil and CO_2 and Sorum and Damsgard (2004) considered that benzocaine anaesthesia had only a minor and insignificant effect on feed intake in Atlantic salmon (*Salmo salar*). On the other hand, Perdikaris *et al*. (2010) investigated the size-relative effectiveness of clove oil for rainbow trout and goldfish (*Carassius auratus* Linnaeus, 1758) and recommended as an effective anaesthetic for both fish species, because it was effective, producing minimum stress and zero mortality. Recently, Doleželová *et al*. (2011) studied the acute toxicity of clove oil to fish *Danio rerio* and *Poecilia reticulate* and reported that no significant difference between LC50 values for *D. rerio* and *P. reticulate*.

The aim of the present study was to determine the optimal dose of clove oil and benzocaine for anaesthesia in Siberian sturgeon fry (*Acipenser baerii*) and to compare their effects on feed intake after anaesthesia. To perform the evaluation of anaesthetics within the context of how they might actually be applied by aquaculturists, the lethal time and the relationship between exposure duration and recovery time at the optimal concentrations of these two anaesthetics were examined as part of the anaesthesia procedure.

Materials and Methods

Preparation of Anaesthetic Solutions

Clove Oil : Commercially available clove oil in 20 ml glass bottles was the preferred anaesthetic. The density of clove oil was 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. The stock solution was freshly made on a day-to-day basis and was kept in the dark at ambient temperature.

Benzocaine : The benzocaine stock solution was prepared by solving 100 g of benzocaine (Sigma, Aldrich) in 1 L of ethanol (95%) (Ross and Ross, 2008). Ethanol has no known anaesthetic properties on fish at low doses (Anderson *et al*., 1997; Munday and Wilson, 1997). A fresh anaesthetic bath was prepared for each test.

Fish : These experiments were conducted on Siberian sturgeon fry with a mean length of 10.0±0.93 (7.2-12.0) cm and weight of 4.1±0.95 (2.1-6.4) g. The main fish stock was maintained in 16square 300 L tanks (110x110 cm^2), provided with a flow-through supply of aerated freshwater at a temperature of 18-20°C in the recirculation system. The fish were starved for approximately 16 h prior to all tests.

Measurement Effective Doses (EC_{50})

To determine the effective doses and the duration of time required to reach a stable level of anaesthesia,10 fish for per dose were individually exposed to seven doses of clove oil ranging from 200 to 1000 mg/L and benzocaine 10 to 50 mg/L in 5 L of fresh water. The water temperature in the buckets was held constant during the experiments by changing the surrounding water of 10 L buckets. The measurement of induction time was modified from earlier studies (Schoettger and Julin, 1967; Summerfelt and Smith, 1990; Keene *et al*., 1998) described in Table 1. All test fish were placed individually in anaesthetic baths supplied with aeration. Induction stages (I-1; loss of equilibrium, I-2; non-moving) of anaesthesia for each fish were measured by using a chronometer to the nearest second.

When the fish reached the syncopal stage (S), they were removed from the anaesthetic bath by a net. The body weight (g) to the nearest 0.01 g and length (cm) to the nearest 0.1 cm were measured. Once recovered, fish were grouped according to dose and transferred to a 300 L tank and monitored for survival and abnormal behaviour. During the experiments, the dissolved oxygen of all test water did not fall below 6.0 mg/L.

Effect of Exposure Duration on Recovery and Mean Lethal Time (MLT_{50})

To determine the mean lethal time, 100 fish were exposed to 370 mg/L of clove oil and 100 fish were exposed to35 mg/L of benzocaine in 30 L solutions provided with pure oxygen. Following exposure for6, 12, 24, 48, 96 and 192 min, ten fish were removed and placed in tanks filled with 300 L of continuously aerated water and provided with a flow-through supply (5-7 L/minute) of freshwater. The recovery time of each live fish was measured with a chronometer according to the criteria shown in Table 1 and the number of dead fish was recorded. This process was repeated three times.

Feed Intake

A total of 1500 fish were used to determine feed intake in one control and six test tanks. The fish were treated with 350 mg/L of clove oil and 35 mg/L of benzocaine in the 20 L anaesthetic bath solutions for three minutes. The fish were removed from the bath solution and immediately placed in one of six tanks with filled 300 L of continuously aerated water and provided with a flow-through supply (5-7 L/minute) of freshwater. Within ten minutes of placement in the tanks, the fish were fed with 0.7% body weight per meal. The duration of feed intake was recorded by chronometer at each meal.

Statistical Methods and calculations

Statistical tests were carried out using SPSS 16.0 software for Windows. One-way Analysis of Variance was performed to test the effect of dose. The EC_{50} values were determined by probit analysis. Differences in recovery times between the two anaesthetics were tested with a t-test. The MLT_{50} was examined with the Kaplan-Meier survival analysis procedure. Two-factor Analysis of Variance was performed to test differentials between feed intakes. Tukey’s procedure was used to make subsequent pairwise comparisons. Significant differences were established at 0.05 levels for differences among the groups.

Results

Effective Doses (EC_{50})

Summary statistics of the induction times for Siberian sturgeon exposed to various levels of clove oil orbenzocaine in the dose response study are given in Table 2. Effective concentrations (EC_{50}) were

Table 1: Evaluation criteria for induction of and recovery time from anaesthesia were modified from earlier studies

Phase of Anaesthesia	Stage	Description	Notable behaviour of fish
Induction	I-1	Sedation	Fish swimming, reaction to external stimuli.
	I-2	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	R-1	Motion	Motion perception and give reaction to external stimuli
	R-2	Regain of equilibrium	Partial recovery of equilibrium, regular opercular ventilation, reaction to strong external stimuli, not swimming correctly.
Normal			Recovery of equilibrium, fish swimming.

Table 2: Time required to reach a stable level of anaesthesia

Doses	Clove oil			Doses	Benzocaine		
	Time (sec.)				Time (sec.)		
(mg/L)	I-1	I-2	I (I-1+I-2)	(mg/L)	I-1	I-2	I (I-1+I-2)
200	174±24.29			25	281±14.19		
300	86±6.04	236±26.07	322±28.28	30	149±11.23	103±16.11	251±14.24
400	72±4.34	107±18.29	178±18.15	35	95±8.14	50±10.43	138±11.26
500	53±4.17	35±4.27	92±3.20	40	92±9.36	43±4.40	134±13.11
600	50±3.02	49±7.23	98±7.07	45	70±6.58	35±6.15	106±6.01
800	37±1.44	32±7.04	69±7.01	50	59±6.10	37±5.30	95±8.06
1000	30±1.89	27±3.13	57±3.12	60	50±4.28	29±4.30	80±5.35

I: Induction, I-1: Sedation and I-2: Loss of equilibrium, Mean±SEM

estimated at 356 (330-381) mg/L of clove oil and 37 (33-40) mg/L of benzocaine for 3 minutes with 95% confidence limits.

Effect of Exposure Duration on Recovery

The recovery times after anaesthesia in fish exposed to a concentration of 356 mg/L (EC_{50}) of clove oil or 37 mg/L of benzocaine for 96 min are shown in Figure 1. The recovery time from clove oil was longerthan for benzocaine and the differences inrecovery times between clove oil and benzocaine at 6, 12, 24 and 48 minutes were statisticaly significent ($P<0.05$). As shown Figure 1, the differences in recovery times with different durations of exposure were statistically significent for clove oil and benzocaine ($P>0.05$).

Mean Lethal Time (MLT_{50})

The mean lethal time was estimated at 37.2 (5.1-69.3) minutes for clove oil and 63.0 (6.1-120.0) minutes for benzocaine with 95% confidence limits. With the Kaplan-Meier Survival Analysis procedure, the distribution of time to effect for the two different anaesthesia methods was examined; the comparison tests showed that there was not a statistically significant difference between them ($P>0.05$).

Feed Intake

Post-anaesthesia duration tofeed intake infish exposed to two different anaesthesia methods for 3 minutes are shown in Figure 2 with control groups. As shown in Figure 2, the duration to feed intake in fish exposed to benzocaine was longer than in fish exposed to clove oil and control ($P<0.05$). There was not a statistically significant difference between clove oil and control ($P>0.05$).

Discussion

This study evaluated the use of clove oil and benzocaine as a tool to immobilise fish for various purposes in aquaculture. In some countries, clove oil is not permitted at the stage of harvesting fish, but it can be used in previous stages of aquaculture procedures such as grading etc. Clove oil and benzocaine are effective fish anaesthetics and there are many examples of their effective dose rates. Endo *et al*. (1972) showed that clove oil is effective in Crucian carp (*Carassius carassius*) and Hisake *et al*. (1986) showed that it provides effective anaesthesia in common carp (*Cyprinus carpio*) at 25-100 mg/L. Soto and Burhanuddin (1995) found a dose of 100 mg/L to be effective in rabbit fish. Perdikaris et al. (2010) observed a size-relative difference in induction time of gold fish, and stated that the rainbow trout and gold fish recovered within 6 min after anaesthesia at 150 mg/L clove oil. Taylor and Roberts (1999) recommended 100 mg/L of clove oil for juvenile and subadult white sturgeon (*Acipenser transmontanus*). Imanpoor *et al*. (2010) recommended a dose of 400 mg/L of clove oil to best anaesthetise Persian sturgeon (*Acipenser persicus*). Akbulut *et al.* (2011b) considered an effective concentration to be 450-670 mg $l^{-1}$of clove oil for juvenile Russian sturgeon (*Acipenser gueldenstaedtii*). Feng *et al*. (2011) stated that an effective dose is 120 mg/L of clove oil for juvenile Siberian sturgeon.

In the present study, the optimal concentration of clove oil was calculated to be 356 mg/L and 37 mg/L of benzocaine for Siberian sturgeon fry. The time required to reach a stable level of anaesthesia ranged from 322 to 57 s in 300-1000 mg/L of clove oil and 251 to 70 s in 30-60 mg/L of benzocaine. These results for the effective concentration of clove oil was higher than some previously documented findings when clove oil was used at different concentrations in other species e.g., white sturgeon, 100 mg/L, 246 s, (Taylor and Roberts, 1999), rainbow trout, 30 mg/L, 3.7 min (Prince and Powell, 2000), sockeye salmon, 50 mg/L, 84 s (Woody *et al*., 2002).

In the case of benzocaine, our induction results are consistent with those from other species. A study on the effective dose of benzocaine noted that it is effective at approximately the same doses as MS-222. For example, Gilderhus (1989) induced anaesthesia in *Oncorhynchus tshawytscha*, *O. mykiss* and *Salmo salar* in 3.5 minutes at 25-45 mg/L. Gilderhus *et al*. (1991) stated that water temperature affected the induction time and effective concentration of

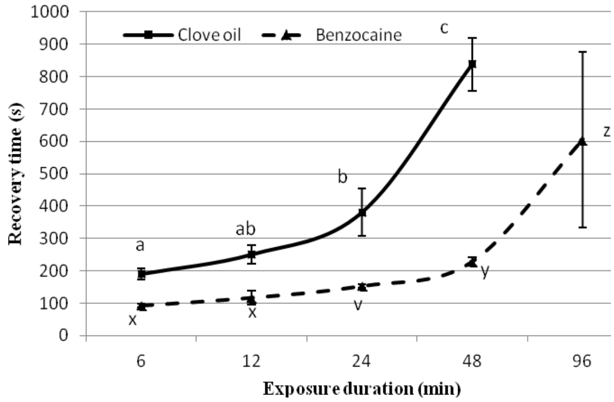

Figure 1. Relationship between the duration of exposure and recovery time after clove oil and benzocaine exposure

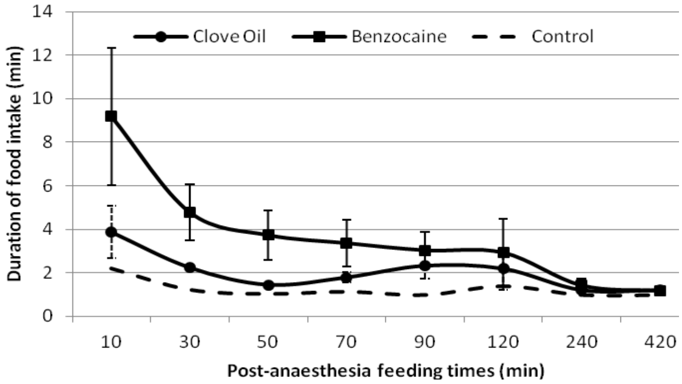

Figure 2. Effect of anaesthesia on feed intake following anaesthesia.

benzocaine as an anaesthetic for striped bass from 55 mg/L at 22°C to 80 mg/L at 11°C, which effectively anesthetised the fish in about 3 min. Oswald (1978) recommended 30-35 mg/L of benzocaine for rainbow trout and 40 mg/L for brown trout (*Salmo trutta*) and Mattson and Riple (1989) suggested 40 mg/L for cod (*Gadus morhua*).

It was observed that the recovery from anaesthesia of fish treated with clove oil was longer than for benzocaine. These results were consistent with the results of Akbulut *et al*. (2001b) in juvenile Russian sturgeon, Mylonas *et al*. (2005) in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), Munday and Wilson (1997) in coral reef fish (*Pomacentrus amboiensis*) and Feng *et al*. (2011) in juvenile Siberian sturgeon.

In addition, increasing the duration of exposure prolonged the recovery time (Figure 1). The duration of survival in fish exposed to benzocaine was longer than in those exposed to clove oil. However, water temperature can have diverse effects on anaesthesia in fish; the nature of these effects depends on the anaesthetic used (Ross and Ross, 2008). Increases in water temperature have been reported to enhance the anaesthetic efficacy of clove oil by shortening the time to induction in Steelhead fry (Woolsey *et al*., 2004) and gilthead sea bream (Mylonas *et al*., 2005). When common carp are anaesthetised with clove oil, higher water temperatures cause enhanced anaesthetic effects and shorter recovery times (Hikasa *et al*., 1986). Zahl *et al*. (2009) reported that after 5 min of exposure to benzocaine in Atlantic cod, induction and recovery times varied in relation to water temperature.

It is clear that the duration of survival in the anaesthetic bath is affected by the concentration of the anaesthetic, the water temperature and also fish size. In the present study, the duration of survival was examined through the analysis of MLT_{50} at the optimal doses and water temperature for Siberian sturgeonfry. The duration of survival of fish treated with clove oil was shorter that of fish exposed to benzocaine.

Studies which have investigated the effect of anaesthesia on feed intake generally support the idea that after anaesthesia, feeding by fish is affected in a minor or major way, regardless of the anaesthetic substance (Soto and Burhanuddin, 1995; Prince and Powell, 2000; Pirhonen and Schreck, 2003; Sorum and Damsgard, 2004). In the present study, it was determined that the effect of both anaesthetics on feed intake continued for 4 h, and feed intake duration at each meal time was higher than in the control. However, their main effect on feeding was observed at 30 minutes (Figure 2).

Comparing the effect of both anaesthetics, it was observed that feed intake in fish treated with clove oil and benzocaine occurred within the first ten minutes, and feed intake duration in both groups was longer than in the controls. However, it was determined that the effect of clove oil was less than that of benzocaine on feed intake.

Eugenol has been widely tested to determine its safety for human use and consumption, and is considered neither toxic nor carcinogenic in humans and other animals (Fisher *et al*., 1990; Zheng *et al*., 1992; Lee and Shibamoto, 2001). A withdrawal period for clove oil is unnecessary and it does not pose an environmental hazard (Cho and Heath, 2000). The results of Doleželová et al. (2011) did not show different sensitivities to clove oil in fish *Danio rerio* and *Poecilia reticulate*. However, its uses as a fish anaesthetic remains under investigation. A withdrawal period of 21 days remains in place for benzocaine.

Eugenol has a multitude of properties which make it useful in a wide variety of applications, including as an antioxidant (Kramer, 1985; Pulla and Lokesh, 1992), as an antifungal agent (Kamble and Patil, 2008), as an antibacterial agent (Karapmar and Aktuğ, 1987; Kouidhi *et al*., 2010) and as an antiparasitic agent (Machado *et al*., 2011). Considering clove oil's antibacterial, antiparasitic and antifungal effects, repeated treatment data may be helpful for its usein aquaculture. Further research is also needed to explore practical techniques for the absorption of both anaesthetic materials from farm effluents.

Acknowledgements

This study was carried out in the recirculation hatchery facilities of the Central Fisheries Research Institute in Trabzon, Turkey. We are grateful to the owner of the Papilla Trout Farm for providing the eggs.

References

Akbulut, B., Çavdar, Y., Çakmak, E. and Aksungur, N. 2011a. Use of clove oil to anaesthetize larvae of Russian sturgeon (*Acipenser gueldenstaedtii*). J. Appl. Ichthyol., 27: 618-621. doi: 10.1111/j.1439-0426.2010.01653.x.

Akbulut, B., Çakmak, E., Aksungur, N. and Çavdar, Y. 2011b. Effect of exposure duration on time to recovery from anaesthesia of clove oil in juvenile of Russian sturgeon. Turkish J. Fish.Aquat. Sci., 11: 463-467. doi: 10.4194/1303-2712-v11_3_17.

Altun, T., Bilgin, R. and Danabaş, D. 2009. Effects of sodium bicarbonate on anaesthesia of common carp (*Cyprinus carpio* L., 1758) juveniles. Turkish J. Fish. Aquat. Sci., 9: 29-31.

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. N. Am. J. Fish. Manage., 17: 301-307.

Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A.B., Rouabhia, M., Mahdouani, K. and Bakhrouf, A. 2007. The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (*Syzigium aromaticum* L. Myrtaceae): A short review. Phytotherapy Res., 21: 501-506.

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

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.

Doleželová, P., Mácová, S., Plhalová, L., Pištěková, V. and Svobodová, Z. 2011. The acute toxicity of clove oil to fish danio rerio and poecilia reticulata. Acta Veterinaria Brno., 80(3): 305-308.

Endo, T., Ogishima, K., Tanaka, H. and Ohshima, S. 1972. Studies on the anaesthetic effect of eugenol in some freshwater fishes. B. Jpn. Soc. Sci. Fish., 38: 761-767.

Feng, G., Liu, J., Zhuang, P., Zhang, L. and Duan, M. 2011. Anesthesia and recovery with clove oil in juvenile Siberian sturgeon (*Acipenser baerii*). Bioinformatics and Biomedical Engineering, (ICBBE) 5th International Conference, 10-12 May, Wuhan, China: 1-4.

Fisher, I.U., von Unruh, G.E. and Dengler, H.J. 1990. The metabolism of eugenol in man. Xenobiotica, 20: 209-222.

Gilderhus, P.A. 1989. Efficacy of benzocaine as an anesthetic for salmonid fishes, N. Am. J. Fish. Manage., 9(2): 150-153.

Gilderhus, P.A., Lemm, C.A. and Woods, L.C. 1991. Benzocaine as an anesthetic for striped bass, Prog. Fish Culturist, 53(2): 105-107.

Gomes, L.C., Chippari-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 Aquac. Soc., 32: 426-431.

Hikasa, Y., Takase, K., Ogasawara, T. and Ogasawara, S. 1986. Anaesthesia and recovery with tricainemethanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. Jpn. J. Vet. Sci., 48: 341-351.

Imanpoor, M.R., Bagheri, T. and Hedayeti, S.A.A. 2010. The Anesthetic Effects of Clove Essence in Persian Sturgeon, *Acipenser persicus*. World J. Fish and Marine Sci., 2 (1): 29-36.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A. and Schmid, E. 2006. Chemical composition and antioxidant properties of clove leaf essential oil. J. Agric. Food Chem., 54(17): 6303-6307.

Kamble, V.A. and Patil, S.D. 2008. Spice-derived essential oils. effective antifungal and possible therapeutic agents. J. Herbs. Spices Med. Plants, 14(3): 129-143.

Karapmar, M. and Aktuğ, S.E. 1987. Inhibitation of food borne pathogens by thymol, eugenol, menthol and anethole. Int. J. Food Microbiol., 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). Aquac. Res., 29: 89-101.

Kouidhi, B., Zmantar, T. and Bakhrouf, A. 2010. Anticariogenic and cytotoxic activity of clove essential oil (*Eugenia caryophyllata*) against a large number of oral pathogens. Ann. Microbiol., 60 (4): 599-604.

Kramer, R.E. 1985. Antioxidants in clove. J. Am. Oil. Chem. Soc., 62: 111-113.

Lee, K.G. and Shibamoto, T. 2001. Antioxidant property of aroma extract isolated from clove buds (*Syzygium aromaticum* (L.). Food Chem., 74(4): 443-448.

Machado, M., Dinis, A.M., Salgueiro, L., Custódio, J.B.A., Cavaleiro, C. and Sousa, M.C. 2011. Anti-Giardia activity of *Syzygium aromaticum* essential oil and eugenol: Effects on growth, viability, adherence and ultrastructure. Exp. Parasitol., 127(4): 732-739.

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

Mattson, N.S. and Riple, T.H. 1989. Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with Benzocaine, MS-222, chlorobutanol, and phenoxyethanol, Aquaculture, 83(1-2): 89-94.

Munday, P.L. and Wilson, S.K. 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboiensis*, a coral reef fish. J. Fish Biol., 51: 931-938.

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.

Oswald, R.L. 1978. Injection anaesthesia for experimental studies in fish, Comp. Biochem. Physiol., 60C: 19-26.

Peake, S. 1998. Sodium bicarbonate and clove oil as potential anesthetics for nonsalmonid fishes. N. Am. J. Fish. Manage., 18: 919-924.

Perdikaris, C., Nathanailides, C., Gouva, E., Gabriel, U.U., Bitchava, K., Athanasopoulou, F., Paschou, A. and Paschos, I. 2010. Size-relative effectiveness of clove oil as an anaesthetic for rainbow trout (*Oncorhynchus mykiss* walbaum, 1792) and goldfish (*Carassius auratus* Linnaeus, 1758). Acta Veterinaria Brno., 79(3): 481-490.

Pirhonen, J. and Shreck, 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.

Prince, A. and Powell, C. 2000. Clove oil as an anesthetic for invasive field procedures on adult rainbow trout. North Am. J. Fish. Manage., 20: 1029-1032.

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. Mol. Cell. Biochem., 111: 117-124.

Ross, L.G. and Ross, B. 2008. Anaesthetic and Sedative Techniques for Aquatic Animals. Blackwell Publishing, 3rd Edition, London: 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.

Soivio, A., Nyholm, K. and Huhti, M. 1977. Effects of anaesthesia with MS222, neutralised MS222 and benzocaine on the blood constituents of Rainbow trout, J. Fish Biol., 10: 91-101.

Sorum, U. and Damsgard, B. 2004. Effects of anaesthetisation and vaccination on feed intake and growth in Atlantic salmon (*Salmo salar* L.). Aquaculture, 232: 1-4.

Soto, C.G. and Burhanuddin, S. 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbit fish (*Siganus lineatus*). Aquaculture, 136: 149-152.

Summerfelt, R.C. and Smith, L.S. 1990. Anaesthesia, surgery, and related techniques. In: C.B. Schreck and P.B. Moyle (Eds.), Methods for Fish Biology. American Fisheries Society, Bethesda, USA: 451-489.

Taylor, P.W. and Roberts, S.D. 1999. Clove oil: an alternative anaesthetic for aquaculture. North Am. J. Aquac., 61: 150-155.

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*). J. Anim. Vet. Adv., 9(14): 1925-1933.

Wagner, E., Arndt, R. and Hilton, B. 2002. Physiological stress responses, egg survival and sperm motility for rainbow trout brood stock anaesthetized with clove oil, tricaine methane sulfonate or carbon dioxide. Aquaculture, 211: 353-366.

WHO 2009. Monographs: pharmaceutical substances: benzocainum-benzocaine. The International Pharmacopoeia. Retrieved 29 September 2009.

Woody, C.A., Nelson, J. and Ramstad, K. 2002. Clove oil as an anaesthetic for adult sockeye salmon: field trails. J. Fish Biol., 60 (2): 340-347.

Woolsey, J., Holcomb, M. and Ingermann, R.L. 2004. Effect of Temperature on Clove Oil Anesthesia in Steelhead Fry. N. Am. J. Aquacult., 66: 35-41.

Zahl, I.H., Kiessling, A., Samuelsen, O.B. and Hansen, M.K. 2009. Anaesthesia of Atlantic cod (*Gadus morhua*)-Effect of pre-anaesthetic sedation, and importance of body weight, temperature and stress. Aquaculture, 295: 52-59.

Zheng, G.Q., Kenney, P.M. and Lam, L.K. 1992. Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. J. Nat. Prod., 55: 999-1003