Efficacy of clove oil, benzocaine, eugenol, 2-phenoxyethanol as anaesthetics on shabbout fish (*Barbus grypus* Heckel, 1843)

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Abstract

To our knowledge, no previous anaesthetic experiments are conducted on shabbout fish. The results from the present study indicated that the induction times decreased significantly as the doses increased in all the anaesthetics (p<0.05). Induction and recovery times were significantly affected by the interaction between concentration and anaesthetic (p<0.05). The effective doses were: 25 and 50 μL L^{-1} at 24°C clove oil and for eugenol, 50 mg L^{-1} for benzocaine and 500 μL L^{-1} for 2-phenoxyethanol. In conclusion, the four anaesthetic agents could be used as sedatives in culture of shabbout fish.

Keywords: Shabbout fish, *Barbus grypus*, Herbal medicines, Chemical anaesthatics.

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Introduction

The anaesthetics are very important for aquaculture because they minimize stress in aquaculture procedures such as the selection of fish, their measurement, sampling, labelling, transportation, artificial insemination and surgery (Hseu *et al*., 1998; Weber *et al*., 2009). Furthermore, it is an important process in terms of various fish enhancement programmes, commercial fisheries and the fish-farming industry without damaging their health or commercial value. In research and aquaculture, chemical anaesthetics have been most widely used (Bell, 1964, 1987; Iwama and Ackerman, 1994; Altun and Danabaş, 2006; Altun *et al*., 2009; Weber *et al*., 2009). 2-phenoxyethanol is widely used for transporting live fish because it is cheap, reliable and efficient and its active ingredient is ethylene glycol monophenyl ether (Gilderhus and Marking, 1987; Weyl *et al*., 1996; Weber *et al*., 2009; Uçar and Atamanalap, 2010). Benzocaine (ethyl-aminobenzoate) the ethyl ester of *p*-aminobenzoic acid, was used as a central nervous system anaesthetic (Hseu *et al*., 1998; Pramod *et al*., 2010; Zahl *et al*., 2011; Ghanawi *et al*., 2013). Eugenol ($C_{10}H_{12}O_2$) is a phenylpropene, an allyl chain-substituted guaiacol and the major constituent (70 to 90 percent by weight) of clove oil (Akbari *et al*., 2010). Clove oil is derived from Eugenia caryophyllate tree, which contains methyleugenol, eugenol and isoeugenol (Isaacs, 1983; Soto and Burhanuddin, 1995). Recently, eugenol and clove oil have been widely used in aquatic animals due to their efficacy and being inexpensive (Weber *et al*., 2009; Akbari *et al*., 2010; Uçar and Atamanalap, 2010; Ghanawi *et al*., 2013)

The shabbout fish (*B. grypus*) is a Cyprinid fish species which inhabits naturally in Euphrates and Tigris Rivers of Turkey, Syria and Iraq (Oymak *et al*., 2009). This large fish is abundant and commercially important for regional fishery. It is commonly called barb or shabbout, also spelled shabboot or shabut (Sahinoz *et al*., 2007). It is a potential freshwater species for aquaculture because of their delicate flavor and rich nutritional value. Thus far, a great deal of past research has focused on anaesthesia of fish species. However, to our knowledge, there are no reports on the effects of anaesthetics on the shabbout fish (*B. grypus*). In this framework, this study was conducted for the comparison of the efficacy of four anaesthetics (clove oil, eugenol, benzocaine, and 2-phenoxyethanol) in the shabbout fish.

Material and methods

Fish

This study was performed in June 2011 at the General Directorate of State Hydraulic Works, Fish Production Station Atatürk Dam Lake, Urfa, Turkey. Six years old fish (n=72; 750.26±126.97 g, 45.73±2.81 cm mean±SD) were obtained from earthen ponds. The first shabut production and adaptation to earthen ponds were done

with this method in 2006 at this farm and fish transferred to the hatchery in an aerated container. The fish were acclimated in twelve 5000 L concrete ponds supplied with constantly running freshwater for 1 week. Each sedative concentration was prepared and tested with 6 fish at a different aquarium. Experiments were performed in triplicate. Each replicate consisted of six fish exposed separately. The fish were fed with commercial pellet feed for 2 weeks and feeding was terminated 24 h before the experiment. No mortality was observed during the acclimatization period. The oxygen and pH were measured with a multiparameter (Hach HQ40D). Temperature was measured with a digital thermometer twice a day (8:00-9:00, 16:00-17:00). The temperature of the incoming water was 24±0.1°C. pH and dissolved oxygen value in the groups were 8.23±0.20 and 8.2±0.11 mg L^{-1}.

Anaesthetic agents

Four anaesthetic agents, clove oil (Biopont, Budapest, Hungary), eugenol (Merck, KGaA, Darmstadt, Germany), benzocaine and 2-phenoxyethanol (Sigma Aldrich Co., St. Louis, USA) were used. Clove oil was initially dissolved in 94% ethanol (ratio of clove oil: ethanol, 1:9) because it is poorly soluble in water. Eugenol is the active ingredient of clove oil which is not completely soluble in water (at low temperatures<15ºC), it is necessary to dilute the product 1:10 in 95% ethanol. 2-phenoxyethanol is soluble in water (26.7 g L^{-1}) at 25°C but readily soluble in ethanol. Benzocaine, being insoluble in water, was dissolved in a few drops of ethanol before mixing into the transporting medium (Zahl *et al*., 2011).

Experimental design

In this study, three stages of induction and three stages of recovery were considered. The different stages (I, II, III) of anaesthesia and recovery in shabbout fish (*B. grypus*) are described in Table 1 (Gullian and Villanueva, 2009). The following doses of each agent were evaluated: clove oil and eugenol (25, 50, 75 μL L^{-1}), benzocaine (25, 50, 75 mg L^{-1}), 2-phenoxyethanol (500, 1000, 1500 μl L^{-1}). Six fish were used for each concentration tested in order to evaluate the time required for the induction of anaesthesia. Animals were considered to have recovered when they demonstrated normal swimming and reaction to the external stimuli (Silva *et al*., 2012).

Statistical analysis

Data are presented as means±standard deviation of the mean (SD). The differences among means were analysed by non-parametric Mann-Whitney's U tests following Kruskall-Wallis's test. Differences were considered significant at $p<0.05$. All analyses were performed with the SPSS 14.0 statistical package.

Table 1: Stages of induction of anaesthesia and recovery in the shabbout fish (Gullian and Villanueva 2009).

Stages	Description	Characteristic behaviour
Induction		
I1	Loss of balance	Partial inhibition of reactions to external stimuli
I2	Total loss of equilibrium	Fish still react to strong stimuli
I3(I)	Total loss of reflexes and movement	Fish lay on bottom of the tank
I4*	Death	Complete cessation of opercula movements
Recovery		
R1	Start of movement	Fish still lay on bottom of the tank
R2	Regular breathing	Reaction to strong stimuli. Irregular balance
R3(R)	Total recovery of equilibrium	Reaction to slight stimuli. Normal swimming

*stage I4 has not been observed in this study.

Results

Induction and recovery times for each anaesthetic agent (clove oil, eugenol, benzocaine, and 2-phenoxyethanol) in the shabbout fish (*B. grypus*) are shown in Fig. 1. Data here reported that the effective doses were: 25 and 50 μL L^{-1} at 24 °C for clove oil and for eugenol, 50 mg L^{-1} for benzocaine and 500 μL L^{-1} for 2-phenoxyethanol. Recovery time of clove oil, eugenol, benzocaine, and 2-phenoxyethanol were 48 to 160 s; 79 to 199 s; 12 to 137 s and 27 to 131s, respectively. Induction and recovery time were significantly affected by the interaction between concentration and anaesthetic (p<0.05).

Discussion

Effective doses of the same anaesthetic often differ in fish species. These differences in principle result from two causes, biological factors (e.g. species, the stage of life cycle and age, size and weight, lipid content and disease status) or environmental factors (temperature, hardness, salinity and pH), or their interaction. Although, there is a lack of information in the literature on the effect of anaesthetic on the shabbout fish (*B. grypus*), numerous investigations have been conducted on different fish species. At the termination of experiments, faster induction and recovery of anaesthesia were obtained from the 2-phenoxyethanol treatment at concentration of 1500 μL L^{-1} and the benzocaine treatment at concentration of 25 mg L^{-1}, respectively. All values in the potential anaesthetic applications for different fish species were compared with the present findings Table 2.

An anaesthetic should act effectively in less than 3 minutes and the recovery should occur within 5 minutes in clean water for farmed fishes (Marking and Meyer, 1985; Bell, 1987).

Table 2: Summary of potential anaesthetic applications for fish species and comparisons with the present study results.

Species	Anaesthetic	Effective dosage	References
Oncorhynchus nerka	Clove oil	50 mg L^{-1}	Woody *et al*., 2002
Sparus aurata	Clove oil	55 mg L^{-1}	Mylonas *et al*., 2005
Cyprinus carpio	Clove oil	30-50 mg L^{-1}	Hajek *et al*., 2006
Anguilla anguilla	Clove oil	0.050 mL L^{-1}	Altun *et al*., 2006
	Eugenol	3.375 mL L^{-1}	
Solea senegalensis	Clove oil	30 mg L^{-1}	Weber *et al*., 2009
	2-phenoxyethanol	600 mg L^{-1}	
Puntius filamentosus	Benzocaine	≥20 mg L^{-1}	Pramod *et al*., 2010
Carasobarbus luteus	Clove oil	75 mg L^{-1}	Gokcek and Ogretmen 2011
Acipenser persicus	Clove oil	400 mg L^{-1}	Bagheri and Imanpour 2011
Acipenser gueldenstaedtii	Clove oil	0.35, 0.50 and 0.75 g L^{-1}	Akbulut *et al*., 2011
Siganus rivulatus	Clove oil	70 mg L^{-1}	Ghanawi *et al*., 2011
Barbus grypus	Clove oil	25 and 50 µl L^{-1}	Present study
	2-phenoxyethanol	500 µl L^{-1}	
	Benzocaine	50 mg L^{-1}	
	Eugenol	25 and 50 µl L^{-1}	

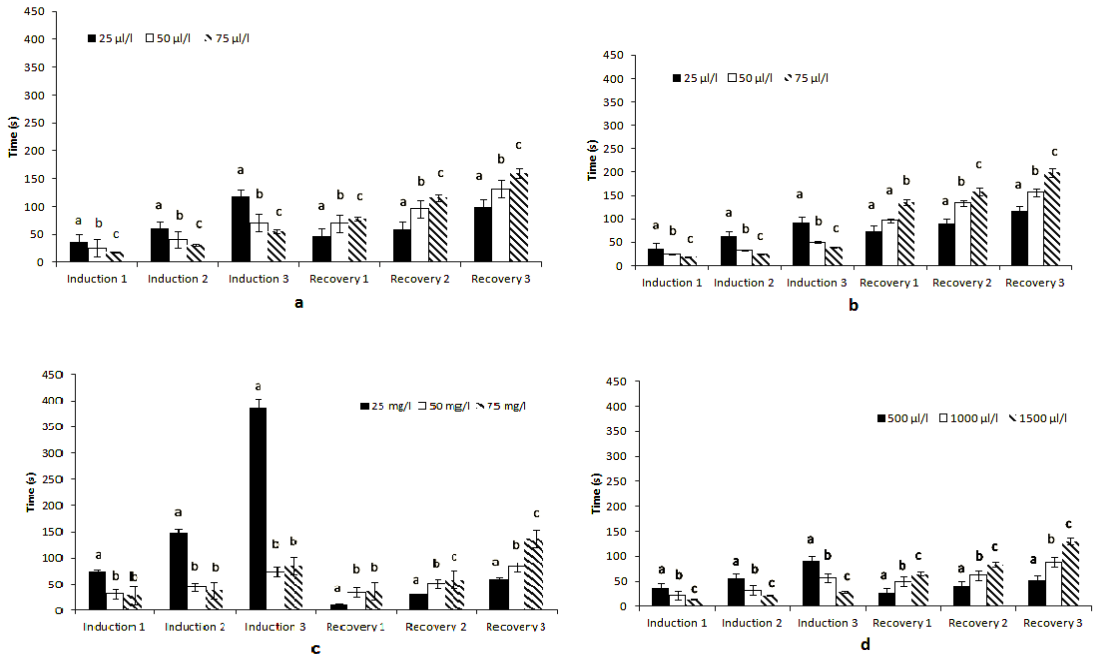

Figure 1: Induction and recovery times for each anaesthetic agent (a) clove oil, b) eugenol, c) benzocaine, and d) 2-phenoxyethanol) in the shabbout fish (*Barbus grypus*).

Our results indicated that induction time at all concentrations of clove oil, eugenol, benzocaine, and 2-phenoxyethanol ranged from 18 to 117 seconds, except for the benzocaine at 25 μL L^{-1} concentration which was considerably more than the upper limit, In addition, the findings indicated that the induction times decreased significantly as the doses increased in all the anaesthetics.

Recovery time were positively correlated with concentration of anaesthetics (Smit and Hattingh, 1979; Limsuwan *et al*., 1983; Hseu *et al*., 1994; Weyl *et al.*, 1996; Velisek *et al*., 2005; Sudagara *et al.*, 2009; Gullian and Villanuera, 2009). Terzioglu (2001) found that recovery times increased with increasing the concentration of 2-phenoxyethanol. On the other hand, some researchers determined that increasing the concentration did not affect the recovery time (Mattson and Riple, 1989; Malmstrom *et al*., 1993). In this study, we found a linear correlation between the concentration value and the recovery time, although higher concentrations of these four anaesthetic agents achieved shorter induction times. Additionally, it was determined that induction and recovery times are related to the anaesthetic concentration.

Anaesthetic should also have non-toxic side effects for either the fish or the handler. In this study, no death or other adverse effects occurred following recovery from anaesthesia. Clove oil and eugenol were harmless for skin and safe handling features as organic anaesthetics. On the contrary, it was observed that Benzocaine and 2-phenoxyethanol could be harmful for the user if contacted with eye or skin.

In conclusion, the results obtained in the present study clearly demonstrate that clove oil, eugenol, benzocaine and 2-phenoxyethanol satisfy these criteria and suggest that they could be considered as a fish anaesthetic. Therefore, our results indicated that all anaesthetics could be used to minimize the stress associated with aquaculture procedures.

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