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COMMUNICATION

Use of Clove Oil and Eugenol to Anesthetize Fingerling Shabut *Barbus grypus*

Fatih Öğretmen*
Faculty of Fisheries and Aquaculture, Muğla Sitki Kocman University, 48000, Muğla, Turkey

Selami Gölbaşi
General Directorate of State Hydzraulic Works, Fish Production Station, 63300, Urfa, Turkey

Burak E. Inanan
Faculty of Science, Department of Biology, Muğla Sitki Kocman University, 48000, Muğla, Turkey

Volkan Kizak and Murathan Kayim
Fisheries Faculty, Tunceli University, 62000, Tunceli, Turkey

Abstract
In this study, the efficacy of four doses of clove oil and eugenol were compared to sedate fingerling Shabut *Barbus grypus* to various stages of sedation and recovery. The results from the present study indicated that effective concentrations were at 50 μL/L for eugenol (induction time: 58 ± 8 s; recovery time: 199 ± 15 s [mean ± SD]) and clove oil (induction time: 76 ± 6 s; recovery time: 161 ± 34 s). The induction times decreased significantly with the increasing concentrations of clove oil and eugenol, while recovery times increased with increasing concentrations of anesthetic ($P < 0.05$). Exposure of fish to 75 or 100 μL/L clove oil or to 50, 75, or 100 μL/L eugenol resulted in mean induction times ≤ 1 min. Fish did not become fully sedated within 3 min when treated with 25 μL/L clove oil. Other concentrations of both anesthetics, fish reached to full induction at ≤ 3 min and recovered from anesthesia ≤ 5 min. Our results showed the clove oil and eugenol are effective anesthetics for fingerling Shabut when used at concentrations of 50–100 μL/L.

Anesthetics play an important role in aquaculture research and production, particularly with regard to facilitating handling while minimizing injury and stress to fish. They are used in many applications, such as measuring or weighing fish, sorting and tagging, vaccination, live transport, broodstock management, sampling for blood or gonadal biopsies, and collection of gametes (Summerfelt and Smith 1990; Mylonas et al. 2005; Ribas et al. 2007; Hegyi et al. 2010). Anesthetics cause different levels of types of behavior in fish. A total loss of consciousness occurs in fish after a state of general sedation. Reflex activity is lost entirely and skeletal muscle tone is also reduced (McFarland 1960). The treatment is terminated when breathing is decreased (Hajek and Kłyszejko 2004: Dziaman et al. 2005). Clove oil (obtained from the clove tree *Eugenia aromatica*) and its derivative eugenol (2-methoxy-4-[2-propenyl] phenol) have been used to anesthetize various fish species (Soto and Burhanuddin 1995; Yıldız 2010). Clove oil or eugenol, as active ingredient, is considered an attractive anesthetic in the aquaculture industry because of efficacy, cost effectiveness, and safety (Keene et al. 1998).

Selection of a suitable anesthetic depends mainly on its effectiveness in immobilizing fish with short induction and recovery times (Gilderhus and Marking 1987; Burka et al. 1997). An ideal anesthetic should possess several attributes, such as being nontoxic, inexpensive, and simple to administer, and resulting in faster induction and recovery (Treves-Brown 2000). It is often advisable to identify the lowest effective doses of different anesthetics in a specified species, as the responses to the same anesthetic may vary considerably among different species (King et al. 2005; Pawar et al. 2011).

Shabut *Barbus grypus* is an indigenous cyprinid in the Mesopotamia basin and is highly valued as a food fish in the region. The Talmud, which was completed in Persia approximately 1500 years ago, contains references to a fish named Shibuta. Probably the most frequently mentioned fish in Talmudic literature is the Shibuta, a species that appears in a variety

*Corresponding author: fatihogretmen@mu.edu.tr
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of contexts and is described as a popular and tasty fish. There have been various attempts to identify it over the last several centuries. It is suggested that the Talmudic Shibuta is a freshwater Iraqi fish bearing the contemporary Arabic, Farsi, and Aramaic common name Shabbout and the scientific name *Barbus grypus* (Zivotofsky and Amar 2006). It is an omnivorous fish species that feeds mainly on detritus, is commercially fished, and reaches nearly 2 m in length and over 50 kg in weight (Coad 1996; Epler et al. 2001). Its reproduction and adaptation to earthen ponds has been performed in General Directorate of State Hydraulic Works, Fish Production Station Atatürk Dam Lake, Urfa, Turkey, since 2006. Shabut production, along with fingerling Common Carp *Cyprinus carpio* and Himri Barbel *Carasobarbus luteus*, has been carried out by hatcheries to enhance freshwater fish stocks in Atatürk Dam Lake and the other dam lakes in the Euphrates basin.

Shabut could be considered as a new species for the regional aquaculture. Some studies have been conducted on the biology of Shabut in Turkey (Sahinoz et al. 2007; Oymak et al. 2008). Given the growing interest in the culture of Shabut and lack of practical information on the administration of anesthetics, the overall aim of the present experiment was to determine induction and recovery times of clove oil and eugenol that could be efficiently used in Shabut.

METHODS

Experimental animals.—The fish ($n = 168$; 30.36 ± 8.47 g, 15.30 ± 1.28 cm [mean ± SD]) were obtained from earthen ponds at General Directorate of State Hydraulic Works, Fish Production Station, Atatürk Dam Lake, Urfa, Turkey. Fish were transferred to the hatchery and acclimated for 2 weeks before the start of the experiment. During the acclimatization period fish were fed ad libitum with 40% protein commercial pellets twice a day. Fish were not fed the day before the start of the study.

Anesthetics.—The two anesthetic agents, clove oil (Biopont, Budapest, Hungary) and eugenol (Merck, KGaA, Darmstadt, Germany), were used for the experiment. Since these anesthetics are poorly soluble in water (Woody et al. 2002), they were initially dissolved in a 94% solution of ethanol (Merck, KGaA, Darmstadt, Germany) to create a stock solution of 1:9 (anesthetic : ethanol). All stock solutions were prepared a few minutes before the start of each experiment, and fresh solutions were prepared for each triplicate.

Measurement of induction and recovery stages of anesthesia.—Shabut fingerlings were transferred to the production unit of National States of Hydraulic Works Department in Urfa and held for a 2-week acclimatization period in flow-through systems before the study began. The fingerlings of Shabut were exposed to clove oil and eugenol concentrations to determine induction and recovery times of anesthetics. Final concentrations of these two anesthetics were adjusted to 25, 50, 75, and 100 μL/L. Each replicate consisted of seven fish exposed separately; fish were exposed to one of four doses of either clove oil or eugenol. After the acclimation period, the fish were randomly transferred to the holding tanks (300 L) filled with fresh and aerated water. The fish were netted and transferred individually to the anesthetic aquarium containing different concentrations of anesthetic solutions (30 L). Each sedative concentration was prepared and tested with seven fish at different aquaria. Experiments were prepared in triplicate. Seven fish were exposed to four concentrations for a total of 84 individuals tested for each anesthetic. The recovery stage was recorded after transferring the fish to aerated water in the recovery aquarium (30 L). Induction and recovery times were measured using a digital stopwatch. Throughout the experiment, the temperature and oxygen concentration were recorded with a multimeter (Hach; Model HQ40d). Water quality parameters during the experiment were temperature 23 ± 0.1°C (mean ± SD), pH 8.23 ± 0.20, oxygen 8.2 ± 0.11 mg/L, and 1‰ salinity.

General fish behavior was assessed for each fish throughout induction and recovery. Changes in the behavior status of the anesthetized fish were assessed using the same method modified by Gullian and Villanueva (2009) as shown in Table 1.

Statistical analysis.—A Kruskal–Wallis test was used to assess the differences in induction and recovery times; specifically, a Kruskall–Wallis test at 0.05 significant levels was used to determine whether significant variation between induction and recovery times of different concentrations of the same anesthetic agent existed. Linear and exponential regression equations were established to explain the relationship between concentration level and induction time as well as concentration level and recovery time. Spearman's rank correlation coefficient was used to assess the relationship between times with increasing

TABLE 1. Behavioral criteria used for evaluating stages of induction and recovery in fingerlings of Shabut (modified from Gullian and Villanueva 2009).

Stages	Description	Characteristic behavior
	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[a]	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

[a]Stage I4 has not been observed in this study.

TABLE 2. Mean times of induction and recovery stages for clove oil and eugenol, the experiments performed in triplicate (n = 7, per group). Within each column and section, values with common letters do not differ significantly ($P > 0.05$); data presented as mean ± SD.

	Induction times (s)			Recovery times (s)		
Concentrations (µL/L)	I1	I2	I	R1	R2	R
Clove oil						
25	50 ± 7 z	108 ± 20 z	776 ± 38 z	58 ± 3 y	110 ± 14 y	150 ± 3 x
50	31 ± 2 y	45 ± 3 y	76 ± 6 y	86 ± 22 z	139 ± 30 zy	161 ± 34 yx
75	18 ± 2 x	31 ± 2 x	56 ± 9 x	105 ± 29 z	153 ± 21 z	189 ± 30 zy
100	14 ± 1 w	28 ± 2 w	48 ± 5 x	102 ± 26 z	135 ± 20 z	202 ± 22 z
Eugenol						
25	35 ± 6 z	84 ± 16 z	231 ± 54 z	41 ± 11 x	96 ± 19 y	148 ± 13 x
50	22 ± 1 y	32 ± 2 y	58 ± 8 y	79 ± 19 y	116 ± 32 zy	199 ± 15 y
75	17 ± 2 x	25 ± 5 x	40 ± 7 x	94 ± 11 zy	138 ± 20 z	202 ± 20 y
100	15 ± 1 w	20 ± 2 w	30 ± 4 w	106 ± 14 z	139 ± 24 z	232 ± 22 z

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concentration levels. The obtained data were presented as mean ± SD and summarized box whisker plots. Statistics were performed using SPSS version 15.0.

RESULTS

In the study, induction and recovery times as well as statistical differences for each anesthetic agent (clove oil, eugenol) in Shabut are shown in Tables 2 and 3. These results demonstrate that the effective dose was 50 µL/L for clove oil and for eugenol. At the end of the experiments, faster induction and recovery of anesthesia were obtained from the clove oil treatment at a concentration of 100 µL/L and from the eugenol treatment at a concentration of 25 mg/L. There is a significant difference between stage R times at concentrations of 25 and 50 µL/L of clove oil and eugenol. No mortality was observed in either group during application time.

There was a negative exponential relation between anesthetic concentration and full induction time for both anesthetics (R1 = −0.924; R2 = −0.947; $n = 28$; $P < 0.05$), while recovery times for both clove oil and eugenol showed positive correlations with increasing concentrations (R1 = −0.732; R2 = −0.815; $n = 28$; $P < 0.05$). Fish induction was faster for eugenol than clove oil at the same concentrations. Recovery times for eugenol occurred over longer periods compared with clove oil, and recovery times for clove oil at all concentration levels were more variable than for eugenol.

The regression equations between induction time (I) and applied concentration (C) for clove oil and eugenol were calculated as

$$I = 972.8e^{-0.035C}(R = 0.733) \quad \text{and}$$
$$I = 320.27e^{-0.026C}(R = 0.858),$$

respectively. Additionally, the regression equations between recovery time (I) and applied concentration (C) for clove oil and eugenol were determined as

$$I = 133.78e^{0.0042C}(R = 0.973) \quad \text{and}$$
$$I = 156.12e^{0.0039C}(R = 0.920),$$

respectively.

TABLE 3. The significance of the pairwise comparison of clove oil and eugenol with their corresponding concentration levels (ns = not significant; an asterisk (*) = $P < 0.05$).

		Induction times			Recovery times		
Concentration (µL/L)	Anesthetics	I1	I2	I	R1	R2	R
25	Clove oil Eugenol	*	ns	*	*	ns	*
50	Clove oil Eugenol	*	*	*	ns	ns	*
75	Clove oil Eugenol	ns	*	*	*	ns	ns
100	Clove oil Eugenol	ns	*	*	ns	ns	ns

DISCUSSION

Biological factors such as species, the stage of life cycle and age, size and weight, lipid content, body content, and disease status affect the metabolic rate and therefore the pharmacokinetics of the anesthetic 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, therefore increasing or decreasing the efficacy of an anesthetic agent (Burka et al. 1997; Ross and Ross 1999; Yıldız 2010). Numerous investigations have been conducted on different fish species. All values in the potential anesthetic applications for

TABLE 4. Summary of potential anesthetic applications for fish species and comparisons with the present study results.

Species	Anesthetic	Effective dosage	References
Sockeye Salmon *Oncorhynchus nerka*	Clove oil	50 mg/L	Woody et al. (2002)
Gilthead Seabream *Sparus aurata*	Clove oil	55 mg/L	Mylonas et al. (2005)
Common Carp *Cyprinus carpio*	Clove oil	30–50 mg/L	Hajek et al. (2006)
European eel *Anguilla anguilla*	Clove oil	0.050 mL/L	Altun et al. (2006)
	Eugenol	3.375 mL/L	
Senegalese Sole *Solea senegalensis*	Clove oil	30 mg/L	Weber et al. (2009)
Carasobarbus luteus[a]	Clove oil	75 mg/L	Gökçek and Öğretmen (2011)
Persian Sturgeon *Acipenser persicus*	Clove oil	400 mg/L	Bagheri and Imanpour (2011)
Russian Sturgeon *A. gueldenstaedtii*	Clove oil	0.35, 0.50, and 0.75 g/L	Akbulut et al. (2011)
Marbled Spinefoot *Siganus rivulatus*	Clove oil	70 mg/L	Ghanawi et al. (2011)
Shabut *Barbus grypus*	Clove oil	50 μL/L	Present study
	Eugenol	50 μL/L	

[a]This species appears to have no common name in English. Within its native region it is known by the Arabic name Himri and the Farsi name Sangal [Ed.].

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different fish species were compared with the present findings (Table 4).

In the present study, the induction times decreased significantly with the increasing clove oil and eugenol concentrations ($P < 0.05$). On the other hand, recovery times increased with increasing concentrations of anesthetic in fingerling Shabut. The application of clove oil at doses of 75 and 100 μL/L, and eugenol at doses of 50, 75, and 100 μL/L resulted in fast induction of ≤1 min. Fish did not become fully sedated within 3 min when treated with 25 μL/L clove oil. Fingerling Shabut were fully induced at other concentrations of both anesthetics at ≤3 min and recovered at ≤5 min. It was demonstrated that clove oil and eugenol act as an effective anesthetic in fingerlings of Shabut and can be used at concentrations ≥50–100 μL/L. Although it results in slightly longer recovery times, the advantage of clove oil is not only the smaller cost for the aquaculturist but also a lesser polluting effect for the environment.

In conclusion, the present study showed that the two anesthetic agents could be used as sedatives in culture of Shabut fish. Clove oil and eugenol have been routinely used as an anesthetic agent in aquaculture. Although Aqui-S (whose main active component is isoeugenol) has been approved for use in food fish production in New Zealand, Australia, Chile, Costa Rica, and the Republic of Korea, its use still remains illegal in the USA and many other countries (Zahl et al. 2012). Obviously, in many countries, there is a need to enact laws that regulate the use of fish anesthetics. Further studies related to effects of different life stages, gender, reproduction state, and sizes on anesthetic application, haematological profile, and respiration rate could provide information for commercialization for both research and industrial use.

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