www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 129-133 (2016) DOI: 10.4194/1303-2712-v17_1_15

RESEARCH PAPER

Efficacy of Clove Oil, 2-Phenoxyethanol and Benzocaine on European Catfish, *Silurus glanis* Linnaeus 1758

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Received 30 June 2016
Accepted 17 August 2016

Abstract

The efficacy of clove oil, 2-phenoxyethanol and benzocaine was tested on captive-bred European catfish (wels), *Silurus glanis* L., 1758 in this study. According to effectiveness criteria, the most optimal implementation results were obtained from 100 µl L^{-1} (induction 69.00±6.48 s and recovery 253.86±14.78 s) clove oil, 1000 µl L^{-1} (induction 88.71±8.50 s and recovery 201.43±12.19 s) PE, and 100 µl L^{-1} (induction 103.00±6.90 s and recovery 215.71±19.99 s) benzocaine. The concentration of anesthetics agent used directly affected the onset of each physiological respond ($p<0.05$). The relationship between doses and induction times was inverse exponential, in spite of that an exponential relationship was confirmed between doses and recovery times for all anesthetics. In conclusion, all these three anesthetics can be effectively used for wels.

Keywords: European catfish, clove oil, 2-phenoxyethanol, benzocaine, anesthesia.

Introduction

The anesthetics are commonly used in aquatic culture activities such as classification, breeding, weighing, broodstock management and drug applications against diseases. Anesthetics cause reduction of fish activity and the total loss of consciousness is the last step of treatment. Furthermore, apnea and low oxygen saturation level in the blood may be caused due to over dose (Tytler and Hawkins, 1981; Gomulka *et al*., 2015).

Clove oil, eugenol, benzocaine (ethyl-p-aminobenzoate), 2-phenoxyethanol (ethylene glycol monophenyl ether) and MS-222 (tricaine methane sulphonate) are the commonly used anesthetics by aquaculture farms and researchers (Velisek *et al*., 2006, 2011). Although clove oil, eugenol, benzocaine and 2-phenoxyethanol are generally used for non-food fish and researches, MS-222 is the only licensed product for cultured fish species in USA and EU (Öğretmen and Gökçek, 2013). On the other hand, although Aqui-S is still illegal for USA and many other countries, it has been approved for use in food fish production in some southern hemisphere countries (Zahl *et al*., 2012). Recently, a new agent which is called Sedanol was practically started to be used in Turkey.

An appropriate anesthetic should possess some features such as being inexpensive, accessible, non-toxic etc. (Treves-Brown, 2000). Each fish species responses to the same anesthetics at considerably varied concentrations, thus determination of the lowest effective doses of different matters is very important (Pawar *et al*., 2011). Also, recovery duration and survival ratio are substantial points on choosing an effective species specific anesthetic (Gilderus and Marking, 1987; Burka *et al*., 1997).

The European catfish or wels, *S. glanis* L., 1758, is a carnivorous and fast growing species. It is commercially produced more than a hundred years in especially central Europe due to high value as food. Although reproduction and hatchery technics, yield efficiency in polyculture and biology were already studied, the effect of commercially used anesthetics on European catfish is still obscure. Recently, intensive culture in cages started and production ratio is expected to be increased in the near future in Turkey. Due to increasing interest on intensive culture of Wels and inconclusive information about anesthetics, the main aim of this study was to reveal the effective doses of three different anesthetics (clove oil, benzocaine and 2-phenoxyethanol) that could be effectively used in European catfish under controlled conditions.

Material and Methods

Fish

Wels were obtained from a commercial fish farm in Kahramanmaraş, Turkey (Tuğçe Ltd.). Fish were transferred to the hatchery of company for acclimation 2 weeks before the study. Six-month old individuals (n=105, average length 15.6±1.5 cm and weight 44,6±6.7 g) were not fed the day before study.

Anesthetic Agents

In this study, clove oil (Aromax, Hungary), benzocaine (Himedia, India) and 2-phenoxyethanol (PE)(Sigma Aldrich Chemic, Germany) were tested as the anesthetic agents. Doses were prepared just before the trials. Clove oil and benzocaine were diluted in ethanol (94%) (1:9) due to low water solubility. In a similar way, PE was also diluted in ethanol (1:1) in a falcon tube before implementation to prevent small droplets in the water (Öğretmen and Gökçek, 2013).

Experimental Design

Five concentrations of three anesthetic agents were tested on Wels to find out the effective dose. The minimum and maximum concentrations were chosen according to previously published studies (Weber *et al*., 2009; Gökçek and Öğretmen, 2011). The concentrations were adjusted for clove oil and benzocaine 25, 50, 75, 100 and 125 µL/L, and for PE 250, 500, 750, 1000 and 1250 µL/L. Each replicate contains seven individuals and all concentrations were tested in triplicate.

During the implementation, fish were netted and transferred to the 20 L aquarium containing different concentration of anesthetic solutions. The induction and recovery times for all agents were measured by a stopwatch under the same conditions. Fish were immediately transferred to the anesthetic free water just after they reached the last anesthesia stage. The recovery stage was recorded after transferring the fish to the aerated clean water in 20 L aquarium. Also, water quality parameters such as dissolved oxygen, temperature and pH were monitored by oxygen meter (Hanna HI-9146) and a pH meter (Hanna) in aquariums and detected as 8.01±0.93 mg/L, 20ºC and 8.63±0.22, respectively.

Four consecutive stages for induction and three stages for recovery were specified according to Theinpoint and Niemegeers (1965). Some modifications were put to use based on behavioral response of Wels (Table 1).

Statistics

The relationship between dosage and induction/recovery times were stated by Regression analyses. Shapiro-Wilk test for normality and Bartlett's test for homogeneity of variance was used. One-way analyzes of variance (ANOVA) was used to determine the differences in time of occurrence of physiological responses to the different doses of the same anesthetic agent compare the means and Duncan test was employed to analyze differences between means. Significance of differences was tested at $P<0.05$ level. All statistical processes were analyzed by the SPSS (SPSS Systems for Windows, Version 15.0).

Results

Physiological responses to the different doses of anesthetic agents were given in Table 2. Induction times significantly decreased with increasing concentrations in all agents, except the lowest concentrations ($P<0.05$). All the lowest concentrations were not efficient enough for fish to get the stages I3 and I4. On the other hand, recovery times increased with increasing concentrations of anesthetics ($P<0.05$).

A significant relation was observed between different concentrations and induction/recovery times for all tested agents ($P<0.05$). While scatter plots yielded an inverse exponential relationship for induction, the scatter plots showed exponential relationships in recovery (Figure 1). The equations of induction times (in seconds) to reach I4 stage of anesthesia and concentrations (c) of anesthetics were $I4= 458.99e^{-0.019c}$ ($R^2=0.9759$) for clove oil, $I4=310.42e^{-0.001c}$ ($R^2=0.9368$) for PE and $I4=240.37e^{-0.009c}$ ($R^2=0.9482$) for benzocaine. The regression equations established for recovery time and concentrations were $R3=48.02e^{0.017c}$ ($R^2=0.9549$) for clove oil, $R3=49.05e^{0.002c}$ ($R^2=0.9478$) for PE and

Table 1. Behavioral stages of anesthesia in Wels, *S. glanis* (modified from Theinpoint and Niemegeers, 1965)

Induction stages
I 1: Respiratory disorder: Operculum excessively comes open
I 2: Loss of balance: partial inhibition of reactions to external stimuli
I 3: Total loss of equilibrium: Fish still react to strong stimuli
I 4: Total loss of reflexes and movement: Fish lay on the 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

$R3=54.80e^{0.014c}$ ($R^2=0.9506$) for benzocaine.

No mortality was observed after treatments.

Discussions

There are several biological and environmental factors which effects to the efficiency of an anesthetic agent such as specie, size, weight, age, tissue fat content, water temperature and pH, etc. (Iversen *et al*., 2003). Furthermore, the efficacy of an anesthetic is directly affected by metabolic rate (Burka *et al*., 1997; Ross and Ross, 1999).

In this study, induction times significantly decreased with increasing of concentrations of clove oil, 2-phenoxyethanol and benzocaine ($P<0.05$). Similar results were gained by previous studies in teleost fish (Hseu *et al*., 1998; Mylonas *et al*., 2005; Gullian and Villanueva, 2009; Weber *at al*., 2009; Heo and Shin, 2010; Gökçek and Öğretmen, 2011; Öğretmen and Gökçek, 2013; Öğretmen *et al*., 2014). On the other hand, recovery times increased with increasing concentrations of three anesthetic agents in wels ($P<0.05$). Prolonged recovery has been reported in Persian sturgeon, *Acipencer persicus* (Bagheri and Imanpour, 2011), Russian sturgeon, A. gueldenstaedtii (Akbulut *et al*., 2011), Himri barbell, *Carasobarbus luteus* (Gökçek and Öğretmen, 2011), African catfish, *Clarias gariepinus* (Öğretmen and Gökçek, 2013), and Shabut, *Barbus grypus* (Öğretmen *et al*., 2014). However, decreasing recovery times with increasing concentrations of clove oil and 2-phenoxyethanol was reported for Sea bass and Sea bream by Mylonas *et al*. (2005). The authors explained this unexpected situation by that the fish did not contact with the highest concentrations of anesthetic agents for long which allows recovered fast (Pawar *et al*., 2011).

The effectiveness criteria for an anesthetic agent is to supply a complete induction in 180 s and recovery in 300 s for teleosts (Marking and Meyer, 1985). In the present study, the application of 75 and 100 µl L^{-1} clove oil, 500, 750 and 1000 µl L^{-1} PE, and 50, 75 and 100 µl L^{-1} benzocaine resulted adequate induction (<180 s), and recovery (<300 s). Although highest concentrations achieved shorter induction time for wels, aforementioned concentrations were presented a good margin of safety when compare against the efficacy criteria above. Fish wasn't fully sedated by the lowest doses of all three agents.

In conclusion, the present study reveals that clove oil, PE and benzocaine can be safely used as sedatives in culture of wels. Although the recovery time was slightly longer than the other, 100 µl L^{-1} clove oil is the most effective agent for wels due to not only smaller cost for fish farms but also a lesser polluting matter for the environment. However, Gomulka *et al*., 2015 emphasized that this agent's therapeutic index is low. On the other hand, it is clear that there is obviously a need to regulate of laws for using of anesthetics in the countries except EU and USA.

Table 2. Physiological responding times (s) for clove oil, 2-phenoxyethanol and benzocaine. Data are presented as mean±sd.

Clove oil	Concentrations (µl L^{-1})				
Stages	25	50	75	100	125
I 1	24.57±3.87^{b}	18.86±2.79^{b}	16.86±4.30^{ab}	13.14±1.68^{a}	10.29±2.18^{a}
I 2	38.00±4.32^{b}	34.85±9.48^{ab}	33.57±5.91^{ab}	31.14±2.85^{ab}	27.86±5.15^{a}
I 3	-	79.14±14.87^{b}	58.71±6.40^{b}	39.43±4.69^{a}	37.14±5.21^{a}
I 4	-	189.00±6.43^{d}	110.57±6.48^{c}	69.00±6.48^{b}	47.42±4.65^{a}
R 1	-	32.43±4.69^{b}	19.29±5.41^{a}	38.43±7.70^{b}	104.67±7.05^{c}
R 2	19.29±2.56^{a}	130.00±26.48^{b}	112.57±10.31^{b}	139.00±21.16^{b}	247.33±16.17^{c}
R 3	69.86±11.04^{a}	139.86±9.67^{b}	170.43±7.98^{c}	253.86±14.78^{d}	475.33±12.27^{e}
PE	Concentrations (µl L^{-1})				
Stages	250	500	750	1000	1250
I 1	43.71±4.03^{b}	32.29±7.57^{b}	17.71±2.75^{a}	16.14±3.02^{a}	14.14±4.60^{a}
I 2	66.29±5.94^{c}	46.00±9.88^{b}	33.29±5.65^{b}	26.29±4.07^{ab}	21.86±5.08^{a}
I 3	-	87.86±14.89^{b}	75.57±16.45^{b}	47.43±4.96^{a}	38.29±8.90^{a}
I 4	-	174.86±10.59^{d}	124.00±11.20^{c}	88.71±8.50^{b}	71.71±6.78^{a}
R 1	-	20.00±7.33^{a}	27.71±3.45^{a}	32.43±6.48^{a}	106.57±35.26^{b}
R 2	-	44.00±11.46^{a}	64.14±13.67^{a}	150.29±25.79^{b}	264.71±45.80^{b}
R 3	-	107.29±8.26^{a}	142.43±20.93^{b}	201.43±12.19^{c}	322.71±7.43^{d}
Benzocaine	Concentrations (mg L^{-1})				
Stages	25	50	75	100	125
I 1	37.29±5.19^{c}	32.29±4.39^{bc}	28.14±2.34^{b}	18.00±4.43^{a}	11.57±2.30^{a}
I 2	-	44.00±8.37^{a}	39.29±6.10^{a}	36.29±3.99^{a}	31.57±3.95^{a}
I 3	-	115.14±27.69^{b}	107.71±22.82^{b}	81.43±12.9^{ab}	65.14±15.89^{a}
I 4	-	156.14±8.75^{d}	126.43±8.00^{c}	103.00±6.90^{b}	81.71±6.90^{a}
R 1	-	13.00±2.77^{a}	14.28±3.04^{a}	16.86±2.34^{a}	17.57±6.88^{a}
R 2	-	83.71±9.70^{a}	135.84±9.26^{b}	191.43±29.17^{c}	258.86±43.83^{d}
R 3	-	120.57±4.86^{a}	149.29±9.48^{b}	215.71±19.99^{c}	352.14±12.20^{d}

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

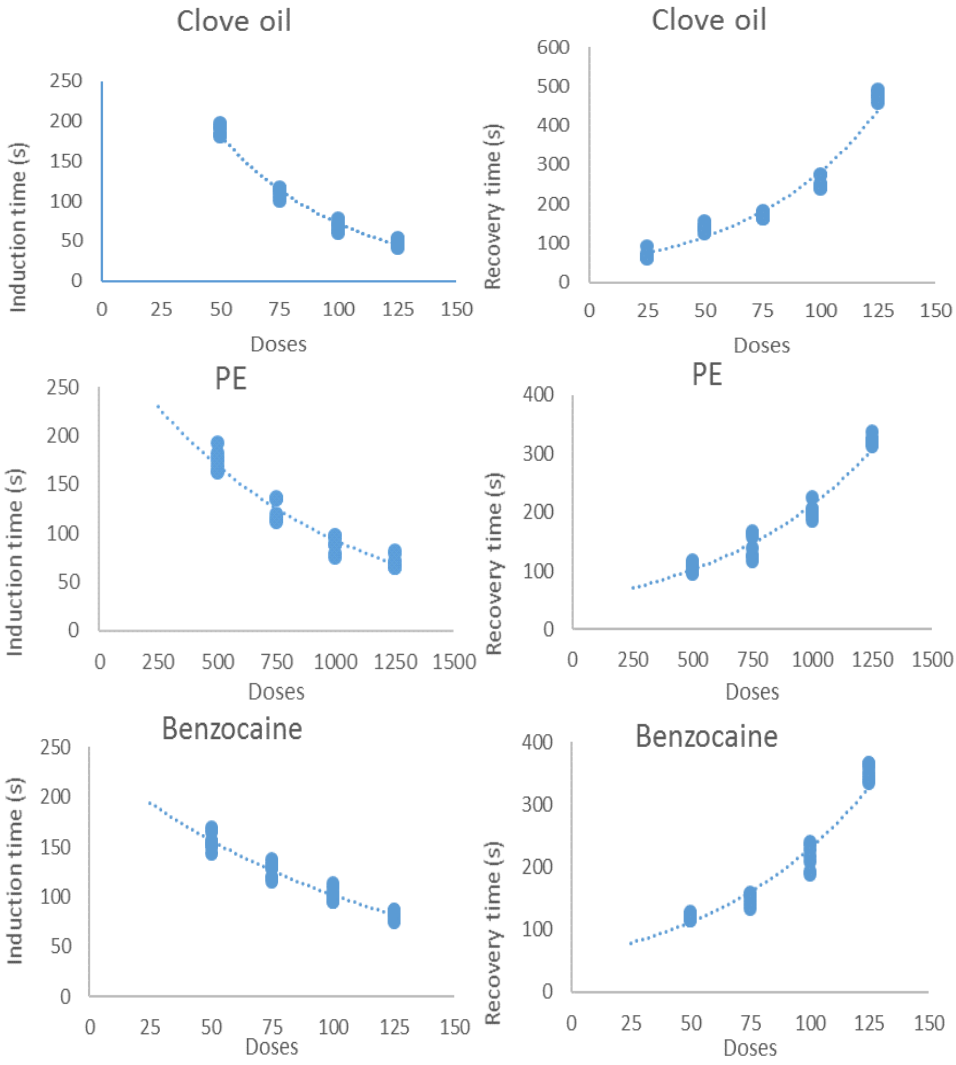

Figure 1. Induction and recovery times (s) relation to anesthetic doses for Wels (n=7 for each trial).

Acknowledgements

Special thanks to owner and staffs of Tuğçe Ltd. for their complimentary helps during the experiment.

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