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Comparative Efficacy of Three Anesthetic Agents in Himri Barbel, *Carasobarbus luteus* (Heckel, 1843) under Controlled Conditions

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Abstract: In this study, the efficacy of three anaesthetic agents (Clove oil, 2-phenoxyethanol and benzocaine) was compared in captive-bred Himri barbel, *Carasobarbus luteus* (Heckel, 1843). The lowest effective concentrations based on the efficacy criteria of complete anesthesia induction within 180 sec and recovery within 300 sec were determined to be 75 mg L^{-1} (induction 143±15 sec and recovery time 149±35 sec) for clove oil, 500 μL L^{-1} (induction 145±27 sec and recovery time 57±16 sec) for 2-phenooxyethanol and 50 mg L^{-1} (induction 152±49 sec and recovery time 88±24 sec) for benzocaine. The onset of individual phases of anesthesia and recovery times depended significantly on the concentration of the anaesthetic used ($p<0.05$). An inverse exponential relationship was observed between concentrations of anaesthetic and induction time whereas exponential relationships were observed between concentrations and recovery times for all anaesthetic agents evaluated.

Key words: Clove oil, benzocaine, 2-phenoxyethanol, induction time, recovery time, Turkey

INTRODUCTION

Anesthetics play an important role in aquaculture research and production. They are used in weight measurements, selection, broodstock management and in fish health (Summer and Smith, 1990; Kazuf and Siwicki, 2001; Hegyi *et al*., 2010). Anesthetics, cause different levels of activity in fish. Fish first obtain a state of general anesthesia which ends in a total loss of consciousness. Reflex activity is lost entirely and skeletal muscle tone is also reduced (McFarland, 1960). Overdose or overexposure during treatments reduces breathing and results in low oxygen saturation in blood and ultimately in respiration and circulation disorders (Tytler and Hawkins, 1981). Reduced breathing is an important warning sign that requires termination of the treatment (Hajek and Klyszejko, 2004: Dziaman *et al*., 2005).

The most commonly used anesthetics in aquaculture are MS-222 (tricaine methane sulphonate), benzocaine (ethly-p-aminobenzoate), methomidate, clove oil and 2-phenoxyethanol (ethylen glycol monophenyl ether) (Velisek *et al*., 2006, 2011). Currently, only MS-222 is licensed for use in food fish in the USA and the European Union. However, compounds such as 2-phenoxyethanol, clove oil and benzocaine have been evaluated experimentally and are being used in non-food fish and in research (Coyle *et al*., 2004).

Choosing an appropriate anesthetic depends mainly on its effectiveness in immobilizing fish with good recovery rates (Gilderhus and Marking, 1987; Burka *et al*., 1997). An ideal anesthetic should possess several attributes such as non-toxic, inexpensive, simple to administer and result in rapid induction and calm 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 (Pawar *et al*., 2011).

Himri barbel is indigenous cyprinid in the basin of Mesopotamia and highly valuable as food in the region. It is omnivorous species that feeds mainly on detritus (Epler *et al*., 2001). Its adaptation to earthen ponds has been noticed when it pumped accidently into carp fish ponds located near the Euphrates river (Al-Daham *et al*., 1991) therefore, Himri barbel could be considered as a new species for the regional aquaculture. Some studies have been done on the biology of Himri barbel in the Iraq, Syria and Turkey (Epler *et al*., 1996; Szypula *et al*., 2001; Al-Hazzaa, 2005; Gokcek and Akyurt, 2008) but the aquaculture potential of the fish has been identified only recently (Al-Hazza and Hussein, 2003; Hazzaa and Hussein, 2003; Gokcek and Akyurt, 2007; Gokcek, 2008; Gokcek and Tepe, 2009a, b; Gokcek, 2011).

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Given the growing interest in the culture of Himri barbel and lack of detailed practical information on the administration of anesthetics, the overall aim of present study was to determine induction and recovery times of three most common fish anesthetic agents (clove oil, 2-phenoxyethanol and benzocaine) that could be efficiently use in Himri barbel, *Carasobarbus luteus* (Heckel, 1843), under controlled conditions.

MATERIALS AND METHODS

Experimental animals: Himri barbel fingerlings were produced from local broodstock in captivity in the aquaculture department of Mustafa Kemal University, Antakya, Hatay, Turkey. About 6 months old fingerlings (average 85±15 g) were transferred to the production unit of National States of Hydraulic Works Department in Adana and held for 2 weeks acclimatization period before the study began.

Anesthetic agents: The anesthetic agents 2-phenoxyethanol (ethylene glycol monophenyl ether, Sigma Aldrich Chemic, Germany) (PE), benzocaine (Ethly-p-aminobenzoate, Himedia, India) and clove oil (Biopont, Budapest, Hungary) were used for the present study. Doses of the anesthetic agents were prepared a few minutes before the experiments. Since, clove oil and benzocaine do not dissolve in water (Woody *et al*., 2002) they were initially diluted in ethanol (ratio of clove oil/benzocaine to ethanol 1:9). PE was initially mixed with water in a reagent bottle and then stirred to disperse the chemical to form small droplets before adding to anesthetic test aquarium.

Induction and recovery stages of anesthetics: The efficacy of three anesthetic agents in fingerling Himri barbel was assessed by testing several doses of each anesthetic. Choice of minimum and maximum doses of each anesthetic was based on previously published information for teleosts (Gomes *et al*., 2001; Weber *et al*., 2009). The following doses of each agent were evaluated; clove oil (25, 50, 75, 100 and125 mg L^{-1}), PE (250, 500, 750, 1000 and 1250 μL L^{-1}) and benzocaine (25, 50, 75, 100 and 125 mg L^{-1}). Five individuals were exposed to five concentrations of each anesthetic totaling 75 individuals. Experiments were prepared in triplicate to verify findings. After 2 weeks of acclimation, the fish were netted from rearing concrete tanks and transferred to the holding aquarium (200 L) filled with fresh and aerated water in the laboratory conditions. Fish were netted and transferred individually to the anesthetic aquarium (10 L). The induction and recovery time for all anesthetics was measured under same experimental conditions using a digital stopwatch. The water conditions were temperature 23°C, pH 7.94±0.56 and oxygen 5.7±1.26 ppm.

Table 1: Signs and stages of anaesthesia in Himri barbel, *Carasobarbus luteus*

Stages	
Induction	Recovery
I1:Loss of balance, partiall inhibition of reactions to external stimuli	R1:Start of movement. Fish still lay on bottom of the tank
I2:Total loss of equilibrium. Fish still react to strong stimuli	R2:Regular breathing. Reaction to strong stimuli.Irregular balance
I3:Total loss of reflexes and movement. Fish lay on bottom of the tank	R3:Total recovery of equilibirium. Reaction to slight stimuli. Normal swimming
I4:Complete cessation of opercula movements, death	

Changes in the physiological status of the anesthetized fish were assessed in four consecutive stages for induction and three stages for recovery described by Theinpoint and Niemegeers with little modifications based on the behavioral response of Himri barbel (Gullian and Villanueva, 2009) (Table 1).

Statistical analysis: A Kruskal-Wallis test was used to assess the differences in induction and recovery times of different concentrations of the same anesthetic agent (Zar, 1999). Regression analyses were used to establish the relationship between dosage and induction time as well as dosage and recovery time. Significance difference was tested at a 5% level, represented $p<0.05$. All results were processed and analyzed with the SPSS computer program (SPSS Systems for Windows, Version 13.0).

RESULTS AND DISCUSSION

Stages of anesthesia: Significant differences ($p<0.05$) in the induction and recovery stages at different concentrations of the three anesthetic agents were identified for himri barbel (Table 2). At certain concentration of 2-phenoxyethanol (250 μL L^{-1}), all stages of induction could not be attained. This may be due to the concentration applied were too low to reach complete anesthetic induction.

Induction and recovery times of anesthesia: Induction times decreased significantly with increasing doses for all the anesthetic agents evaluated. Induction time (I 3) ranged from 1126±45 sec (25 mg L^{-1}) to 71±19 sec (125 mg L^{-1}) for clove oil from 145±27 sec (500 μL L^{-1}) to 32±3 sec (1250 μL L^{-1}) for PE and from 207±34 sec

Table 2: Induction and recovery times (sec) for Himri barbel anaesthetized with five concentrations of three anaesthetic agents. Data are presented as mean±SD. Hyphen denotes non-attainment of stage

Stages	25	50	75	100	125
Clove oil (concentrations (mg L^{-1}))					
I1	83±21^{a}	56±13^{b}	46±3^{b}	33±2^{c}	25±4^{c}
I2	183±23^{a}	87±17^{b}	77±13^{b}	60±5^{c}	41±8^{d}
I3	1126±45^{a}	237±113^{b}	143±15^{c}	116±19^{c}	71±19^{d}
R1	37±18^{a}	46±12^{a}	46±19^{a}	54±14^{a}	44±11^{a}
R2	60±19^{a}	106±37^{b}	69±29^{a}	90±19^{ab}	70±15^{a}
R3	132±20^{a}	148±11^{a}	149±35^{a}	198±8^{b}	287±8^{b}
Stages	250	500	750	1000	1250
PE (concentrations (µL L^{-1}))					
I1	128±11^{a}	46±2^{b}	28±6^{c}	23±3^{cd}	18±1^{d}
I2	-	86±10^{a}	62±9^{b}	43±6^{c}	26±4^{d}
I3	-	145±27^{a}	85±21^{b}	59±7^{c}	32±3^{d}
R1	-	25±5^{a}	26±3^{a}	31±7^{a}	57±22^{b}
R2	-	41±6^{a}	51±6^{ab}	49±12^{ab}	71±25^{b}
R3	-	57±16^{a}	71±10^{a}	79±8^{ab}	90±5^{b}
Stages	25	50	75	100	125
Benzocaine (concentrations (mg L^{-1}))					
I1	39±4^{a}	31±4^{b}	26±2^{c}	20±2^{d}	13±3^{e}
I2	75±12^{a}	83±19^{bc}	58±14^{cd}	52±12^{d}	27±6^{e}
I3	207±34^{a}	152±49^{b}	72±8^{c}	62±12^{c}	32±4^{d}
R1	23±4^{a}	30±15^{a}	35±3^{a}	44±7^{a}	I4
R2	31±5^{a}	41±24^{a}	47±4^{a}	52±4^{a}	I4
R3	69±21^{a}	88±24^{a}	95±6^{ab}	114±16^{b}	I4

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

(25 mg L^{-1}) to 32±4 sec (125 mg L^{-1}) for benzocaine. Recovery times increased with increasing concentrations of anesthetic agents ($p<0.05$). Recovery time ranged from 132±20 sec (25 mg L^{-1}) to 287±8 sec (125 mg L^{-1}) for clove oil from 57±16 sec (500 µL L^{-1}) 90±5 sec (1250 µL L^{-1}) to 90±5 sec (1250 µL L^{-1}) for PE and from 69±21 sec (25 mg L^{-1}) to 114±16 sec (100 mg L^{-1}) for benzocaine.

Induction and recovery in relation to concentration: A significant correlation was observed between anesthetic concentration and induction time for all tested anesthetic agents ($p<0.05$) whereas scatter plots yielded an inverse exponential relationship (Fig. 1). The regression equations of times to reach I3 and Concentrations (C) of three anesthetic agents in Himri barbel were $I3 = 1260.50e^{-0.0183c}$ ($R^2 = 0.91$) for clove oil, $I3 = 348.40e^{-0.0018c}$ ($R^2 = 0.92$) for PE and $I3 = 332.53e^{-0.0183c}$ ($R^2 = 0.91$) for benzocaine. Similarly, a significant correlation ($p<0.05$) was observed between anesthetic concentration and times to reach R3 for all anesthetic agents whereas scatter plots showed exponential relationships (Fig. 1). The regression equations established for recovery time and concentrations were $R3 = 99.63e^{0.0074c}$ ($R^2 = 0.72$) for clove oil, $R3 = 53.19e^{0.006c}$ ($R^2 = 0.93$) for PE and $R3 = 35.54e^{0.0136c}$ ($R^2 = 0.96$) for benzocaine.

Post-treatment survival: Himri barbel reared in post-treatment tanks recovered well after the anesthetic experiment. Mortality was observed in the highest dose of benzocaine (1250 µL L^{-1}) with the ratio 60%. No other mortality was observed during post-treatment period. Anti-stress agents form an integral component of modern day aquaculture (Pawar *et al*., 2011). Biological factors include species, the stage of life cycle and age, size and weight, lipid content, body content and disease status. All these factors affect the metabolic rate and therefore, the pharmacokinetics of the anaesthetic 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 and therefore, increase or decrease the efficacy of an anaesthetic agent (Burka *et al*., 1997; Ross and Ross, 1999).

In the present study, the induction times decreased significantly with the increasing clove oil, 2-phenoxyethanol and benzocaine concentrations ($p<0.05$). The results are in agreement with previous studies in teleost fish (Mattson and Riple, 1989; Hseu *et al*., 1998; Mylonas *et al*., 2005; Gullian and Villanueva, 2009; Weber *et al*., 2009; Heo and Shin, 2010). On the other hand, recovery times increased with increasing concentrations of anaesthetic in fingerling Himri barbel. Prolonged recovery with increased anaesthetic dosage has been reported in sockeye salmon (Woody *et al*., 2002) and cobia (Gullian and Villanueva, 2009). However, decreasing recovery times with and increase in concentration of clove oil and 2-phenoxyethanol for European sea bass and gilthead seabream has been reported by Mylonas *et al*. (2005). The explanation put

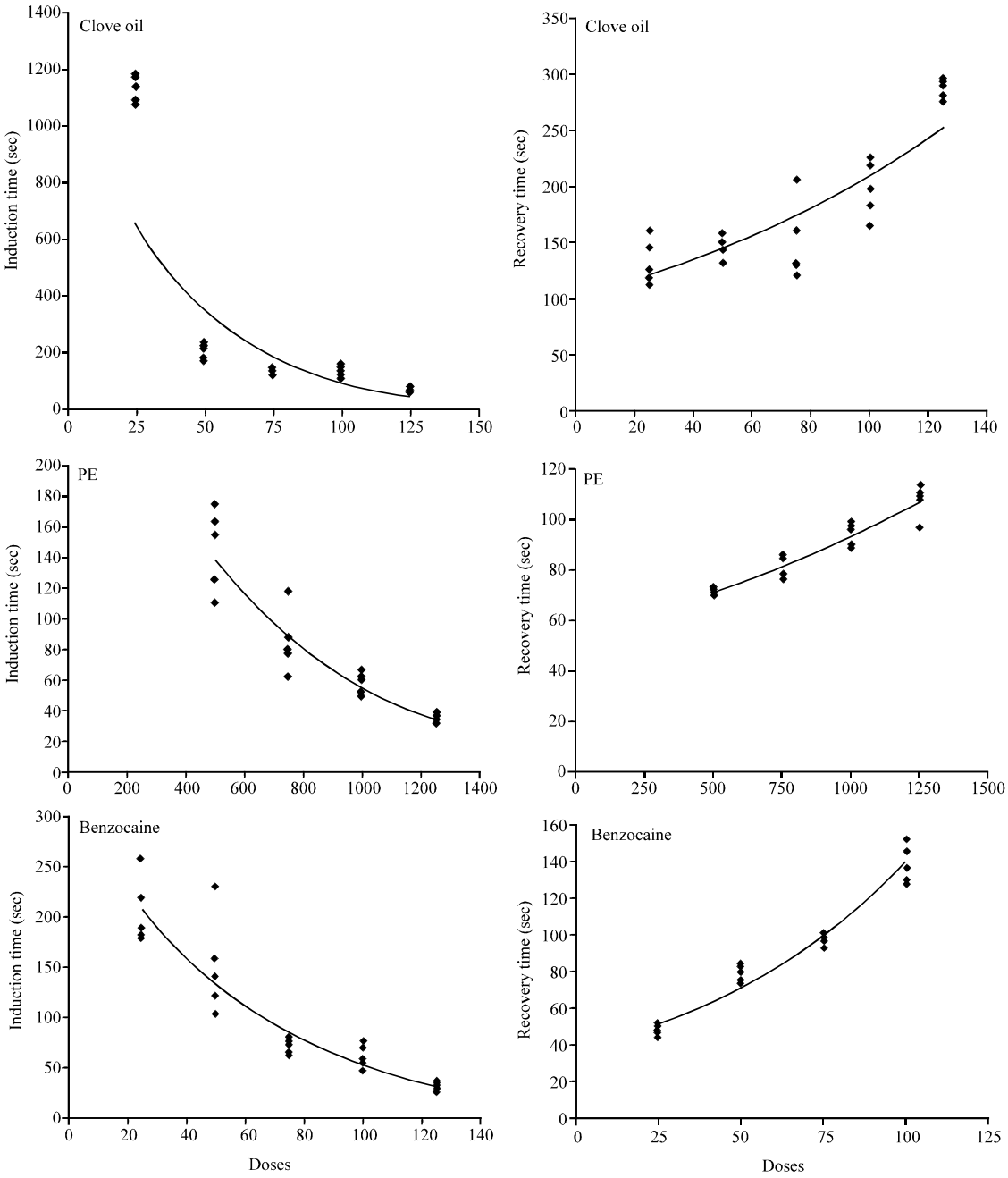

Fig. 1: Induction and recovery times (sec) relation to anaesthetic concentrations for Himri barbel 8n = 5 for each trial)

forward by these researchers is that with the highest doses the fish is not contact with the anaesthetic for long which allow faster recovery (Pawar *et al*., 2011). Also, differences in the physiological responses of fish to the anaesthetic agents also influence this trend (Weber *et al*., 2009).

According to Marking and Meyer (1985), the anaesthetic agent is considered effective if it produces a complete induction within 180 sec and recovery with 300 sec for fish. In this study, application of clove oil at dose of 75 mg L^{-1}, 2-phenoxyethanol at dose of 500 μL L^{-1} and benzocaine at dose of 50 mg L^{-1} resulted in quick induction, total immobilization and fast recovery in Himri barbel fingerlings. Although, higher concentrations of three anaesthetic agents achieved shorter induction times, aforementioned doses were

effective and presented a good margin of safety when compared against the above efficacy criteria. The present study demonstrated that clove oil acts as an anaesthetic in fingerlings of Himri barbel and can be used at concentrations ≥75 mg L^{-1}. Although, it results in slightly longer recovery times, the advantages of clove oil is not only smaller cost for aquaculturist but also a lesser polluting effect for the environment. On the other hand, commercial dosage of 2-phenoxyethanol (500 μL L^{-1}) was also effective dose for Himri barbel. Benzocaine was effective as anaesthetic for Himri barbel at concentrations ≥50 mg L^{-1}. However, at concentration of 125 mg L^{-1} benzocaine, Himri barbel reached stage I4 with the ratio 60% during post-treatment period. Mattson and Ripple (1989) found that cod entered stage I3 within 2 min at dosage of 50 mg L^{-1} benzocaine. A concentration of 35 mg L^{-1} was considered an adequate dose to induce fish in rainbow trout (Gilderhus and Marking, 1987). For other fish species, concentration of 80-200 mg L^{-1} benzocaine was required to induce anesthesia (Dawson and Gilderhus, 1979).

CONCLUSION

In many countries, the use of fish anesthetics is a matter of concern as there are no specific laws regulating their use (Pawar *et al*., 2011). Clove oil, 2-phenoxyethanol and benzocaine have been extensively used as an anaesthetic agent in aquaculture of freshwater and marine fishes. Further studies on different life stages, gender, reproduction state and sizes followed by assessments of the effects of anesthetics on haematological profile and respiration rate will advance the understanding of anesthesia of Himri barbel, *Carasobarbus luteus*.

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