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Anaesthetic Qualities of Eugenol and 2-Phenoxyethanol and Their Effect on Same Haematological Parameters in Farmed European Sea Bass (*Dicentrarchus labrax* L.)

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Abstract: To gain more knowledge on the anaesthetic characteristics of 2-phenoxyethanol and eugenol in fish, this study investigated induction and recovery when administering these substances in increasing concentrations to 240 adult farmed European sea bass (*Dicentrarchus labrax*). Levels of serum cortisol, serum glucose and haematocrit value were recorded in order to evaluate the effects of the anaesthetic substances on haematological indicators of stress in fish. The anaesthetic responses showed the notable power of Eugenol; the induction phase was rapid and subjects reached deep anaesthesia in significantly shorter times compared to 2-phenoxyethanol. The subjects anaesthetized with Eugenol tended to recover very slowly even at low concentrations in comparison with the fish treated with 2-phenoxyethanol which always recovered rapidly. The blood parameters showed few statistically significant differences between control and anaesthetized fish with an irregular trend between the groups tested except for the haematocrit values which were in three of the four trials with Eugenol, statistically higher in the control fish compared with the anaesthetized fish. The results of this study suggest that the anaesthetics used do not substantially affect the evaluated blood profile in the sea bass.

Key words: Fish, anaesthesia, serum cortisol, serum glucose, haematocrit value, Italy

INTRODUCTION

Fish anaesthesiology has evolved away from the traditional anaesthetic practices borrowed from those commonly used on warm-blooded animals. Over time, this discipline has developed numerous methods of employment and has adopted various substances in order to facilitate handling fish in aquaculture (Summerfelt and Lynwood, 1990; Ross and Ross, 1999; Mylonas *et al*., 2005) for example when measuring or weighing fish during the administration of vaccines, sampling for blood or gonadal biopsies, transport and minor and major surgical operations including paracentesis, gonadectomies, exeresis of skin tumours and parasitic nodules, laparotomies, enterotomies and enucleation of the eyeball (neoplasms) (Harms and Lewbart, 2000; Mylonas *et al*., 2005).

The anaesthetics used in fish work directly on the central nervous system, depressing it both pharmacodynamically and pharmacokinetically according to the concentration used. Such substances initially reach the cerebral cortex and then the basal ganglia, the cerebellum and finally the spinal cord and medulla (Ross and Ross, 1983; Imamura-Kojima *et al*., 1987).

The anaesthetic methods used include injectable anaesthetics, water-soluble anaesthetics and inhaled anaesthetics. On fish farms, dissolvable anaesthetics are the preferred method because of their great simplicity of use, their reliability and their wide safety margins for the operator. The dissolvable anaesthetics most frequently used in fish farming are: MS-222 (tricaine methane sulphonate), Propiscin (ethomidate), Quinaldine sulphate, Benzocaine hydrochloride, Methomidate, 2-Phenoxyethanol and Eugenol.

Eugenol is the major component in clove oil (70-90% by weight) and is obtained by distillation of the flowers, stems and leaves of the clove tree (*Eugenia aromatica* or *caryophyllata*). In addition, besides meeting the necessary anaesthetic requirements (Keene *et al*., 1998; Pirhonen and Schreck, 2003; Woody *et al*., 2002; Mylonas *et al*., 2005; Guenette *et al*., 2007), it does not seem to pose any threat to public health if it is used in aquaculture as it has also been employed for centuries as a topical analgesic in dentistry (Curtis, 1990; Soto and Burhanuddin, 1995). Moreover, Eugenol is cheaper and more potent than other anaesthetics used in fish and the US Food and Drug Administration has classified it as a Generally considered as Safe Q (GRAS) compound (Summerfelt and Lynwood, 1990).

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It is also of importance to understand what effect the possible use of anaesthetics on farmed fish could have on the conditions of stress and on the modification of haematological indicators of stress.

It is well known that farmed fish are often subjectetd to adverse stimuli that cause acute (Pickering, 1981; Pottinger *et al*., 1999) or chronic (Pickering, 1981; Montero *et al*., 1995) stress. External (environment) and internal (disease and metabolic unbalance) stressors are perceived by fish and react with a primary neuroendocrine response, represented by an increase in corticosteroids and catecholamines (Pickering, 1981). As a direct consequence of their high levels in the circulatory system, a wide range of secondary stress responses can be observed such as the increase in blood glucose (Pickering, 1981; Melotti *et al*., 1992) from tissue reserves of glycogen. Moreover, during the adaptive stress response, the haemopoietic activity of the spleen increases which encourages the production of red blood cells for oxygen transport (Franklin *et al*., 1993). This condition leads to an increase in the levels of blood corpuscular components such as haematocrit value, one of the most reliable indices (Buscaino *et al*., 2010).

2-Phenoxyethanol and Eugenol tend to modify the blood parameters of stress in particular by acting on serum glucose and plasma cortisol dynamics (Jinn-Rong *et al*., 1994; Cho and Heath, 2000; Ortun *et al*., 2002; Pirhonen and Schreck, 2003; Holloway *et al*., 2004; Velisek *et al*., 2005). As reported in the research serum glucose and cortisol tend to rise after the administration of these anaesthetic substances. However, the existing literature does not contain data on the dynamics of these blood parameters in *Dicentrarchus labrax* after administration of 2-Phenoxyethanol and Eugenol.

The European sea bass (*Dicentrarchus labrax*) is one of the major Mediterranean species produced by the aquaculture industry in Italy with the annual production of about 9.200 tons (ISMEA, 2009).

Anaesthetics are commonly used in juveniles of these species when evaluating the occurrence of skeletal deformities (Chatain, 1994; Koumoundouros *et al*., 1997) and during vaccinations. Moreover, these substances are also used in adult fish during the evaluation of reproductive status and spawning induction therapies (Mylonas *et al*., 2003).

The aim of the present research was to evaluate the anaesthetic characteristics of Eugenol and to compare its efficacy to 2-Phenoxyethanol which is commonly used in fish farms, administering these substances in four increasing concentrations to adult farmed European sea bass. In addition in order to contribute to the body of knowledge regarding the effects of fish exposure to anaesthetics, the researchers recorded the levels of the main blood parameters that are indicators of stress in fish after the sea bass had reached deep anaesthesia.

MATERIALS AND METHODS

Fish: A total of 240 farmed European sea bass (*Dicentrarchus labrax*) aged around 2 years, weighing 223.3±95.1 g (Mean±SD) and measuring 24.2±3.1 cm (Mean±SD) were examined in this study. The fish came from the off-shore fish farm Hippocampus (Villafranca Tirrena-Messina, Italy).

Anaesthetics: The experiments required the use of 2-Phenoxyethanol (99%, MERCK, Whitehouse station, NJ, USA) and Eugenol (99%, ALDRICH, St. Louis, MO, USA). 2-Phenoxyethanol is ethylene-glycol-monophenyl-ether. Its summary formula is $C_8H_{10}O_2$, the molar weight 138.17 g L^{-1}, density 1.107-1.108 g dm^{-3} and the boiling temperature is 245°C. The anaesthetic is slightly soluble in water (26.7 g L^{-1}) at 25°C but readily soluble in ethanol. Eugenol (2-methoxy-4-(2-propenyl) phenol) chemical formula is $C_{10}H_{12}O_2$, the molar weight 164.20 g L^{-1}, density 1.06 g cm^{-3} and the boiling point temperature is 265°C. It is slightly soluble in water and soluble in organic solvents. Eugenol concentrations of 30, 40, 50 and 60 mg L^{-1} and 2-Phenoxyethanol concentrations of 200, 300, 400 and 500 mg L^{-1} were used (Table 1).

These concentrations were selected after reference to other studies (Soto and Burhanuddin, 1995; Anderson *et al*., 1997; Hseu *et al*., 1998; Griffiths, 2000; King *et al*., 2005; Mylonas *et al*., 2005; Palic *et al*., 2006).

Experimental design: The study was designed to include eight experimental trials (from I-VIII), one for each concentration of the anaesthetics chosen. Four increasing concentrations of 2-Phenoxyethanol were used in trials I-IV and four increasing concentrations of Eugenol were used in trials V-VIII (Table 1). The entire study was performed during the month of July 2007.

Table 1: 2-Phenoxyethanol and Eugenol concentrations (mg L^{-1}) in the eight experimental trials

Anaesthetic agent	Experimental trial	Concentration (mg L^{-1})
2-Phenoxyethanol	I	200
	II	300
	III	400
	IV	500
Eugenol	V	30
	VI	40
	VII	50
	VIII	60

A diagram with a schematic representation of the experimental design is shown in Fig. 1. Each trial was carried out in part on a boat and in part at the Department of Experimental Sciences and Applied Biotechnologies of the Faculty of Veterinary Medicine, University of Messina.

For each trial, the researchers approached a pen containing seabass by boat and 30 fish were randomly caught with a net. All the fish were immersed in a tank (on the boat) containing 400 L of water taken from near the pen (in order not to cause any osmotic or thermal shock to the subjects). About 10 of the 30 fish caught were randomly selected to act as a control group for the haematological parameters and immediately underwent venipuncture for blood collection (using a 2.5 mL syringe with a 22 G X 1½ needle). Approximately 2 cc. of venous blood from each fish were collected and stored in tubes with EDTA and refrigerated for transport to the laboratory.

The remaining 20 sea bass were placed in another tank, containing 400 L of water to which was added the anaesthetic at the selected concentration as shown in Table 1.

The sea bass undergoing this study were carefully observed in order to note the characteristics of both anaesthesia induction and recovery; the data collected are shown in a table on the anaesthetic stages based on t he physiological responses of the fish following administration of the anaesthetics (Table 2).

After the fish had reached the fourth stage of anaesthesia, blood samples were taken from the caudal

Table 2: Phases and stages of the physiological responses caused by the anaesthetics during anaesthesia induction and recovery. Data from Summerfelt and Lynwood (1990) modified by the researchers

Anaesthetic phase	Stage	Characteristics
Induction		
Normal	0	Physiological position; reactive to external stimuli; opercular rate and muscle tone normal
Sedation	1	Slight loss of reactivity to stimuli; slight decrease in opercular rate and equilibrium normal
Light excitement	2	Increased locomotor activity, very high and erratic opercular rate and equilibrium normal
Partial loss of equilibrium	3	Partial loss of muscle tone; swimming erratic; uncoordinated locomotion, high and erratic opercular rate
Total loss of equilibrium and reflex reactivity	4	Flank position, immobilization, total loss of reactivity; opercular movements slow and irregular; heart rate slow and loss of all reflexes
Recovery		
Equilibrium and reflex reactivity loosed	0	Flank position, immobilization, total loss of reactivity; opercular movements slow and irregular; heart rate slow and loss of all reflexes
Recovery from partial loss of equilibrium	1	Slight tilting on the flank, uncoordinated locomotion, low locomotor activity, slight increased in opercular rate
Recovery from sedation	2	Physiological position, increased locomotor activity and increased opercular rate
Normal	3	Physiological position; normal locomotor activity and normal opercular rate

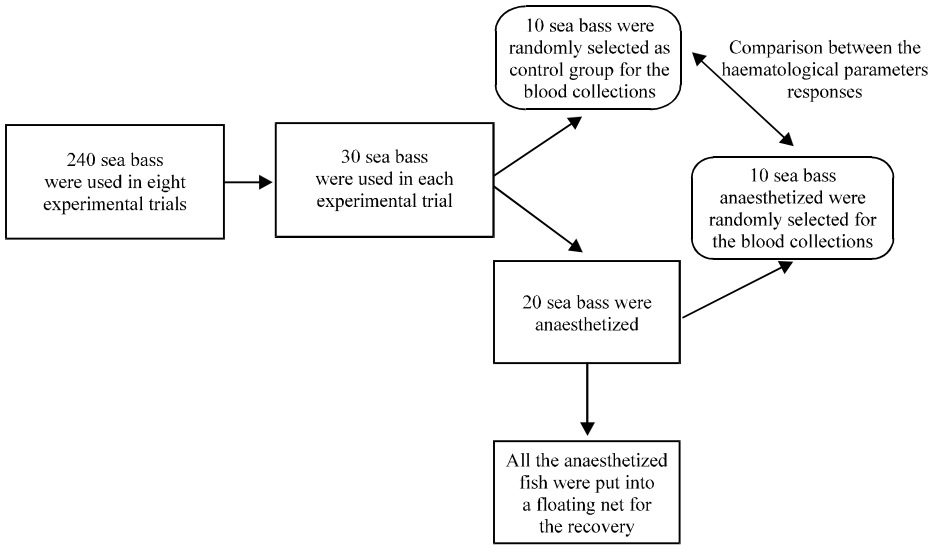

Fig. 1: The experimental design of the study. The diagram shows the schematic representation of a single experimental trial

vein of ten fish randomly chosen from the 20 fish anaesthetized in each experimental trial. The approximately 2 cc. of venous blood taken from each fish collected were treated. All blood samples were analysed as in haematological analysis.

After this phase, the anaesthetized fish were put in to the sea within a small floating net where they were able to recover gradually.

The criteria employed by Summerfelt and Lynwood (1990) and modified by the researchers of this study were used for the classification of the stages during anaesthesia induction and recovery.

The evaluations of the anaesthetics were based on a comparison of the substances during the phases of induction and recovery with a direct comparison being made between the concentrations of 200 and 30, 300 and 40, 400 and 50, 500 and 60 mg L^{-1} of 2-Phenoxyethanol and Eugenol, respectively for both phases, the Time to Reach the Anaesthetic Stage (TRAS) expressed in seconds and Opercular Movements (OM) expressed in beats/min were evaluated for each stage of anaesthesia induction and recovery.

The protocols of animal experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and EEC Directive 86/609.

Haematological analysis: After having completed the first phase of each experiment on board the boat, the blood samples were taken to the Department of Experimental Sciences and Applied Biotechnologies of the Faculty of Veterinary Medicine, University of Messina. The levels of serum cortisol, serum glucose and the haematocrit value were measured in all blood samples after centrifugation. The quantitative analysis of serum cortisol was carried out in a double-blind test using the immunoenzymatic method (SEAC-Radim group, Florence, Italy) the analysis of serum glucose was carried out using the spectrophotometric method (SEAC-Radim group, Florence, Italy); a Select-A-Fuge model 24 blood micro haematocrit centrifuge (Bio-Dynamics, Inc., Indianapolis, United States) running at 3600 rpm for 5 min was used to assess the haematocrit value.

Statistical analysis: The statistical analysis of the results obtained from the TRAS and OM of each stage of induction and recovery using 2-Phenoxyethanol and Eugenol was carried out using the Mann-Whitney U-test for non-parametric values. An unpaired t-test was used to determine significant differences in serum cortisol, serum glucose and haematocrit levels between the control fish and the anaesthetized fish from each experimental trial. All statistical analyses were performed using the STATISTICA 7.0 software package.

RESULTS AND DISCUSSION

Evaluation of anaesthetic qualities: The median Time to Reach the Anaesthetic Stage (TRAS) and the Opercular Movements (OM) during each stage of induction and recovery with 2-Phenoxyethanol and Eugenol are shown in Table 3 and 4.

Comparison between the substances showed higher values of TRAS after administration of Eugenol at the first

Table 3: Comparison between the median values (25, 75 percentiles) of the Time to Reach the Anaesthetic Stage (TRAS) expressed in seconds (sec) of the four concentrations of 2-Phenoxyethanol (PHE) and Eugenol (EUG) and between the median values (25, 75 percentiles) of the Opercular Movements (OM) expressed in beats/min of the four concentrations of 2-Phenoxyethanol (PHE) and Eugenol (EUG) during each stage of anaesthesia induction. The comparison between the four concentrations was made as shown: I concentration: Comparison between 200 mg L^{-1} of PHE and 30 mg L^{-1} of EUG; II concentration: comparison between 300 mg L^{-1} of PHE and 40 mg L^{-1} of EUG; III concentration: comparison between 400 mg L^{-1} of PHE and 50 mg L^{-1} of EUG; IV concentration: comparison between 500 mg L^{-1} of PHE and 60 mg L^{-1} of EUG. For each concentration, the presence of statistically different responses between TRAS or OM of PHE and EUG (Mann Whitney U test) is indicated by. *The statistically significant level was set at $p<0.001$

	Induction							
	Stage I		Stage II		Stage III		Stage IV	
Concentrations	TRAS (sec)	OM (b min^{-1})	TRAS (sec)	OM (b min^{-1})	TRAS (sec)	OM (b min^{-1})	TRAS (sec)	OM (b m^{-1})
I concentration								
PHE	147.5 (145, 160)	80 (80, 84.5)*	192.5 (180, 200)*	80 (76.25, 84.2)*	382.5 (360, 390)*	93 (93, 93)*	460 (460, 480)*	52 (52, 52)*
EUG	140 (130, 160)	63 (62, 64)	180 (176.3, 193.8)	64.5 (63, 67.5)	300 (290, 310)	65.5 (65, 66.75)	560 (540, 580)	48 (48, 48.7)
II concentration								
PHE	60 (55, 60)*	72 (72, 75)*	90 (80, 100)*	72 (70, 74.75)	120 (120, 130)*	87 (85, 88)*	180 (170, 187.5)*	66.5 (65, 67.75)
EUG	15 (15, 18)	68 (68, 72)	20 (18.5, 23.5)	70 (68, 70)	25 (23, 25)	74 (70.5, 74)	28 (27.25, 28)	65 (65, 65)
III concentration								
PHE	30 (30, 33.75)*	72 (72, 72)	44 (40, 45)*	75 (73, 77.75)*	80 (70, 90)*	83 (80, 83)*	110 (100, 120)*	67 (65, 68)*
EUG	5 (5, 7.75)	72 (72, 74)	13 (10, 13)	70 (70, 72)	18 (15, 18)	65 (63, 66.5)	21 (18, 22)	59 (58, 62)
IV concentration								
PHE	5 (5, 7)	72 (72, 74)	8 (8, 10)	85 (84, 92)*	12 (12, 14)	83 (83, 83.75)*	18 (18, 22)*	59 (58, 61)
EUG	5 (4, 5)	73 (72, 73)	10 (9.25, 10)	68 (68, 68)	12 (12, 13)	68 (67, 68)	17.5 (16, 18)	65 (60, 68)

Table 4: Comparison between the median values (25, 75 percentiles) of the Time to Reach the Anaesthetic Stage (TRAS) expressed in seconds (sec) of the four concentrations of 2-Phenoxyethanol (PHE) and Eugenol (EUG) and between the median numbers (25, 75 percentiles) of the Opercular Movements (OM) expressed in beats/min of the four concentrations of 2-Phenoxyethanol (PHE) and Eugenol (EUG) during each stage of the recovery from anaesthesia. The comparison between the four concentrations was made as shown: I concentration: comparison between 200 mg L^{-1} of PHE and 30 mg L^{-1} of EUG; II concentration: comparison between 300 mg L^{-1} of PHE and 40 mg L^{-1} of EUG; III concentration: comparison between 400 mg L^{-1} of PHE and 50 mg L^{-1} of EUG; IV concentration: comparison between 500 mg L^{-1} of PHE and 60 mg L^{-1} of EUG. For each concentration, the presence of statistically different responses between TRAS or OM of PHE and EUG (Mann-Whitney U test) is indicated by*. The statistically significant level was set at $p<0.001$

	Recovery					
	Stage I		Stage II		Stage III	
Concentration	TRAS (sec)	OM (b min^{-1})	TRAS (sec)	OM (b min^{-1})	TRAS (sec)	OM (b min^{-1})
I concentration						
PHE	15 (15, 15)	60 (60, 65)*	20 (20, 20)*	75 (75, 76.5)*	30 (30, 30)*	83 (83, 85)*
EUG	16.5 (15, 20)	52 (50, 52)	40 (40, 41.25)	65 (63, 65)	87.5 (85, 95)	70 (70, 72)
II concentration						
PHE	20 (20, 22)*	68 (68, 69)*	40 (40, 45)*	75 (73, 75)*	70 (60, 70)*	73 (72, 74)*
EUG	210 (210, 240)	48 (45, 48)	270 (270, 280)	58 (56, 60)	300 (300, 312.5)	68 (68, 70)
III concentration						
PHE	17.5 (15, 20)*	65 (63, 65.5)*	37.5 (33.75, 40)*	75 (74.5, 77)*	57.5 (50, 62.5)*	71 (70, 72)
EUG	420 (420, 420)	59 (58, 60)	450 (450, 450)	65 (63.5, 67)	495 (480, 500)	70 (70, 71.5)
IV concentration						
PHE	85 (80, 90)*	59.5 (59, 60)*	130 (120, 130)*	63 (62, 64)	140 (130, 150)*	73 (72, 74)
EUG	300 (300, 310)	56 (53.5, 56)	335 (330, 340)	65 (64, 68)	380 (360, 380)	73 (72, 74)

three concentrations and higher values of TRAS after administration of 2-Phenoxyethanol at the highest concentration. Moreover, higher values of OM were recorded with 2-Phenoxyethanol administration at all concentrations.

In the recovery phase, comparison between the substances revealed higher values of TRAS at all stages at all concentrations after administration of Eugenol; OM showed higher values in subjects treated with 2-Phenoxyethanol.

Mortality following anaesthesia: The mortality rate in the fish undergoing anaesthesia was 3.2%. Of these, there was only one subject treated with 2-Phenoxyethanol at a concentration of 200 mg L^{-1} and three with Eugenol (two at a concentration of 50 mg L^{-1} and one at a concentration of 60 mg L^{-1}).

Haematological parameters: The results of the serum cortisol levels, serum glucose levels and haematocrit levels are shown, respectively in Fig. 2-4.

In all of the eight experimental trials, there were a few differences in the blood parameters assessed between the two groups of subjects (control and anaesthetized fish). However, the trends and the statistical evidence did not identify substantial effects on blood parameters during anaesthesia with 2-Phenoxyethanol and Eugenol, except for the haematocrit values in the experimental trials with Eugenol. In fact, during three of the four trials, the haematocrit values were statistically higher ($p<0.01$) in the control fish compared with the fish treated with Eugenol while the opposite was observed during the remaining trial.

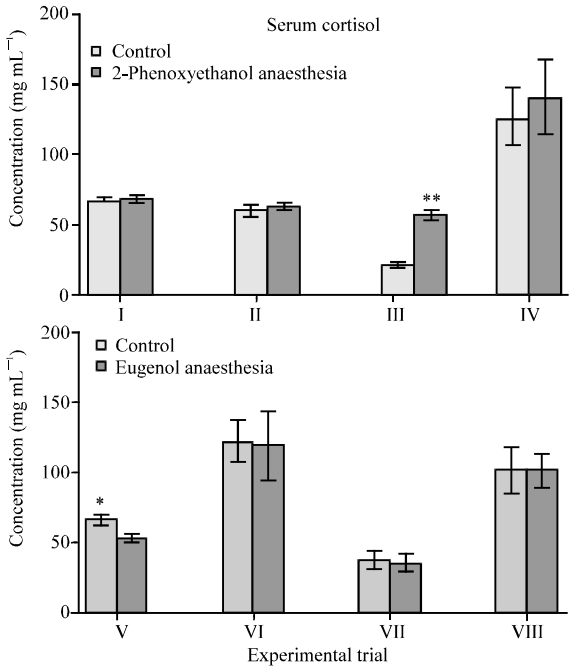

Fig. 2: Mean±standard deviation of serum cortisol levels (ng mL^{-1}) of control fish and those anaesthetized with 2-Phenoxyethanol and Eugenol (n = 160); * = $p<0.05$, ** = $p<0.01$

The researchers believe that this study has contributed to knowledge regarding the anaesthetic properties of the substances employed and the impact of these substances on the haematological indicators of stress in fish.

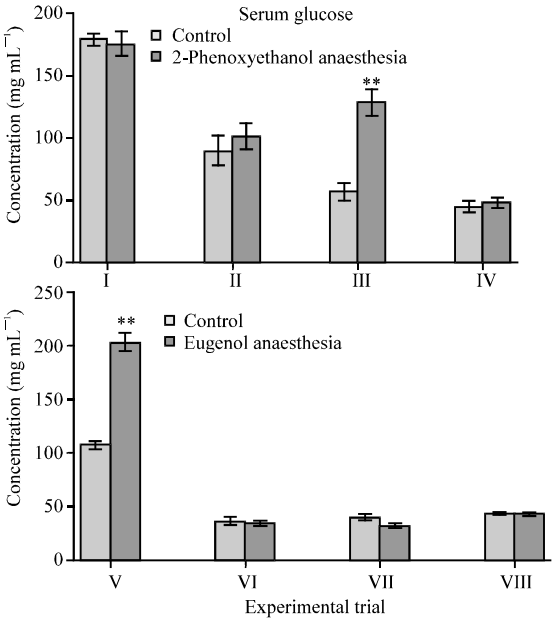

Fig. 3: Mean±standard deviation of serum glucose (mg dL^{-1}) of control fish and those anaesthetized with 2-Phenoxyethanol and Eugenol (n = 160); * = p<0.05, ** = p<0.01

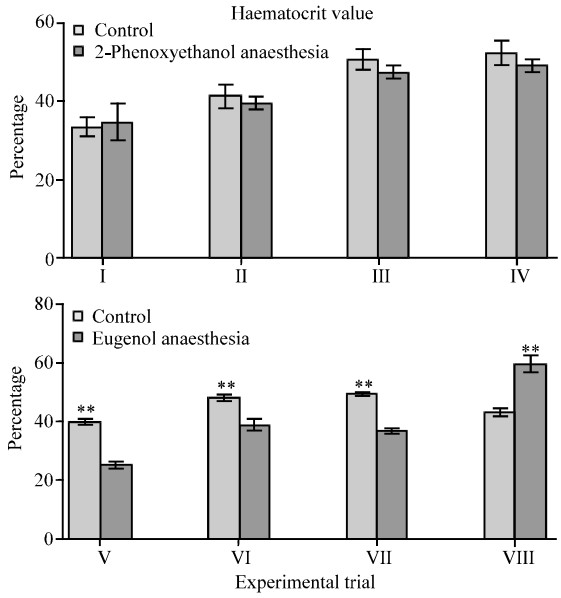

Fig. 4: Mean±standard deviation of haematocrit value levels (%) of control fish and those anaesthetized with 2-Phenoxyethanol and Eugenol (n = 160); * = p<0.05, ** = p<0.01

Evaluation of anaesthetic qualities: The results showed that higher anaesthetic concentrations resulted in shorter induction times both with Eugenol and 2-Phenoxyethanol administration as reported in other studies (Griffiths, 2000; Tort *et al*., 2002; Hamackova *et al*., 2004; Mylonas *et al*., 2005).

Comparison of the substances revealed the notable anaesthetic power of Eugenol as reported by other researchers (Keene *et al*., 1998; Griffiths, 2000; Pirhonen and Schreck, 2003; Woody *et al*., 2002; Cooke *et al*., 2004; Hamackova *et al*., 2004; Mylonas *et al*., 2005; Guenette *et al*., 2007). In fact, after administration of Eugenol the induction phase was rapid and subjects reached deep anaesthesia in significantly shorter times compared to 2-Phenoxyethanol at all concentrations except the lowest.

The frequency of opercular movements also assumed values that were generally lower after administration of Eugenol, revealing once again its notable anaesthetic power and its capacity to induce very deep and long-lasting anaesthesia even at intermediate concentrations. In general and in agreement with other researchers (Peake, 1998; Griffiths, 2000; Tort *et al*., 2002), the recovery times tended to increase with increasing concentrations of the two anaesthetics except for the highest concentration of Eugenol which in contrast to 2-Phenoxyethanol showed a faster recovery time than at the lower concentrations.

This is consistent with the study of Mylonas *et al*. (2005) in which European sea bass and gilthead sea bream treated with clove oil and 2-Phenoxyethanol showed similar or shorter recovery times at higher anaesthetic doses. They suggested that this may be explained by the fact that higher doses induced anaesthesia more rapidly and thus the fish were removed from the anaesthetic bath and placed in clear water earlier than fish exposed to lower doses. The shorter exposure time to the anaesthetic bath, the smaller amount of anaesthetic absorbed by the body and the faster its removal from the blood of the fish could explain the reduction in the recovery times. A similar phenomenon has also been reported in red pacu (*Piaractus brachypomus*) (Sladky *et al*., 2001) and in largemouth bass (*Micropterus salmoides*) using low concentrations of clove oil (Cooke *et al*., 2004).

The comparison of substances during the recovery phase showed particularly significant differences in the characteristics of the two anaesthetics, confirming the capacity of Eugenol to induce very deep anaesthesia in fish (Pirhonen and Schreck, 2003; Tort *et al*., 2002; Woody *et al*., 2002; Cooke *et al*., 2004; Hamackova *et al*., 2004; Mylonas *et al*., 2005). In fact, the subjects anaesthetized with Eugenol tended to recover very slowly even at low concentrations in comparison with the fish treated with 2-Phenoxyethanol which always recovered rapidly even at higher concentrations.

The frequency of opercular movements in the fish at recovery however, did not show any differences. In connection with the above, it seems opportune to refer to the data regarding the number of mortalities among the anaesthetized fish during the trials although, the percentages were low. Of these, there was only one sea bass that died as a consequence of anaesthesia with 2-Phenoxyethanol at the lowest concentration leading to the question of predisposition. The condition was different for Eugenol because the three subjects that died at the two highest concentrations confirm its capacity to induce markedly deep anaesthesia which in some cases could cause death.

In the light of such considerations and while highlighting the very high anaesthetic qualities of both substances, anaesthetics are a valid tool for facilitating the handling of fish in aquaculture. Moreover, the researchers feel that it is right to recommend their use in relation to the type of application to be performed as reported by other researchers (Hseu *et al*., 1998; Woody *et al*., 2002; Cooke *et al*., 2004). In fact, given the excellent inductive characteristics of Eugenol and the equally valid characteristics at recovery of 2-Phenoxyethanol, the researchers believe that the former is more suited to long handling practices or to major surgery and the latter more suited to short manipulation, minor surgery or transport.

Haematological parameters: In the present study, the statistically significant differences in the levels of blood parameters observed indicate an irregular trend between the values of the parameters recorded in the control and anaesthetized fish. Indeed, in particular regarding cortisol and glucose, some experimental trials recorded higher levels of the blood parameters in control subjects and in other trials higher levels in the anaesthetized subjects, precluding the identification of a clear and constant trend of the effects of the anaesthetic treatment on these parameters. The haematocrit differed in this respect being significantly lower in fish treated with Eugenol compared with control fish during the first three experimental trials and then the reverse in the last trial. No significant differences in the levels of haematocrit were recorded during the trials with 2-Phenoxyethanol.

The absence of reference data on the haematological parameters of this fish species treated with 2-Phenoxyethanol and Eugenol did not allow us to perform a clear comparison of the results with other studies.

However, in similar studies with different fish species, Holloway *et al*. (2004) and Velisek *et al*. (2005) detected an increase in glucose concentration in common carp (*Cyprinus carpio*) after Clove oil anaesthesia but no changes in haematocrit values. Moreover, Ortun *et al*. (2002) showed that in gilthead seabream, 2-phenoxyethanol anaesthesia produced a stress response (increase in cortisol and plasma glucose) in the fish although, the effects were greater with the narcotic dose (200 mu l L^{-1}) after an hour. Jinn-Rong *et al*. (1994) in yellowfin porgy showed significantly lower haematocrit values 24 h after anaesthesia as also reported by Gomulka *et al*. (2008) in the Siberian sturgeon treated with 0.075 mL L^{-1} of Eugenol.

These studies showed a significant change in the main blood indicators of stress following administration of the anaesthetics. This conflicts with the present study. However, as demonstrated by the study cited above in many cases the anaesthetics modify some haematological indicators of stress only a few hours after the anaesthetic treatment. In this regard, in this study, the blood samples were collected immediately after the final stage of anaesthesia induction to assess the real-time impact of anaesthesia on the fish. This is because the objective of this research was to verify the possible changes in blood parameters during deep anaesthesia with substances that may be used during common farming practices.

Finally, the results regarding the haematocrit values observed in the trials with Eugenol were interesting. In fact, during the first three trials, the haematocrit values were significantly lower in anaesthetized fish compared to control fish. However, the inverse condition that was observed during the last experimental trial (the highest concentration of Eugenol) allow us to hypothesize that in the experimental conditions of this study, the concentration of 50 mg L^{-1} of Eugenol could represent an end point for raising the levels of haematocrit in this species.

CONCLUSION

The results of this study suggest that the anaesthetics under consideration do not affect these blood parameters during the deep stages of anaesthesia and show no evidence at least during the anaesthetic stages considered that these substances are a valid tool for stress reduction in fish or a direct source of stress as described by other researchers (Cho and Heath, 2000; Ortun *et al*., 2002; Holloway *et al*., 2004; Velisek *et al*., 2005).

RECOMMENDATION

In future, in order to evaluate the impact of anaesthetic techniques on the haematological dynamics of farmed fish, it would be desirable to undertake further

studies on blood stress indicators through the evaluation of other reference parameters in addition to those considered in this study and for these parameters to be measured some hours after the anaesthetic treatment and in long-term stress conditions.

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