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Efficacy of phenoxyethanol and clove oil as anaesthetics in marine finfish cobia, *Rachycentron canadum*

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Abstract
The use of anaesthetics facilitates work with fish at the research level for weighing, sampling, disease diagnosis, cannulation and transport where the fish must be held immobile for extended periods of time. Many chemicals have proved effective in anesthetization of fish. In this study, we selected two anaesthetics, phenoxyethanol and clove oil to test the efficacy in anesthetization of cobia *Rachycentron canadum* .The efficacy of clove oil and phenoxyethanol as anesthetics was evaluated in the marine finfish cobia, *Rachycentron canadum*. Significant differences were found in the induction and recovery stages at different concentration levels of the anesthetics. Fishes exposed to 0.01-0.04 ml/L clove oil and 0.1-0.4 ml/L phenoxyethanol, had a 100% survival rate in the experimental period. The cobia exposed to the lowest concentration of 0.01 ml/L clove oil and 0.2 ml/L phenoxyethanol did not reach the stage 3 due to the insufficient concentration to obtain complete loss of reflexes and immobilization. The concentration at 0.02 ml.l^{-1} clove oil and 0.2 ml/L phenoxyethanol showed the complete loss of equilibrium at 4 min.07 sec. and 2 min.56 sec. respectively. After transferring them into the recovery tanks normal locomotor activity was observed. Based on our results both 2-phenoxyethanol and clove oil can be recommended for cobia, *Rachycentron canadum*. Our results indicate that the induction time of the fish exposed to the two anaesthetic solutions generally decreased with increasing concentrations, but the recovery time was increased with increasing concentrations. The effective concentration which produced anaesthesia within 3 min and allowed recovery within 5 min for phenoxyethanol is 0.2 ml/L and clove oil is 0.03 ml/L.

Keywords: anaesthetics –phenoxyethanol – clove oil- cobia.

1. Introduction
Anesthesia is a biological state induced by an external agent, which results in the partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non-chemical means [1]. In aquaculture, very often different manipulations should be done with the fish, concerning their artificial reproduction, surgical operations, marking, transport, blood sampling, etc., which can lead to stress, traumatism, or even to their death. All the manipulations are difficult due to the energetic resistance of the fish, as a result of which injuries, superficial and internal bleeding, incomplete spawn suckling and other negative effects can be reduced to a minimum by using different types of anesthetics. Application of anesthetics immobilizes the fish, decreases the stress and guarantees humane treatment of the fish. A number of chemicals have proved effective in anaesthetization of fish. Each has its own advantages and drawbacks. For example, only MS-222 (tricaine methanesulfonate) is registered by the Food and Drug Administration (FDA) for use with food fish in the USA but it is more expensive than a number of anesthetics. Clove oil received attention as an alternative fish anesthetic for a variety of fish species [2, 3, 4, 5, 6] as well as for crustaceans [7]. Clove oil has recently been suggested as an alternative fish anesthetic. Clove oil is a pale yellow liquid derived from the leaves, buds and stem of the clove tree (*Eugenia sp*.). Its active ingredients are eugenol (4-allyl – 2-methoxyphenol) and iso-eugenol (4-propeny1-2-methoxyphenol), which can comprise 90-95% of clove oil by weight. Clove oil is recognized as GRAS (Generally Regarded as Safe) substance by the US FDA for use in humans. Clove oil has a slightly faster induction time and a longer recovery time than similar concentration of TMS [4, 6].
The anaesthetic efficacy of 2-phenoxyethanol has been documented for many fish species including rainbow trout [8] and cod [9]. 2- phenoxyethanol was more suitable than either quinate or MS-222 to sedate non-food (ornamental) fishes during live transport [10].

Phenoxyethanol (PE) is a mild toxin and may cause some irritation to the skin, therefore any contact with eyes should be avoided [11]. Based on human toxicology data, it may also cause liver and kidney damage [1]. Phenoxyethanol does not block the stress response of fish and low doses have been shown to cause changes in plasma levels of cortisol, glucose, and lactate, with glucose and lactate levels being affected for over 24 hours post exposure [12].

2-PE is also known as phenyl cellosolve, phenoxyethanol, phenoxetol, ethylene glycol mono phenyl ether, and beta-hydroxy ethyl phenyl ether. The purpose of the present study was to determine the efficacy of clove oil and 2-phenoxyethanol as anaesthetics in cobia.

2. Materials and Methods

Clove oil (density 1.04g.ml/L) was purchased from Himedia laboratories Pvt Ltd, Mumbai, India). 2-phenoxyethanol (minimum assay 99.0%, maximum limits of impurities 0.005%) was obtained from Loba chemicals Pvt Ltd, Mumbai, India). Clove oil was dissolved in ethyl alcohol (95%) at a ratio of 1:9 and then added into the anesthetic tank with the volume of 100 litre water. For clove oil, the concentrations experimented were: 0.01 ml/L , 0.02 ml/L, 0.03 ml/L, and 0.04 ml/L. Phenoxyethanol did not require any preparation and it was directly mixed into the water. Phenoxyethanol efficacy was determined with four concentrations of 0.1 ml/L, 0.2 ml/L, 0.3 ml/L, and 0.4 ml/L. The cobia, *Rachycentron canadum* with a mean weight of 226±42 g was used for the experiment. The salinity and pH of experimental tanks ranged from 34-35ppt and pH of 8.2-8.3 respectively. Water temperature and dissolved oxygen were maintained at 27.8 ºC – 28.4 ºC and 5-8 mg/L respectively. For recovery from anesthetic effect, the fishes were transferred into recovery tank and monitored for 24 hours. The time for induction of anesthesia and recovery was measured with a stop watch, and the behavior of the fish was observed and analyzed according to the stages described elsewhere in this article [13].

Signs and Stages of Anesthesia in *Rachycentron Canadum* (Modified from Theinpoint and Niemegeers, 1965)

Induction stages

- Loss of balance, partial inhibition of reactions to external stimuli
- Total loss of equilibrium. Fish still react to strong stimuli.
- Total loss of reflexes and movement, Fish lay on bottom of the tank
- Complete cessation of opercula movements, death.

Recovery stages

- Start of movements. Fish still lay on the bottom of the tank
- Regular breathing. Reaction to strong stimuli. Irregular balance
- Total recovery of equilibrium. Reaction to slight stimuli. Normal swimming

ANOVA was used as a tool for statistical analysis. Duncan Multiple Range Test was used to find out the differences if any between various concentrations of both clove oil and phenoxyethanol tested in this experiment.

3. Results

The results obtained from the experiment are shown in Table 1 and 2. Significant differences were found in the induction and recovery stages at different concentration levels of the anaesthetics. Fishes from the rearing tank exposed to 0.01-0.04 ml/L clove oil and 0.1-0.4 ml/L phenoxyethanol, had a 100% survival rate in the experimental period. No deaths were occurred throughout the experiment. Fish exposed to 0.01 ml/L clove oil and 0.1 ml/L phenoxyethanol reached stage 2 of the induction phase. The cobia exposed to the lowest concentration of 0.01 ml/L clove oil and 0.1 ml/L phenoxyethanol did not reach the stage 3 due to the insufficient concentration to obtain complete loss of reflexes and immobilization. Complete loss of equilibrium was observed at 0.02 ml/L clove oil and 0.2 ml/L phenoxyethanol in 4 min. 07 sec.and 2 min.56 sec. respectively. Immediately after transferring them into the recovery tanks, normal locomotor activity was observed.

At a concentration of 0.03 ml/L of clove oil and 0.3 ml/L of phenoxyethanol, cobia lost equilibrium in 2 min.12 sec. and 2 min.12 sec. and they were completely immobilized after 2 min.56 sec.and 2 min.53 sec. respectively. At a concentration of 0.04 ml/L clove oil, fish reached stage 3 within 2 min. 19 sec. and the recovery and returning to normal swimming position was observed in 2 min.23 sec. Phenoxyethanol at the highest concentration of 0.4 ml/L, the stage 3 was attained in 1 min.56 sec. and recovery was found in 3 min. 23 sec.

Table 1: Induction and recovery time of cobia (*R. canadum)* exposed to clove oil

Clove oil (ml/L)	0.01		0.02		0.03		0.04	
	Mean Min'Sec''	SD	Mean Min'Sec''	SD	Mean Min'Sec''	SD	Mean Min'Sec''	SD
Induction time								
Stage 1	2'37'' ^{a}	0.03	2'12'' ^{b}	0.01	1'50'' ^{c}	0.02	1'32'' ^{c}	0.02
Stage 2	6'34'' ^{a}	0.01	3'43'' ^{b}	0.02	2'12'' ^{c}	0.02	1'56'' ^{c}	0.01
Stage 3	-		4'07'' ^{a}	0.02	2'56'' ^{b}	0.03	2'19'' ^{b}	0.01
Recovery time								
Stage 1	-		-		0'48'' ^{a}	0.01	0'52'' ^{a}	0.01
Stage 2	1'10'' ^{a}	0.01	1'12'' ^{a}	0.01	1'23'' ^{b}	0.02	1'27'' ^{b}	0.01
Stage 3	1'56'' ^{a}	0.02	1'39'' ^{a}	0.01	2'14'' ^{b}	0.02	2'23' ^{b}'	0.03

Each value is the mean and SD of duplicate tanks. Different superscript letters in the same row indicate significant statistical differences ($P < 0.05$).

Table 2: Induction and recovery time of cobia (*R. canadum)* exposed to phenoxyethanol

Phenoxyethanol ml.l^{-1}	0.1		0.2		0.3		0.4	
	Mean Min'Sec''	SD	Mean Min'Sec''	SD	Mean Min'Sec''	SD	Mean Min'Sec''	SD
Induction time								
Stage 1	0'53'' ^{a}	0.02	1'17'' ^{b}	0.02	1'24'' ^{b}	0.03	1'33'' ^{b}	0.02
Stage 2	4'50'' ^{a}	0.02	2'32'' ^{b}	0.02	2'12'' ^{b}	0.02	1'52'' ^{c}	0.02
Stage 3	-		2'56'' ^{a}	0.02	2'53' ^{a}'	0.01	1'56'' ^{b}	0.03
Recovery time								
Stage 1	-		-		0'43'' ^{a}	0.02	0'50'' ^{a}	0.03
Stage 2	1'26'' ^{a}	0.01	1'35'' ^{b}	0.02	1'45'' ^{c}	0.04	1'47'' ^{c}	0.02
Stage 3	2'51'' ^{a}	0.01	2'39'' ^{a}	0.01	3'45'' ^{b}	0.02	3'23'' ^{b}	0.02

Each value is the mean and SD of duplicate tanks. Different superscript letters in the same row indicate significant statistical differences ($P < 0.05$).

The recovery of cobia from anesthesia, after transferring them in clean water, was relatively quick when compared to the induction time in each concentration. For a short period of time they lay on the bottom of the tank and after a while they start to move their fins and make uncoordinated movements. They regain equilibrium and complete recovery as shown in Table 1 and 2.

4. Discussion

Efficacy of clove oil and phenoxyethanol on cobia was studied in this investigation. The onset of individual phases of anesthesia and recovery time depended significantly on the anesthetic concentrations used. In general, induction time decreased and recovery time increased with the increasing levels of anesthetic concentration. Alteration in physiological signs characteristics of cobia was observed similar to the stages of anesthesia described [13].

R. canadum reached the anaesthesia stage (3) more quickly at a lower concentration of clove oil than phenoxyethanol, and the recovery time of phenoxyethanol at the state (3) was significantly longer. Similar observations have been reported in other species of fish, when the efficacy between clove oil and TMS was compared in zebrafish *Danio rerio* [14], rainbow trout *O. mykiss* [6, 15] and red pacu *P. brachypomus* [16]. Result of this study demonstrated that when *R. canadum* was administered with phenoxyethanol at a concentration of 0.3 ml/L the fishes reached stage 3 of anaesthesia with complete loss of reflexes after 3 min. exposure. Therefore this dose could be recommended for procedures that require total fish immobilization in a short span of time. Clove oil at 40 mg/L was found better dose to obtain complete immobilization in rainbow trout *O. mykiss* (47.4±0.45 g) at 3 min. exposure time [15]. Similar concentration was reported safe to anesthetise rainbow trout (20.5 ± 0.73g) at 20 min. exposure time [6]. When compared to the concentration of phenoxyethanol, the effective concentration of clove oil was low. The induction time of cobia exposed to two anesthetics, clove oil and phenoxyethanol generally decreased with increasing concentrations. The efficacy of clove oil with 2-phenoxy ethanol at different temperature was compared and found that clove oil is efficient at 10 times lower dosages in comparison to those of 2-phenoxyethanol [17]. The efficacy of clove oil as an anesthetic was previously reported in a variety of fish including rainbow trout juveniles and adults [4], *Signus lineatus* [3] and non-salmonid fishes [18]. The anaesthesia and recovery were achieved with 4.3 and 10.9 min, respectively with a concentration of 60 mg/L at 10 ºC. This author indicated that exceeding the time of anaesthesia over 5 min. had a considerable influence on the time of recovery [18].

Based on our results, both phenoxyethanol and clove oil can be recommended as anesthetics in cobia. However, clove oil requires less than 10 fold concentration of phenoxyethanol. Moreover it is a natural anaesthesia. Clove oil is an efficient and relatively safe anesthetic [19]. In general, the main considerations in the anaesthetization of fish are safety, efficiency, price, toxicity and physiological response, duration of withdrawal and restriction of use [11, 20, 21]. The main ingredient of clove oil, eugenol can be regarded as highly safe for human use. Because it is 'generally recognized as safe (GRAS)' it is used as a food additive and an analgesic and disinfectant in dentistry [22]. But 2-phenoxyethanol was easy to use since it did not need any preparation as that of clove oil. Despite 2-phenoxyethanol requires comparatively high anesthetic doses in fishes. The range of effectively anesthetic doses of 2-phenoxyethanol in most fishes are from about 200 to 600 µl/l [1, 8, 9, 23].

The effective concentration of anesthesia is one which provides anaesthetic effect within 3 min. and allowed recovery within 5 min [13]. Based on this fact, it was concluded that phenoxyethanol at 0.2 ml/l and clove oil at 0.03 ml/l could be used to anaesthetic the fish.

5. Acknowledgement

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6. References

1. Summerfelt RC, Smith LS. Anaesthesia, Surgery and related techniques. In: Methods for Fish Biology (ed. by C. B. Schreck & P.B. Moyle). American Fisheries Society 1990, 213-272
2. Hikasa Y, Takase K, Ogasawara T, Ogasawara S. Anaesthesia and recovery with tricaine methanosulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*, Japanese Journal of Veterinary Science 1986; 48: 341-351.
3. Soto CG, Burhanuddin CG. Clove oil as a fish anaesthesia for measuring length and weight of rabbit fish (*Siganus lineatus)*. Aquaculture 1995; 135:149-152.
4. Anderson WG, Mckinlay RS, Colaveccia M. The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management 1997; 17:301-307
5. Munday PL, Wilson SK. Comparative efficacy of clove

oil and other chemicals in anesthetization of *Pomacentrus amboinensis*, a coral reef fish. Journal of Fish Biology 1997; 51:931-938.
6. Keene JL, Noakes DLG, Mocccia RD, Soto CG. The efficacy of clove oil as an anesthetic for rainbow trout *Oncorhychus mykiss* (walbum). Aquaculture Research 1998; 29:89-101.
7. Gardner C. Options for humanely immobilizing and killing Crabs. Journal of Shellfish Research 1997; 16:219-224.
8. Gilderhus PA, Marking LL. Comparative efficacy of 16 anesthetic chemicals in rainbow trout. North American Journal of Fisheries Management 1987; 7:288-292
9. Mattson NS, Riple TH. Metomidate, a better aneasthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol and phenoxyethanol. Aquaculture 1989; 83(1-2):89-94.
10. Guo FC, Teo LH, Chen TW. Effects of anaesthetics on the water parameter in a simulated transport experiment of platyfish, *Xiphophorus maculates* (Gunther). Aqua Res 1995; 26:265-271.
11. Bell G. An outline of anesthetic and anesthesia for salmonids, a guide for fish culturists in British Columbia. Canadian Technical Report of Fisheries Aquatic Sciences 1987; 1534:16.
12. Molinero A, Gonzalez J. Comparative effects of MS 222 and 2-phenoxyethanol on gilthead sea bream (*Sparus aurata L.*) during confinement. Comparative Biochemistry & physiology A-comparative physiology 1995; 111(3):405-414.
13. Theinpoint D, Niemegeers CJE. R7464-a new potent anaesthetic in fish. International Zoo Yearbook 1965; 5:202-205.
14. Grush J, Noakes DLG, Moccia RD. The efficacy of clove oil as an anaesthetic for the Zebra fish, *Danio rerio* (Hamilton). Zebra fish 2004; 1:46-53.
15. Pirhonen J, Schreck CB. Effects of anaesthesia withMS-222, clove oil and CO2 on feed intake and plasma cortisol in steelhead trout (Oncorhynchus mykiss). Aquaculture 2003; 220:507-514.
16. Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA. Comparative efficacy of tricaine methanesulfonate and clove oil anaesthetics in red pacu (*Piaractus brachypomus*). American Journal of Veterinary Research 2001; 62:337-342.
17. Mylonas CG, Cardinaletti G, Sigelaki I, Polzonetti-Magni A. Comparative efficacy of clove oil and 2-phenoxyethanolas anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. Aquaculture 2005; 246:467-481.
18. Peake S. Sodium bicarbonate and clove oil as potential anaesthetics for non salmonid fishes. North American Journal of Fisheries Management 1998; 18:919-924.
19. Taylor PW, Roberts SD. Clove oil: An alternative anaesthetic for aquaculture. North American Journal of Aquaculture 1999; 61:150-155.
20. Park IS, Hur JW, Song YC, Im JH, Johnson SC. Anaesthetic effect of lidocaine hydrochloride-sodium bicarbonate on the winter flounder *Pleuronectes americanus*. Ocean and Polar Research 2004; 26:475-480.
21. Kang EJ, Kim EM, Kim YJ, Lim SG, Sim DS, Kim YH *et al.* Effect of lidocaine hydrochloride and clove oil as an anaesthetic on Korean rose bitterling, *Rhodeus uyekii* and oily bitterling. *Acheilognathus koreensis* Journal of Aquaculture 2005; 18:272-279
22. Schnick RA, Meyer FP, Walsh DF. Status of fishery chemicals in1985. Progress Fish Culture 1986; 48:1-17.
23. Hseu JR, Yeh SL, Chu YT, Ting YY. Comparison of efficacy of five anesthetics in gold lined seabream. Sparus sarba Acta Zool Taiwan 1998; 9:11-18