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INDUCTION AND RECOVERY FROM ANAESTHESIA IN PIKE (*ESOX LUCIUS* L.) EXPOSED TO CLOVE OIL

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Abstract

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The aim of this experiment was to investigate the possibilities for using clove oil as anaesthetic in pike (*Esox lucius*). The experiments were carried out under controlled laboratory conditions. The following four concentrations were experimented: 0.02 ml.1^{-1} 0.04 ml.1^{-1} 0.06 ml.1^{-1} 0.08 ml.1^{-1}. Each concentration was tested with 12 pikes having an average body weight of 244.4 to 284.9 g, and the anesthesia was applied separately for each pike. The fish behavior was traced and analyzed, and the time for inducing anesthesia and recovery was timed with a stopwatch. At concentration of 0.02 ml.1^{-1} complete immobilization of the pikes was not achieved.At 0.04 ml.1^{-1} the fish were completely immobilized in 5'50" to 9'50" min. At concentration of 0.06 ml.1^{-1} the fish were anaesthetized for 4'15" - to 9'20" min, at 0.08 ml.1^{-1} the fish were anaesthetized for a period of 3'30" to 9'50". There was an inverse proportional dependence between recovery time and clove oil concentration.

Key words: pike (*Esox lucius*), clove oil, anaesthesia

Introduction

In aquaculture, very often different manipulations should be done with the fish, concerning their artificial reproduction, surgical operations, marking, measuring, 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 occur. To a great extent these negative effects can be reduced to a minimum by using different types of anaesthetics. Their application immobilizes the fish, decreases the stress and guarantees humane treatment of the fish.

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There is a relatively wide range of anaesthetic substances (Marking and Mayer, 1985; Gilderhus and Marking, 1987; Coyie et al., 2004; Hamackova et al., 2004; Hamackova et al., 2006), however only a few of them have found a practical application. The most widely spread are the following, MS-222, chinaldin, benzocain, carbon dioxide, 2-phenoxyethanol, etc. Recently special attention is paid to clove oil as an anaethetic substance in the aquaculture, and there is a good reason that this substance is considered as alternative of MS-222 (Anderson et al., 1997).

The Clove oil is a natural product, used for a long time in medicine, cosmetics and food industry as a food aromatizer. It has been applied in human medicine as a mild anaesthetic from ancient times (Ross and Ross, 1999; Taylor and Roberts, 1999). It has an antibiotic, antiseptic, antimicotic and antibacterial effect (Hamackova et al., 2006).

Clove oil is a dark brown liquid, which is distilled from the buds, leaves and stems of the clove tree *Eugenia caryophyllata* ((Briozza et al., 1989; Soto and Burhanuddin, 1995; Keene et al., 1998).

The eugenol is the active component of clove oil, and it represents 70-90% of its weight. Clove oil contains also eugenol acetate (>17%) and caryophyllene 5 (12%). There is a growing interest in clove oil as an anaesthetic in the aquaculture. Its advantages are: low price, pleasant aroma, quick induction and recovery from the anaesthesia, application of low concentrations and last but not the least, the fact that it is a safe natural product. The eugenol was tested for food industry and it was established that it is safe up to 1500 ppm (USFDA 1978, cit after Hamackova et al., 2006). It was used in Indonesia as an analgetic and pain-relief medicine against headache, toothache and arthritis pains (Ross and Ross, 1999).

Taylo (1999) established an effective dosage of 25 mg.1^{-1} of clove oil for inducing anaesthesia to *Oncorhynchus tshawytscha*, *Oncorhynchus kisutch*, *Oncorhynchus mykiss* and *Acipencer transmontanus*, as well as 10 min LC_{50} for the same species. Waterstrat (1999) and Small (2003) give information for an effective concentration of 100 mg.1^{-1} for *Ictalurus punctatus*. Woody et al. (2002) recommend a concentration of 50 mg.1^{-1} for inducing anaesthesia to *Oncorhynchus nerka*, in fish 400-500 mm in length and water temperature of 9-10^{o}C. Anderson et al. (1997) tested clove oil as an anaesthetic to *Oncorhynchus mykiss* and made the conclusion that this substance is an alternative to the most widely spread anaesthetic in fish-breeding – MS-222 (tricain). Hamackova et al. (2004) investigated the effects of 3 types of anaesthetics for inducing anaesthesia on *Tinca tinca*, including clove oil. The research was done at only one concentration of clove oil 0.033 ml.1^{-1}.

Hoskonen and Pirhonen (2004) experimented with 3 concentrations of clove oil (20-200 mg.1^{-1}) at water temperature of 5, 10, 15 and 20^{o}C for the following fish species: Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), whitefish, powan (*Coregonus lavaretus*), roach (*Rutilus rutilus*) and rainbow trout (*Oncorhynchus mykiss*).

Velisek et al. (2005b) studied the anaesthetizing effect of clove oil on carp (*Cyprinus carpio*), with the purpose to investigate its toxic effect including LC_{50}. Similar investigation was carried out with rainbow trout (*Oncorhynchus mykiss*) by Velisek et al. (2005a). Data for the toxic effect of clove oil concerning wels (catfish) (*Silurus glanis*) was indicated by Velisek et al. (2006).

Mylonas et al. (2005) compared the efficiency of clove oil with 2 phenoxyethanol at different temperatures and established that it is efficient at 10 times lower dosages in comparison to those of 2 phenoxyethanol. Similar comparison was made by Tamaru et al. (1996), who stressed upon the better efficiency of clove oil.

Similar data concerning clove oil activity as an anaesthetic in the aquaculture was given by Hamackova et al. (2006). The results from the researches by many other authors, as well as their own experimental data obtained for various fish species, were cited in this paper. The authors accentuated on the great potential of clove oil application as fish anaesthetic, during different manipulations and recommended concentrations of 0.033 ml.1^{-1} for most of the species, for the Siberian sturgeon (*Acipenser gueldenstaedti*) – 0.07 ml.1^{-1}, especially.

The researches on clove oil anaesthetic effect upon pike (*Esox lucius*) are limited and insufficient. Peake (1998) indicated the concentration of 60 ml.1^{-1} (approximately, 0.057 ml.1^{-1}), as efficient for pike. Hamackova et al. (2006) recommended the concentration of 0.025-0.033 ml.1^{-1}, as efficient for pike.

Materials and Methods

The experiments for determining the efficiency of clove oil as an anaesthetic, were carried out under controlled laboratory conditions. Clove oil as a commercial product was purchased from “Bulgarska Roza” Ltd., Karlovo. Its anaesthetic effect was tested at water temperature of 10°C, at which most often the different manipulations related to fish propagation process are carried out. The following 4 concentrations were experimented: 0.02 ml.1^{-1}, 0.04 ml.1^{-1}, 0.06 ml.1^{-1}, and 0.08 ml.1^{-1}. Before the preparation of the working solution the clove oil was dissolved in ethyl alcohol (95%) at a ratio of 1:9, and then was added into the experimental tanks with volume of 20 l of water. Each one of the concentrations was tested with 12 pikes, having an average body weight of 244.4 to 284.9 g. The pikes were separately placed for anaesthesia. 48 pikes in total ware used for this experiment.

For recovery from the anaesthetic effect of clove oil, the fish were transferred into 1 m^3 tanks and there they were monitored for 24 h.

The time for induction of anesthesia and recovery was measured with a stopwatch, and the behavior of the fish was observed and analyzed according to the phases described in Table 1.

Table 1
Phases of induction and recovery form anesthesia

Anesthetizing		Recovery	
Phase	Behavior of the fish	Phase	Behavior of the fish
1.	Acceleration of the opercular movements, increased respiratory activity	1.	Weak, uncoordinated locomotion.
2.	Decresed respiratory activity accompanied by uncoordinated locomotion	2.	Regaining of the normal position. Decreased locomotor activity
3.	Loss of equilibrium, decreased opercular movements, the fish still react to strong external stimuli	3.	Normal position of the body. Normal locomotor activity is
4.	Complete immobilization, the fish lie on the bottom and do not react to handling		
5.	Complete cessation of opercular movements, the fish die if they remain in the solution.		

In this study the main focus was on the time needed for the catfish to reach phase 3 and 4 of anesthesia and respectively the period needed for recovery.

In order to determine the toxicity of clove oil, OECD 203 for "Fish, Acute Toxicity Test" was used. Fish were exposed to concentrations of 0.02-0.2 ml.1^{-1} for 10 min, at concentration increase for each following exposition by 0.02 ml.1^{-1}. For each concentration, as well as for the control group, 12 pikes were used. On the basis of the results obtained, the average value of LC_{50} for a period of time of 10 min was calculated.

Results and Discussion

The results obtained from the experiment are shown in Table 2. The pikes exposed to the lowest concentration of 0.02 ml.1^{-1} did not reach stage 4–complete immobilization. They partially lose equilibrium for 2'42'' to 8'. Immediately after transfring them into the recovery tanks normal locomotor activity is observed.

At a concentration of 0.04 ml.1^{-1}, pikes lose equilibrium in 1'40'' to 4'00'', they are completely immobilized in 5'50'' to 9’50'' min. The recovery from anaesthesia and returning to normal position is done within 0'20'' to 9'30''.

At concentrations of 0.06 ml.1^{-1} fish reach phase 4 within 4'15'' - 9'20'', and the recovery and returning to normal swimming position is done in 2'33'' to 7'50''.

At the highest concentration of clove oil of 0.08 ml.1^{-1} fish are anaesthetized (phase 4) in 3'30'' to 9'50'', they regained their normal position in 1'50'' to 10'30''. On the basis of the experiment, LC_{50} (0.18 ml.1^{-1}) value was determined when exposing the pikes to clove oil for a 10 min period.

Fish absorb the anaesthetic substances mainly

Table 2
Time for induction and recovery from anaesthesia of pike (*Esox lucius*) exposed to diferent concentrations of clove oil

Dose ml. l^{-1}	Feature	Body weight /BW/, g	Body length /SL/, cm	Induction of anesthesia			Recovery from anasthesia		
				Decreased locomotor activity, min' sec"	Total loss of equilibrium, min' sec"	Imobilization, min' sec"	Uncoordinated locomotion, min' sec"	Decreased locomotor activity, min' sec"	Normal position, min' sec"
0.02	x	244.4	28.7	5'45"					
	SD	38.38	2.07	1.75					
	Cv%	15.7	7.21	30.57					
0.04	x	284.9	29.35	2' 37"	3' 58"	7' 45"	2' 31"	3' 57"	12'00"
	SD	144.05	3.87	0.81	1.18	1.53	1.59	3.04	1.96
	Cv%	50.56	13.19	31.68	29.73	19.77	63.38	77.17	16.28
0.06	x	263.1	29.9	2' 11"	3' 35"	7'00"	2' 38"	4' 42"	11' 42"
	SD	58.21	1.96	0.51	1.1	1.76	1.8	1.7	2.36
	Cv%	22.12	6.58	23.09	30.75	25.01	68.09	36.22	20.12
0.08	x	251.3	28.93	1' 38"	2' 20"	6'00"	2' 14"	4' 54"	12' 04"
	SD	72.78	2.07	0.22	0.46	1.98	1.32	2.45	2.09
	Cv%	28.96	7.16	13.75	19.77	33.09	58.92	50.26	17.37

through their gills (Locke, 1969; Ferreira et al., 1984). Regarding this, the gills area related to body weight has a significant importance for the efficient concentrations of anaesthetic substances. Together with this, the different fish species have different metabolic rate, which reflects upon the rate of anaesthetics absorption, and respectively upon anaesthesia induction. In this respect, for different fish species the adequate effect of anaesthetics is observed at different concentrations.

When pikes are placed in the working solution, they move vigorously for about 1 minute, after which they calm down. The visual effect of clove oil can be established at first by the acceleration of opercular movements and by the partial loss of reaction to external stimuli, but it can be best observed in phase 3 – loss of equilibrium. In this stage fish lie on their back or sideways and turn over periodically. Gradually, the intensity of their movements decrease and they enter phase 4. Pikes lie at the bottom, they do not move and they do not react to external stimuli. Different manipulations can be done on them at this stage, including surgical operations, injections, etc.

The recovery of pikes from anaesthesia, after transferring them in clean water is relatively quick. 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. In time they regain equilibrium, which they could loose several times until their complete recovery.

The analysis of data from Table 2 shows that at the lowest concentration – 0.02 ml.1^{-1}, fish reach phase 2, without passing into phase 3. From this point of view, the concentration of 0.02 ml.1^{-1} cannot be used for anaesthetizing of pike, it has only sedative effect. Similar data for the same and for lower concentrations of clove oil was announced by Hajek et al. (2006) regarding common carp. The period of time in witch pikes reach phase 1 and 2 when exposed to clove oil, as well as their recovery at the given concentration varies within a comparatively small degree as far as individual fish are concerned.The effect of clove oil on the pike at the next two concentrations of 0.04 and 0.06 ml.1^{-1} is approximately the same. Fish lose their equilibrium after 2'36" and 2'11", respectively and reach phase 4, at an average of 7'45"for the first concentration and 7'0" for the second (higher) concentration. Their recovery of equilibrium and normal body position is done at an average in 3'57" at 0.04 ml.1^{-1} and 4'43" at 0.06 ml.1^{-1}.

At the highest concentration of 0.08 ml.1^{-1}, pikes lose their equilibrium in the shortest time and again in the shortest time reach stage 4 (complete loss of reactivity) at an average of 6'0", but at the highest degree of time variation (Cv, 33.09%) for reaching it. The average time values for regaining a normal position are highest one - 4'52" min and is near that of the concentration of 0.06 ml.1^{-1}, but at considerably grater variation (Cv, 50.26%).

It should be noticed that when the concentration of clove oil is increased, the necessary time for loosing the equilibrium, as well as for reaching stage 4 (complete anaesthesia) is decreased. The reverse dependency is observed when pikes recover from anesthesia and regain normal swimming position. At higher concentrations, this process takes longer, and the fish at the lowest concentration recover in the shortest time. Similar dependencies were established by other authors for the application of clove oil for anaesthesia of different fish species (Keene et al., 1998; Hoskonen and Pirhonen, 2004).

Conclusions

The experiment confirmed the good possibilities of using clove oil as an anaesthetic when carrying out different manipulations on the pike (*Esox lucius* L.). A concentration of 0.02 ml.1^{-1} has only a sedative effect. Pikes loose their equilibrium but do not reach phase 3. The best results in regard to the time for reaching phase 4 (complete anaesthesia) and the time for complete recovery are observed at a concentration of 0.06 ml.1^{-1}. At this concentration pikes reach phase 4 of anaesthesia – a complete loss of reactivity in 7'00", and recover in 4'43".

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